Paediatric High-Grade Glioma: Biologically and Clinically in Need of New Thinking

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[Background]

- Paediatric high-grade gliomas are classified as World Health Organisation (WHO) astrocytoma grades III and IV.
- High-grade gliomas in children are different from those that arise in adults. (E.g. DNA copy number and gene expressions differ)
- It is important to avoid therapeutic strategies developed purely using data obtained from studies on adult glioblastoma since this approach has resulted in repetitive trials and ineffective treatments being applied to these children with limited improvements in clinical outcome.
- Our understanding of the origin and biological features of childhood brain tumours has been revolutionized through the application of genome- and epigenome-wide molecular profiling techniques.
- Age at presentation, anatomical location, and prognosis have clear impact on distinct cellular origins and biological drivers.

[Unique Biological Drivers and Distinct Therapeutic Targets]

- The unique biology of tumours in children is best illustrated by the identification of somatic histone mutations.
- Specific recurrent mutations in genes encoding:
  - **H3.3 (H3F3A)**
  - **H3.1 (HIST1H3B, HIST1H3C)**

    Mutations in these histone variants result in AA substitutions at 2 key residues in the histone tail:
  - **K27M** (lysine-to-methionine at position 27)
  - **G34R/V**

    (glycine to arginine or valine at position 34).
- These mutations are not yet found in other cancer types (e.g. glioblastoma in the elderly populations); similar variants have been reported in rare childhood bone tumours: H3F3A G34WL and H3FB K36M).
- These mutations are mutually exclusive. (Cannot happen at the same time).
- These mutations rewire the epigenome and deliver potent and distinct oncogenic insults to susceptible pools of progenitor cells, likely originating early in neurodevelopment.
- Harbour distinct/exclusive anatomical distributions of tumours:
  - H3.3 G34R/V in the cerebral hemispheres.
  - H3.3 K27M throughout the midline structures (including thalamus, brainstem, cerebellum, and spine).
  - H3.1 K27M restricted to the pons.

*****DIPGs*****

DIPGs: a non-surgically resectable glial tumour of the pons usually displaying histological features ranging from grade II to grade IV.

More than 85% of DIPGs harbour a K27M mutation in one or the other histone variant (H3.1 and H3.3).

H3.1 mutant tumours display a younger age, distinct clinicopathological and radiological features, and a slightly longer survival time.

H3.3 mutant tumours display a older age. G34R/V mutations also usually occur at an older age group.

- K27M mutation confers loss of the transcriptionally repressive trimethyl mark at lysine 27 on the histone tail – easily detected by IHC.

- The relationship of histone Mut and histone WT DIPG is uncertain.

- Both histone H3 mutations (H3.3 and H3.1) lead to a reduction in DNA methylation throughout the epigenome.
  - DNA methylation usually inhibits transcription.
  - K27M globally, G34R/V mostly at subtelomeric regions.

MGMT gene promoter region encodes a DNA repair enzyme which acts by removing alkyl groups from the O6 position of guanine.

The newly alkylated MGMT protein (suicide enzyme) is then marked for degradation by ubiquitination.

Epigenetic silencing of the MGMT gene by methylation of the CpG islands of the promoter region => shown to correlate with loss of gene transcription and protein expression.

Loss of MGMT protein expression results in decreased DNA repair and retention (accumulation) of alkyl groups - this allows alkylating agents such as

temozolomide to have greater efficacy in patients whose tumours exhibit hypermethylation of the MGMT promoter and reducing the MGMT protein concentration.

= This hypermethylation is associated with resistance in adult glioblastoma to alkylating agents such as temozolomide (TMZ).

HOWEVER,

Whilst MGMT hypermethylation is one of the markers of adult glioblastoma, this is not as relevant in children.

This may be due to:

- MGMT methylation being predominantly found in the H3.3 G34R/V subgroup and less frequent in tumours with K27M mutations.

  = This might be likely to be contributing to the lack of clinical response to TMZ in most HGG including DIPG across numerous trials.

- Aside from possessing a distinct epigenetic profile, these histone-defined subgroups frequently co-segregate with differential secondary genetic alterations:
  - H3.3 G34R/V group with ATRX.
  - H3.3 K27M (Thalamic) with FGFR1.
  - H3.1 K27M (DIPG) with ACVR1.
    *Common TP53 mutation is absent in H3.1 K27M subgroup.
    (But for ICR_B301 and SU_DIPG_4, they have H3.1 K27M mutation + ACVR1 missense mutation + TP53 missense mutation altogether).

    Other patient-derived cell H3.1 K27M mutant cell lines such as SU_DIPG_36, OPBG_DIPG_015/018 are ACVR1 mutant and TP53 WT.

As seen above, despite the ability to subclassify tumours based on histone H4 mutations,

!!More than half of all childhood diffuse infiltrating gliomas do not fall into this these categories!!

Examples:

- < 5% harbour hotspot mutations in the IDH1/2 genes associated with global hypermethylation ("G-CIMP").
  = Likely to represent the younger tail of an age distribution for these tumours
- 5%-10% of predominantly cortical tumours harbour activating BRAFV600E mutation.
  - ➔ Have histological and epigenetic similarities to pleomorphic xanthoastrocytoma.
  - ➔ Better clinical outcome.
  - ➔ Frequently co-segregate with CDKN2A/B (p16) deletion.

*Low-grade gliomas have mitogen - activated protein kinase pathway activation.

Patients whose tumours have these mutations are candidates for target-driven clinical trials and provide a paradigm for translational progress in this disease.

The remaining tumours form a heterogenous group with numerous potential driver events, which are poorly defined in terms of distinct molecular and clinicopathological features.

Examples:

- SETD2 mutations
  (SETD2 = histone methyltransferase)
  - May extend the proportion of tumours with dysregulated H3K36 trimethylation (=likely consequence of H3.3 G34R/V mutation).
  - Frequently harbour alternations associated with RTK activation, most commonly through PDGFRA Amp and/ or mutation (frequently in association with H3.3 K27M mutation).
    *Whilst adult glioblastoma frequently involves EGFR, children's glioma doesn't.
- Gene fusions involving the NTRKs 1-3; associated with LGG rather than HGG in many cases.
- MYCN amplifications are also seen.

= > A high risk group involves K27M mutation and/or amplification of PDGFRA, MYCN, etc.
= > An intermediate group is enriched for G34R/V and IDH1 mutations.

There is a significant proportion of non-histone, non-IDH1, non-BRAF mutated tumours with remarkably stable genome profiles …

[Tumour Microenvironment of the Childhood Brain]

Several observations highlight:
pHGGs 'hijack' mechanisms of development and plasticity in the childhood brain.
(May elucidate novel therapeutic strategies in the future)

While the cell of origin for pHGGs is still controversial, multiple lines of evidence point to a neural precursor cell, possibly in the oligodendroglial lineage.

Consistent with this hypothesis, pHGGs occur in relatively discrete spatial and temporal patterns that coincide with waves of developmental myelination in the childhood and adolescent brain.

It was observed that the elevated neuronal activity promotes the proliferation of oligodendroglial lineage precursor cells

=> Hypothesis: Active neurons may play a role in the pHGG microenvironment.

Results:
Excitatory neuronal activity was found to robustly promote the growth of pHGG, including paediatric cortical glioblastoma and DIPG.

Discussion:
Neuronal activity-regulated pHGG growth depends upon activity-regulated secretion of neuroligin-3 and brain-derived neurotrophic factor.

*Neuroligin: A synaptic adhesion molecule that promotes glioma proliferation through stimulation of the PI3K – mTOR pathway.

These observations highlight the manner in which pHGGs "hijack" mechanisms of development and plasticity in the childhood brain and may elucidate novel therapeutic strategies in the future.

Immune cells, particularly tumour-associated macrophages (TAMs) are known to play an important role in the microenvironment of low-grade paediatric gliomas and in adult high-grade gliomas.

- Mouse models of low-grade glioma reveal a tumour growth-promoting effect of TAMs.
- Similarly, in adult gliomas, the number of cells in the tumour mass immunopositive for CD68 expression (CD68+) increases with increasing tumour grade and M2 phenotype microglia appear to promote glioblastoma growth.
  *CD68: Marker for activated microglia/ macrophage.

- The effects of TAMs on glioma growth and progression appear to depend on the TAM activation state along the M1-M2 spectrum:

  M2 Phenotype TAMs = Promote tumour growth.

  M1 Phenotype TAMs = Potentially inhibit growth.

- Gene expression data from paediatric infiltrating astrocytoma demonstrate enrichment in expression of M1 and M2 microglia/macrophage gene signatures. => Different TAMs from Adult:
- The differences in TAM phenotype compared to the adult is represented from the observation that in pilocytic astrocytomas of childhood, tumour recurrence correlates inversely with CD68+ TAMs.

- Microglia account for approximately one third of the cellular mass of DIPG.

= > The functional role of TAMs may play in DIPG and other infiltrating gliomas of childhood remains to be determined.

Our understanding of TAMs in paediatric infiltrating gliomas remains incomplete, so variety of immune-modulatory strategies are currently being explored presently.

- Blockade of programmed cell death protein 1 (NCT01952769 and NCT02359565)
- Tumour vaccine strategies (NCT01400672 and NCT00874861)

*NCT=trial number

** Unfortunately, one blocker trial of programmed cell death protein 1 using pembrolizumab was stopped for safety concerns, but still highlighted precarious location of the pons for inflammatory and subsequent edema.

[A Clinical Conundrum and a Way Forward]

A significant number of prospective clinical trials for children with HGG for the past 4 decades, but little improvement in the patient outcome.

Note that whilst most studies studying adult brain tumours are limited to WHO grade IV astrocytoma (i.e. glioblastoma), the majority of pHGG clinical tirals have included both WHO grade III (anaplastic astrocytoma) and grade IV tumours.

***The first prospective, randomized clinical trial for children with HGG was published in 1989 by the Children's Cancer Study Group.

Result:
= Showed a significant improvement in outcome using adjuvant radiation therapy (RT) followed by pCV chemotherapy (prednisone, chloroethyl- cyclohexyl nitrosourea [CCNU], and vincristine) compared with RT alone.

RT + pCV > RT alone.

Addition of chemotherapy led to a dramatic increase in 5-year progression-free survival from 16% to 46%, and as a result no subsequent cooperative group study used an "RT only" arm going forward.

However, the survival rates on both treatment arms on this particular trial far exceeded what has been observed in more recent studies. The discrepant results were concluded to be due to including of large numbers of low-grade gliomas. (Majority of the differences were from inclusion of low-grade tumours).

Contemporary trials using updated neuropathology criteria and central review have shown 3-year event-free survival (EFS) and overall survival (OS) rates of ~10% and 20%, respectively. The patients with HGGs in this study had a similar poor OS rate of 20% at 5 years.

These studies demonstrated that the strongest factors associated with more favourable outcome were:

- Extent of resection (>90% resection)
- Low methylation-inhibited binding protein 1 indices
- Non-overexpression of p53.

Single agent Temozolomide (TMZ), when administered during and after RT, has been shown to significantly prolong EFS and OS in <u>adults</u> with glioblastoma compared with RT alone.

HOWEVER, in the Children's Oncology Group (COG) single-arm study (ACNS0126), Children's outcomes were not improved when treated with similar adjuvant chemotherapy regimens.
= The predictive value for benefit from TMZ has not been demonstrated as in the adult setting.

In the subsequent COG HGG trial, ACNS0423, CCNU was added to TMZ during maintenance.
(Not published)

Result:
1 year OS = 100% was reported in patients with isocitrate dehydrogenase 1 (IDH1) mutant tumours vs. 81% in those with IDH WT tumours. (P = 0.03)

Underscored the strong prognostic value of IDH1 mutations in paediatric HGG patients, similar to adults.

The most recent COG HGG trial ACNS0822 – compared 2 different experimental arms with vorinostat or bevacizumab during maintenance therapy post RT.
However, study permanently closed in 2014 during phase II since no arm showed any clear superiority.

German HIT-GBM-Cooperative group study – Not much retrieved from the results. Not meaningful.

The use of adjuvant pCV + RT provided no survival benefit in patients with brainstem tumours, including DIPG. No subsequent DIPG trial has shown convincing benefit of any adjuvant therapy beyond RT alone (independent of whether the chemotherapy is given before, during, and/or after RT).

The use of higher doses of RT, given in hyperfractionated regimens, to cumulative doses as high as 7800 cGy (absorbed organ dose), has shown no survival benefit and increased neurotoxicity.

= The studies mentioned above are but a small number of those that have attempted to improve the outcome of non- brainstem HGG and DIPG. Unfortunately, based on a limited understanding of the biology and heterogeneity of these tumors, most studies resulted in added toxicity and little activity.

Problem:

DIPGs arise in an eloquent brain area for which resection cannot be accomplished, and biopsy was considered to carry substantial risk. DIPGs frequently are associated with typical radio- logical features on MRI; a decision to forgo surgical biopsy resulted in limited tissue being available for subsequent molecular analysis. Excessive caution and the inability to use biological information to guide clinical studies resulted in a very limited understanding of the biology of DIPGs.

BUT!

Efforts of centres that performed surgical biopsies on patients prior to treatment, coupled with increased emphasis on rapid obtainment of autopsy tis- sue for molecular analysis, did the field advance more in 5 years than in the prior 50 years combined.

The identification of the chromatin mutations, their association with aberrant pathway activation that appears to segregate into different groups, and the identification of a new target not previously associated with any cancer has changed the very landscape by which we think about these tumours.

pHGG a biologically diverse group of tumours than a single disease -> clinical behaviours more closely follow tumour biology (i.e. molecular genetics and epigenetics) rather than histological grading
= > Novel and emerging drugs for treatment of HGG will likely target only a subset of pHGG, resulting in relatively small eligible populations for targeted clinical trials.

Problem:
The issue of poor drug delivery, as a result of the blood–brain barrier, may be a major reason for failure in both adult and paediatric diffuse HGG.

To date, direct measurement of tumour drug concentrations in DIPG has not been reported, and it is postulated that the blood–brain barrier is more intact and hence a greater barrier than in other non-brainstem CNS tumours.

As promising agents with a strong biological rationale emerge, it is essential that the ability to achieve required target drug concentrations is studied in both accurate pre- clinical models and, if appropriate, confirmed in proof of mechanism clinical trials measuring tumour drug concentrations in post-biopsy/surgical resection.
In DIPG,

Efforts have demonstrated the potential therapeutic efficacy of epigenetic modifying agents targeting histone demethylases and histone deacetylases.

Both classes of agents promote restoration of histone-3 K27 trimethylation through differing mechanisms and demonstrate synergy when used in combination.

Example:
Menin inhibition showed decreased DIPG proliferation. Menin is a member of trithorax group, which is a complex that antagonises the K27me3, depositing polycomb repressive complex.

[Conclusions]

The general challenges for future clinical trial design in pediatric HGG are 4-fold: (i) lack of currently actionable alterations in a large proportion of patients, (ii) intratumor heterogeneity and molecular pathway redundancy, (iii) issues with drug delivery including poor blood–brain barrier penetration of many molecular targeted agents, and (iv) small subsets of patients for each given biology and target expression. While novel clinical trial designs are needed, large-scale studies using adaptive clinical trial designs as proposed for adult glioblastoma86 will only be feasible in the much smaller paediatric population through international collaboration.

Additional layers of complexity that remain poorly understood but will need to be addressed include intratumoral heterogeneity and clonal evolution at the single cell level, as well as the role of the tumour microenvironment, tumor metabolism, and tumour immunology, and how to exploit them therapeutically.

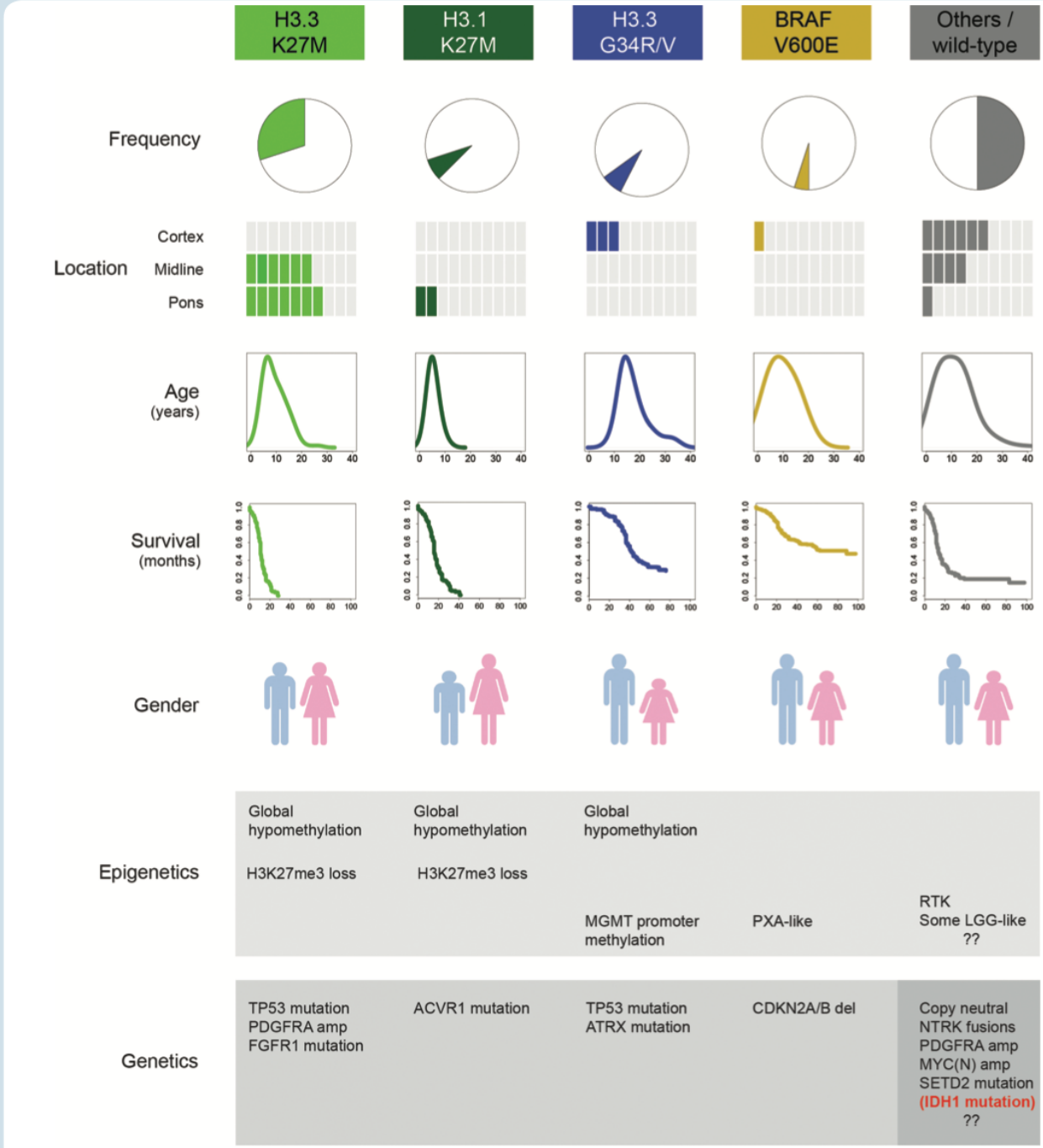

Fig. 1 *Biologically and clinically defined subgroups of pediatric infiltrating glioma.* Specific, selective, recurrent, and mutually exclusive mutations in the genes encoding the histone H3.3 (*H3F3A*) and H3.1 (*HIST1H3B*, *HIST1H3C*) variants, along with *BRAF*V600E, mark distinct subgroups of disease in children and young adults. There are clear differences in location, age at presentation, clinical outcome, gender distribution, predominant histology and concurrent epigenetic and genetic alterations. The remaining half of tumors comprising these diseases harbor numerous, partially overlapping putative drivers or other (epi)genomic characteristics, but as yet do not form well-validated biological and clinical subgroups. The small proportion of children (mostly adolescents) with *IDH1* mutations represent the lower tail of age distribution of an otherwise adult subgroup, and are excluded here.