



Diffuse Intrinsic Pontine Gliomas. DIPG

The DIPG “ranks first amongst
pediatric brain tumors with a poor
prognosis.”

J. Neurosurgery Peds 3:257-258, 2009



History

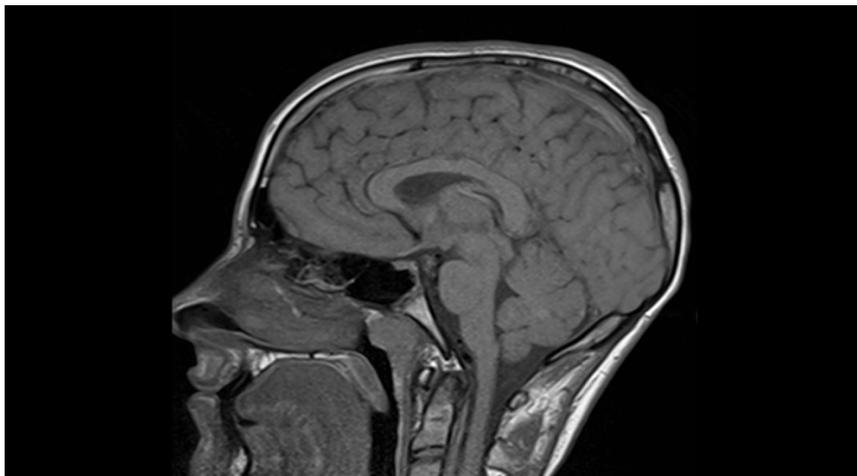
- Tumors of the brain stem were recognized in childhood during the CT era
- However the brain stem is a complex structure with 3 basic parts, the midbrain, the pons and the medulla.
- CT could not distinguish accurately where a tumor was in the brain stem.



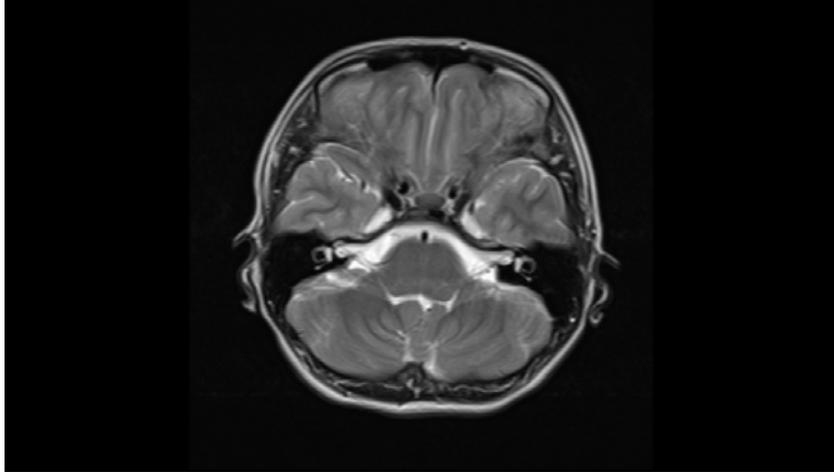
Early Optimism

- With radiation to brain stem gliomas in the CT era there was a high response rate and a roughly 20% survival.
- Much optimism that these results could be improved by either modifying radiation or adding chemotherapy.
- However in the MRI era, it became obvious that the 65 to 70% who died from brain stem gliomas were in fact mostly diffuse intrinsic tumors of the pons.
- Tumors that were in the medulla, in the mid-brain or focally in the pons were usually low grade tumors that were either did not need therapy or were amenable to treatment.

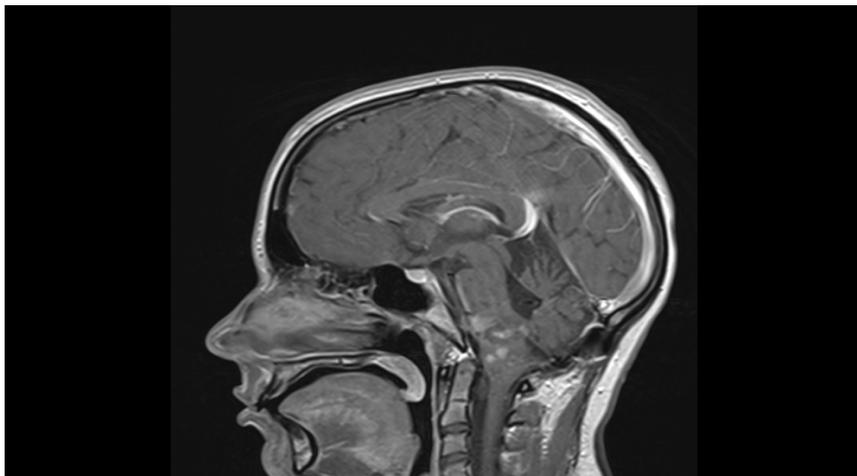
Normal Pons, T1



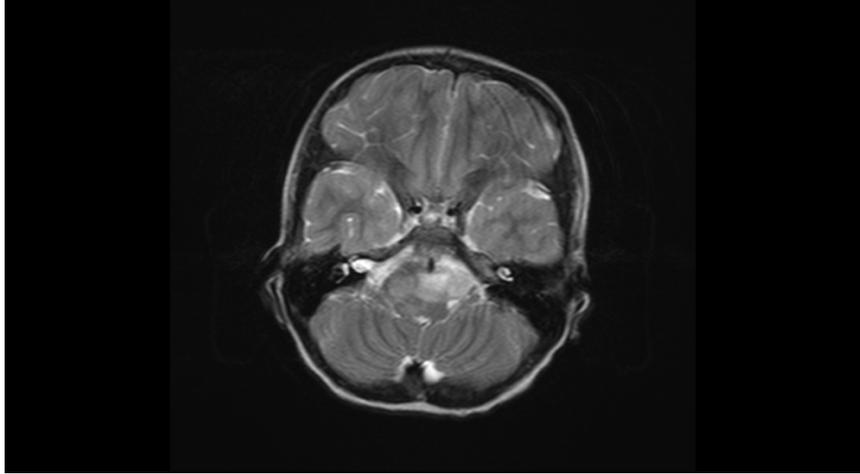
Normal Pons, T2



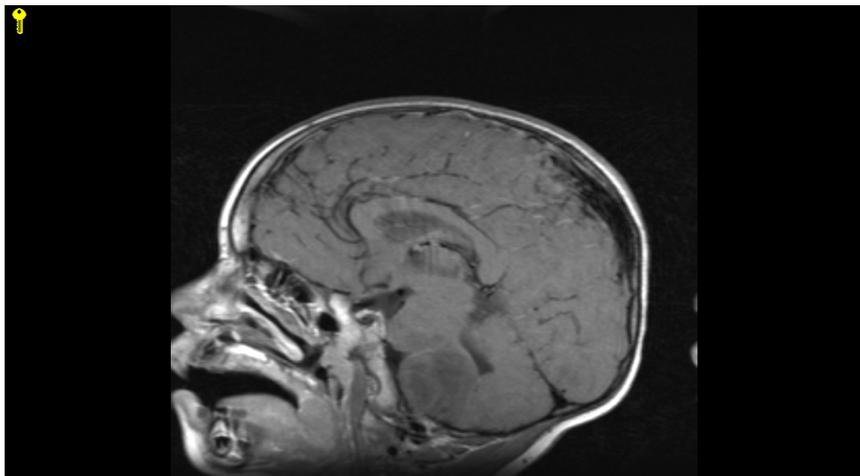
Low grade tumor of the Medulla



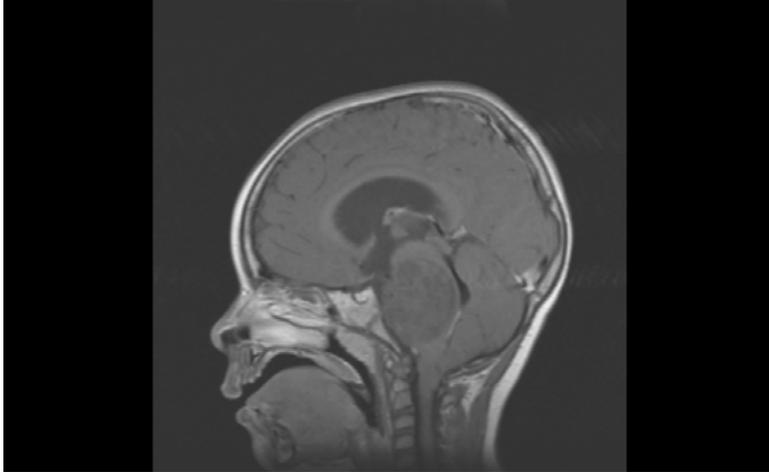
Focal pontine tumor



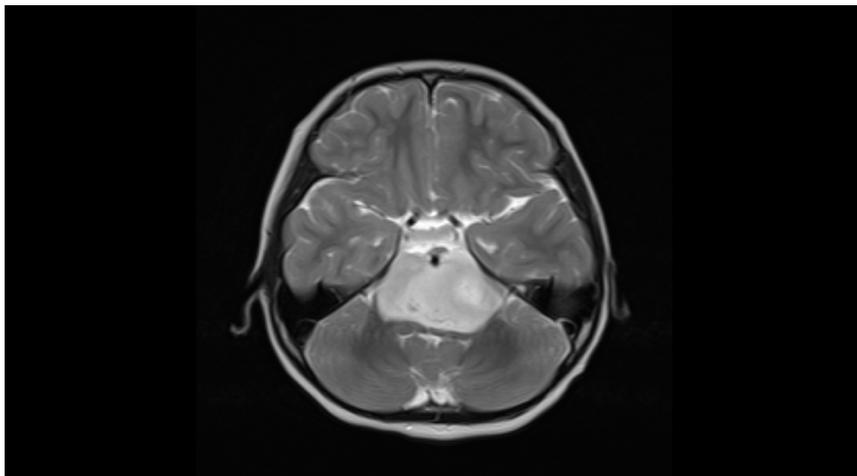
Diffuse Medullary tumor



Diffuse pontine, T1



Diffuse pontine





DIPG

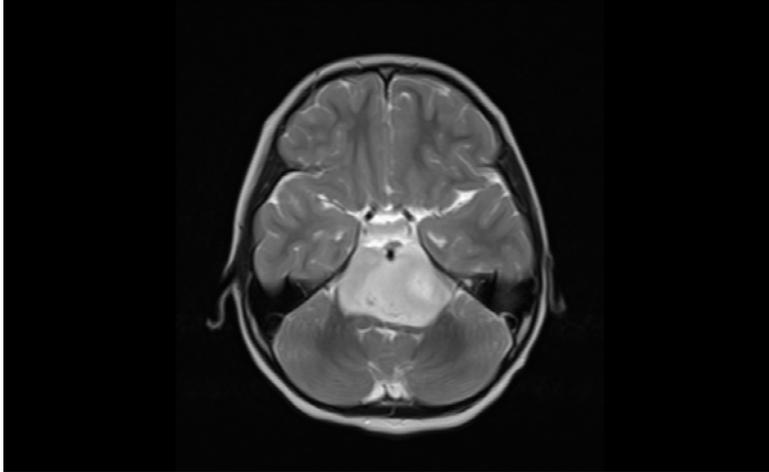
- There are approximately 200 children per year with this diagnosis
- Mean age at diagnosis is 7 to 9 years
- Children under the age of 3 years and young adults over the age 18 may have a better prognosis and any study containing these patients may be biased in its results.
- Resection is impossible as they diffusely involve a structure which is vital to life.



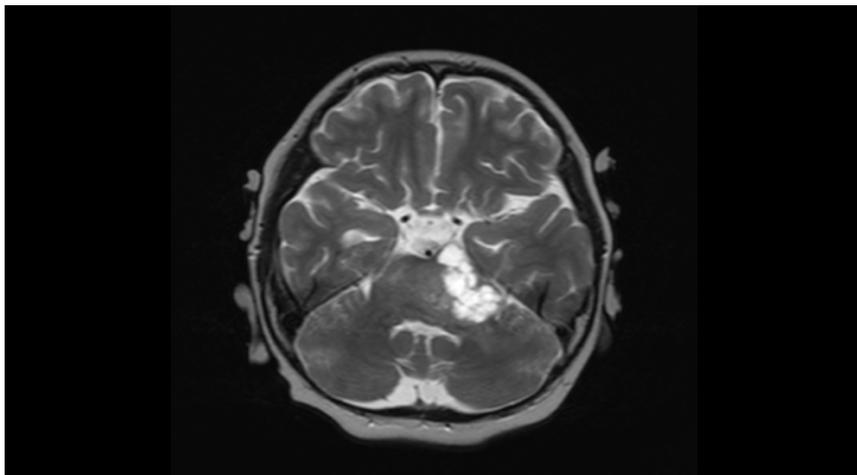
DIPG in the MRI era

- Defined and diagnosed by MRI
- These children present neurologically devastated with multiple cranial nerve palsies and severe limb weaknesses. Often with speech that can not be understood, unable to swallow their own saliva, unable to walk and sometimes unable even to sit.
- Most DIPGs responded well to radiation with often dramatic improvement in the scan and clinically. However 90% died by 2 years and less than 2% survived on long term follow-up. Median survival only 9 to 10 months

Pre-radiation



Post-radiation





Attempts to improve outcome by intensifying therapy

- Series of studies looking at increasing intensity and/or fractionation of radiation. If responses with radiation then more radiation might cure.
- Instead of the conventional once a day to 54 Gy, there was escalation up to 78 Gy in twice daily fractions. No improvement in outcome but with more toxicity (COG 1994, UKCCSG 1997, POG 1999).
- Necrosis of the pons is a potentially devastating complication toxicity.



Conventional chemotherapy

- Conventional chemotherapy was tried both before and after radiation in institutional and group setting without significant improvement. Indeed the UKCCSG stated in their last trial that this approach should be abandoned as toxic without benefit and with toxicity. The most recent COG experience with temodar was negative.
- At the extreme, high dose therapy with autologous bone marrow rescue was tried without any improvement in outcome

Bouffet et al, 2000; Dunkel et al, 1998



Concurrent therapy during radiation

- This was tried by a number of cooperative groups. A large number of agents were tried (carboplatin, cisplatin, etoposide, troposphamide, toptotecan) with no benefit. Indeed POG 8495 showed poorer survival with radiation and cisplatin versus radiation alone.
- There are now a large number of agents that could be tried and are being tried during radiation. However there is no biology to guide which agents and there is a real possibility of harm.



Experimental therapy

- There are at any time a number of Phase I and II studies in this tumor.
- It is a "ideal" tumor for experimental therapy in that there is no conventional agent which has any impact on survival. Studies can be performed with new agents at diagnosis with radiation or at relapse. A secondary end-point of efficacy is also easily assessable given the short median survival time.
- No information from a phase I or II has lead to a therapy with even minimal efficacy.



What is it like on the “ground”

- Diffuse pontine gliomas make up 10%-15% of pediatric brain tumors. However in any one year they make up to 20 to 30% of the deaths from brain tumors.
- Diagnosis made on the MRI by three MDs, radiology, neuro-oncology and neurosurgery.
- Diagnosis told to parents and it is explained that surgery is not necessary. The grimmest of all diagnostic talks in neuro-oncology.
- Conventionally fractionated radiation as standard. All offered experimental therapy if available study.



Why Biopsy

- Biopsy currently only recommended for atypical cases
- However, no progress in cure for this tumor by the current approach to choosing new agents.
- Experimental therapy rationalized often by work on glioblastoma multiforme (GBM). Yet DIPGs look different by scan, behave very differently and relapse very differently.
- Why are they not as different from glioblastomas as ependymomas. Why not as different as pediatric glioblastomas from adult glioblastomas.



Why not biopsy

- The most influential paper in making MRI the standard method of diagnosis was that by Albright and al in 1993.
- This paper analyzed results from CCG-9882. This was a trial for brainstem gliomas from 1988 to 1991. There were 120 children on the trail of which 56 had surgery.
- Of the 56 who had surgery, 5/56 had complications. Of those who had stereotactic biopsies 2/20 had complications. No information on reversibility of complications. There was no mortality



Why not biopsy

- However Albright et al recommended that biopsies not be done because it added nothing to the diagnostic precision of MRI, not because of the complication rate.
- Sensitivity of MRI confirmed by a German group
- As stated by Fred Epstein in a commentary on the Albright paper "*routine* biopsy should be relegated to neurosurgical history".

Neurosurgery 33:1026, 1993



Why biopsy now

- We would like it to be clear that we agree with the authors of the 1993 paper that there is a low utility in *routine* biopsies of this tumor to make a diagnosis. Diagnosis by scan is of a high degree of accuracy in experienced hands.
- However what is proposed is not to do routine biopsies for diagnosis but to establish molecular information which will allow us to understand this tumor and to rationally choose between experimental therapies.



Why can autopsy studies not substitute for biopsy

- Autopsy studies have been done. The group in Toronto offered limited post-mortems to all children with DPG. 9 /24 had autopsies. 2/9 were PNETs. Time to autopsy was variable but usually many hours. High resolution single nucleotide polymorphism arrays were done and results are in abstracts (Supplied by Eric Bouffet).
- Limitations, all of the autopsied children had radiation at diagnosis and 8/9 had responded. Recurrent DPG is notoriously fast growing and aggressive. Radiation may be clonal selective. May be why autopsy studies seem to categorize more children as PNET than biopsy studies
- Almost all children with DIPG die at home. Autopsy is invariably delayed. We studied an aggressive brain tumor at relapse and at a 3 hour autopsy and found marked changes in mRNA and in protein expression.



Have Biopsy Studies been done

- There are no molecular biology biopsy studies in print.
- A biopsy study by the European consortium ITCC is to be presented at ASCO. This was a mandated procedure prior to study entrance on a phase I trial. This group biopsied 20/21 children and of the 20 with pathology all were high grade gliomas.
- Complication rate is preliminary but appears 10% with all complications being transient. They decided prior to the study which targets were important (eg EGFR, PTEN, etc) and looked for them with IHC, FISH and PCR successfully.
- This study confirms safety and feasibility of biological studies on small stereotactic biopsies.

(Personal communication Darren Hargrave)



What we propose

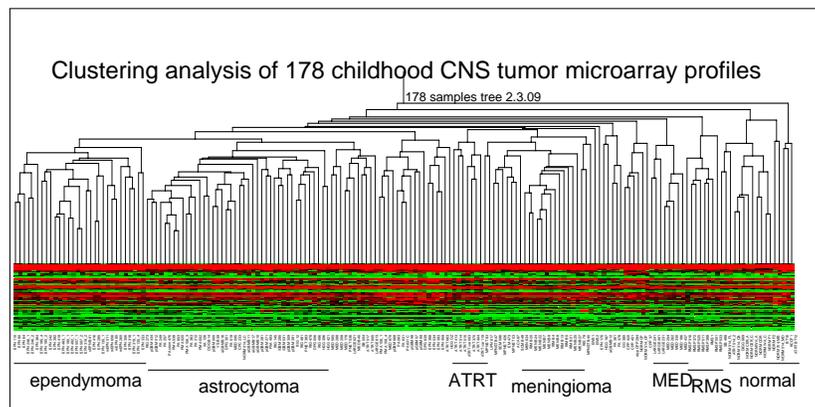
- To biopsy 10 children with typical diffuse pontine gliomas.
- Mandated witnessed consent process.
- The Children's Hospital Denver has agreed to pay for any expense secondary to a complication
- To exclude children under the age of 3 years and adults over the age of 18. There is a suggestion these groups has a more favorable outcome and probably a different biology.



Why is this study unique

- We are not pre-supposing what a DIPG tumor is or what the potential targets may be. The ITCC selected what to study based on what is known about the biology of (largely adult) GBMs.
- We are not making any assumptions about the biology of DIPGs or even about which tumor they most closely resemble.
- We will do a genome-wide mRNA screen looking at more than 18000 genes and comparing these results to the other 178 tumors of childhood that we have characterized by microarray.

- Our existing microarray data bank will provide a foundation for comparative evaluation of the biology of DIPG.
- We are continually adding tumors to this data bank





- What can you do with such a limited number of samples using genomics?
- You can hopefully answer the fundamental question: what is this tumor?



Example

- Previously we had taken a pediatric brain tumor, ectomesenchymoma (ECM) and did genomics on a limited number of samples. The genomics showed a significant overlap in gene expression with malignant peripheral nerve sheath tumors (MPNST) and not with medulloblastomas and AT/RTs.
- We concluded for this tumor that there should be consideration of developing a common therapy for ECMs and MPNSTs.

Acta Neuropathol. 2007;113(6):695-703



For this study

- We will “chip” 10 specimens and use an unbiased hierarchical analysis to identify which tumors these specimens most closely resemble.
- The most interesting comparisons will be between these DIPG samples and the gene signatures of adult, pediatric, “infantile” and radiation-induced GBMs. These GBMs have different gene signatures.

JNEN. 2007;66(8):740-799



Why is this analysis important

- If there is no significant difference between the genomic signature of DIPGs and either pediatric or adult GBMs, then the development of future therapy does not require further characterization of the biology of DIPGs. DIPGs are then GBMs of the pons.
- If on the other hand the gene signature is very different then developing therapy on the basis of the biology of GBMs may be fundamentally wrong. The current approach would then be questionable.



Surprises are possible

- An analysis (manuscript in preparation) by our group of the genomics of GBMs in the under 5s suggest that these tumors have more in common with AT/RTs than GBMs in older children.
- It is possible that DIPG have more in common with other brain tumors of childhood than GBMs. There is the curious characterization by histology of some of these as PNETs at autopsy.



Improving the analysis

- We are the biology center for a POETIC study which in one arm aims to recruit 60 children with GBMs. POETIC has proven an excellent consortium at providing biological specimens. This large group of childhood GBMs will be important to compare to the DIPGs. It is possible that DIPG genomics resemble a subgroup of childhood GBMs.
- This study is currently at the POETIC institutions' IRBs.



Can we do this on small biopsies?: experience with atypical pontine tumors

- Two children were judged to be atypical and biopsies were considered clinically necessary
 - **UPN506**: a single biopsy yielded 6 milligrams tissue. RNA extraction (Qiagen RNeasy) gave a yield of 14.8 micrograms total RNA
 - **UPN639**: a single biopsy yielded 6 milligrams tissue. Dual RNA/DNA extraction (Qiagen Allprep) gave a yield of 3.2 micrograms total RNA and 7.9 micrograms of DNA
- Both samples passed stringent quality control criteria (Agilent Bioanalyzer)
- Gene expression microarray analysis (Affymetrix HG-U133plus2) was successfully performed on both samples.



Proof of principle - genomic analyses can be performed on limited biopsy material

- RNA yields were easily sufficient to perform Affymetrix HG-U133 plus 2 gene expression microarray analysis
 - Affymetrix HG-U133 plus 2 Gene expression microarray requires 300 nanograms of RNA amplified using Ambion magnetic bead system.
- We also obtained sufficient DNA to perform further analysis.
 - Affymetrix 6.0 SNP analysis requires 250-500 nanograms unamplified DNA
 - Nimblegen 2.1M arrayCGH analysis requires 2.5 micrograms of unamplified DNA



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Further studies on the DNA

- In the study as submitted to our IRB almost 2 years ago, we thought that proteomics would prove the best confirmation of the genomic results.
- We are now inclined to compare the genetics of DIPGs with other closely related tumors (defined by our genomic analysis) by another technique.
- We plan to use aCGH (array comparative genomic hybridization) and SNP (single nucleotide polymorphism) analysis.
- Would use a blinded comparison by our genetics core.

- UC Denver campus is well equipped to perform the proposed analyses
 - pediatric neuro-oncology laboratory with established expertise in gene expression microarray analyses
 - On campus Genomics Core Facility
 - NCI cancer center Bioinformatics collaborators
- We have used comparative analysis of microarray data to address a number of clinical problems in pediatric neuro-oncology
 - **Predicting which children are at risk for ependymoma relapse.** Sowar K, Straessle J, Donson A, Handler M, Foreman NK. J Neuro-Oncol. 78(1):41-6; 2006.
 - **Paired overexpression of ErbB3 and Sox10 in pilocytic astrocytoma.** Addo-Yobo SO, Straessle J, Anwar A, Donson AM, Kleinschmidt-Demasters BK, Foreman NK. J Neuropathol Exp Neurol. 65(8):769-75;2006
 - **Molecular array analyses of 51 pediatric tumors shows overlap between malignant intracranial ectomesenchymoma and MPNST but not medulloblastoma or atypical teratoid rhabdoid tumor.** Kleinschmidt-DeMasters BK, Lovell MA, Donson AM, Wilkinson CC, Madden JR, Addo-Yobo SO, Lillehei KO, Foreman NK. Acta Neuropathol. 2007;113(6):695-703.
 - **Unique Molecular Characteristics of Radiation-Induced Glioblastoma.** Donson AM, Erwin NS, Kleinschmidt-DeMasters BK, Madden JR, Addo-Yobo SO, Foreman NK. JNEN. 2007;66(8):740-799.
 - **Radiation-induced meningiomas: clinical, cytogenetic, and microarray features.** Lillehei KO, Donson AM, Kleinschmidt-Demasters BK. Acta Neuropathol. 2008;116(3):289-301.
 - **Rare Nerve Lesions of Non-Nerve Sheath Origin: A 17-Year Retrospective Series.** Strom T, Kleinschmidt-Demasters BK, Donson AM, Foreman NK, Lillehei KO. Accepted, Arch Path Lab Med. 2008.
 - **Claudin 6 is a positive marker for atypical teratoid/rhabdoid tumors.** Birks DK, Kleinschmidt-DeMasters BK, Donson AM, Barton VN, McNatt SA, Foreman NK, Handler MH. Brain Pathology, Epub Feb 2009



What benefit

- To the *individual child*, the prospect that the biology may suggest benefit from a particular trial or targeted agent.
- At the recent AACR, a study presented at the plenary session, showed for adults with metastatic cancer that using molecular biology to guide choice of therapies, including experimental, increased progression free survival.

Hoff et al, AACR 2009



What benefit

- To *the family*, the prospect that their child's life is not "wasted".
- To *future children* with DIPG, the prospect that knowledge of the tumor's biology will allow progress towards a cure.



There is a controversy about biopsies in these tumors.

- Best seen in the recent very contradictory reviews in J Neurosurgery and the British J Neurosurgery.
- Dunkel and Souweidane, in a brief review, felt to advocate for biopsies at this point of time was "premature". This was in commentary on article by Frazier et al which was in broadly in favor of biopsies.
- They point to the availability of "seemingly unlimited" amounts of tissue in GBMs and the lack of progress in therapy.
- They also felt that the potential of autopsy studies and CSF studies have not been exhausted.

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- The lack of progress with GBMs as a whole is discouraging. Progress is likely to be made with sub-groups (e.g. children under 5). We need to establish whether DIPGs are a sub-set of GBMs and if so which subset do they belong to before progress can be made.
- The insights from autopsies are limited by the prior therapy and few will be as extensive as the Toronto study.
- We are the biology site organizing analysis of CSF for Dunkel's new trial that includes DIPG. However making sense of the CSF biology will require information from the tumor. CSF biology may be a way of avoiding future biopsies if we can correlate CSF findings with the biology of these tumors.



- The British Journal Of Neurosurgery review was exhaustive and included three commentaries one by a neuro-oncologist, one by a neurosurgeon and one by two ethicists.
- The main review makes the point that morbidity and mortality of biopsy is low and that diagnosis by scan has the possibility of error.
- They point out a series by Schumacher in 2007 that found, with MRI, poor specificity of tumor versus non-tumor and correct grading of tumor in only 2/3 of cases



- They point out that in the French series 2/24 children had unexpectedly low grade tumors and their therapy was altered as a result of the biopsy
- However their strongest argument is that new targets can only be identified and validated by tissue
- Summarized by their statement that “with an increasing knowledge of tumor biology and genetics there is the potential for specific therapies tailored for individual tumors based on their biological or genetic characteristics”.
- British Journal of Neurosurgery 22:619, 2008



Summary

- The risk of harm of biopsies is low. The risk is probably not different from the risk of the biologically “blind” phase I/II studies dozens of these children are exposed to every year.
- There is a low, but possible, benefit to the child. There may be mistakes in diagnosis and grade. There may be information from the biology which might suggest potential benefit from one experimental therapy versus another. Certainly the benefit possibility *to the individual* is of the same order as that of phase I/II therapies in this tumor.



- There is a real possibility of benefit to future children with this tumor. All precious biological information obtained by us will be posted on the web so that all investigators may studied it. Such information, we believe, may guide the choice of future agents. It will enable us to explore what CSF and blood will tell us about the biology of the tumor.
- For the children and parents of children on the study, it will bring comfort that death was not meaningless. That they contributed to a fundamental study of the biology of this terrible tumor to benefit future children.



- This study may answer the question about the value of biopsying this tumor.
- The time is now for an attempt to understand the biology of this tumor. We have new biological tools. We should use them now “given the desperation of the families who are afflicted and the frustration of the clinicians called upon to care for them”.



Conclusion

It is unlikely that progress in curing DIPGs will be made by “biologically blind” multiple phase I/II studies without knowing what the tumor is.