AKT1 E17K MUTATIONS IN GRADE I & II MENINGIOMAS

Britney Pitter
Luis Fernandez
12 August 2016

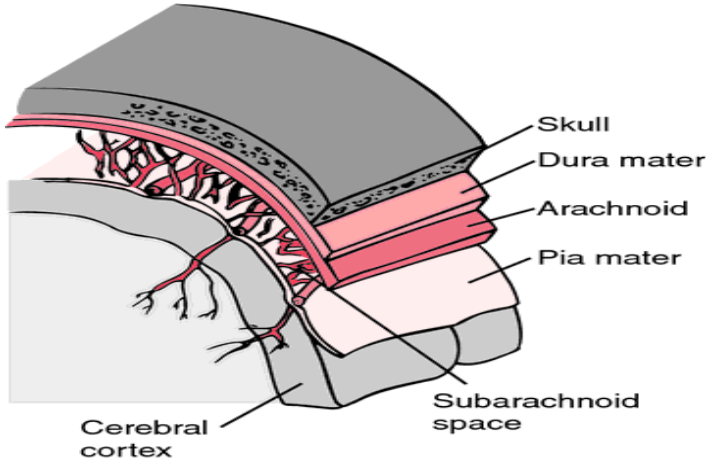
UNIVERSITY of WASHINGTON

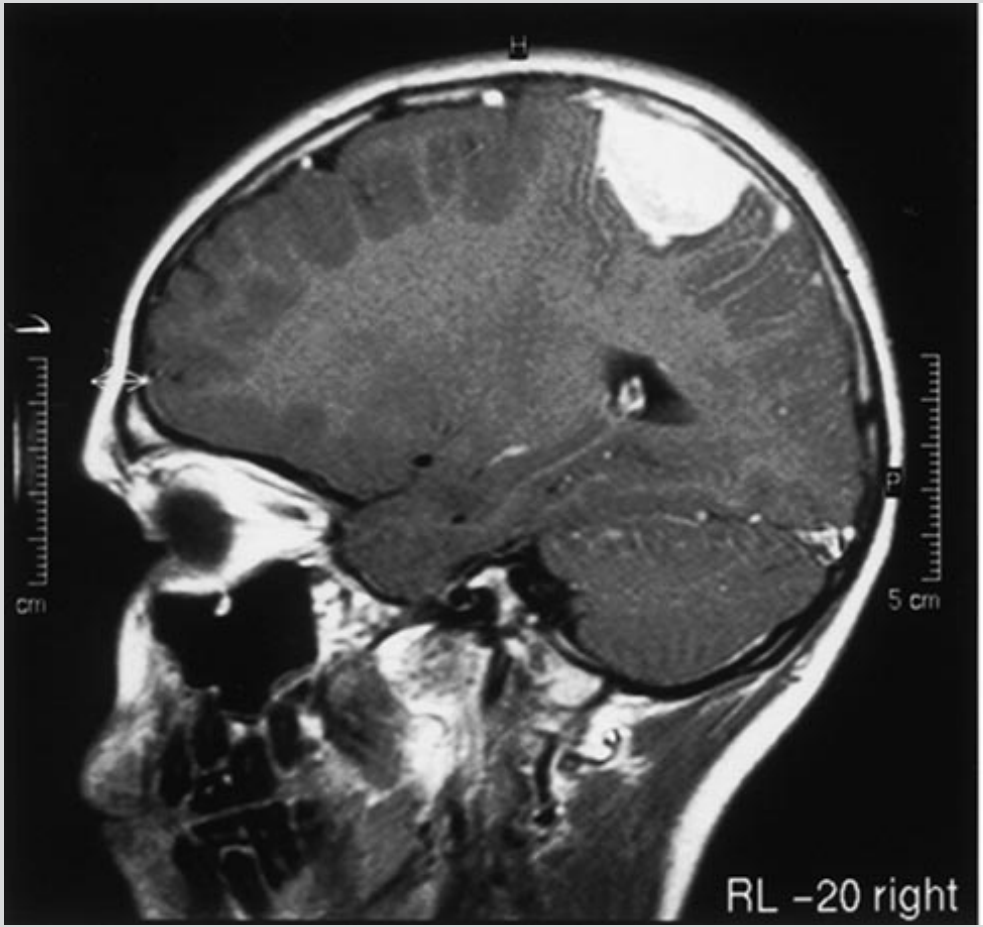
Overview

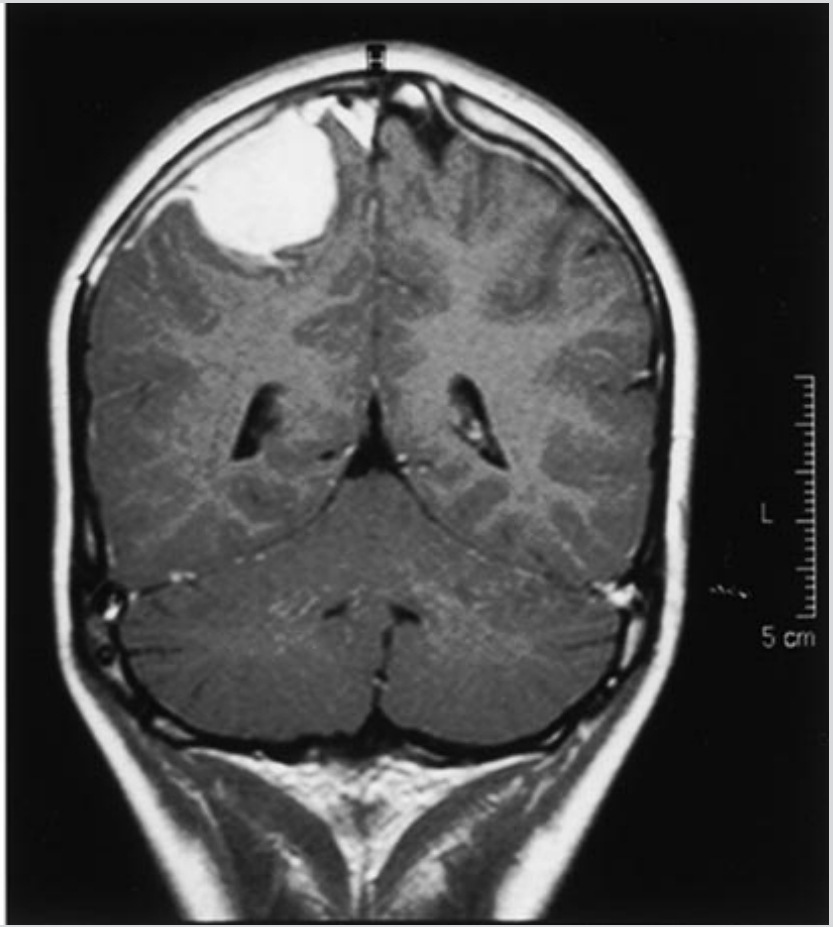
- Ferreira Lab Meningioma Project:
  - Definition
  - Grading Scale
  - AKT E17K Mutation
- DNA Extraction Technique
- Next Generation DNA Sequencing
- Meningioma Data Analysis
- Conclusion

Meningiomas

- ➔ Tumor in the meninges: dura mater, arachnoid, pia mater
- ➔ Meningiomas are the most common primary nervous system tumor. Most are benign (Grade I).
- ➔ Meningiomas linked to mutations in AKT1 E17K & NF-2




H
A
P
5 cm
RL −20 right


H
L
5 cm

Grading scale according to WHO 2007

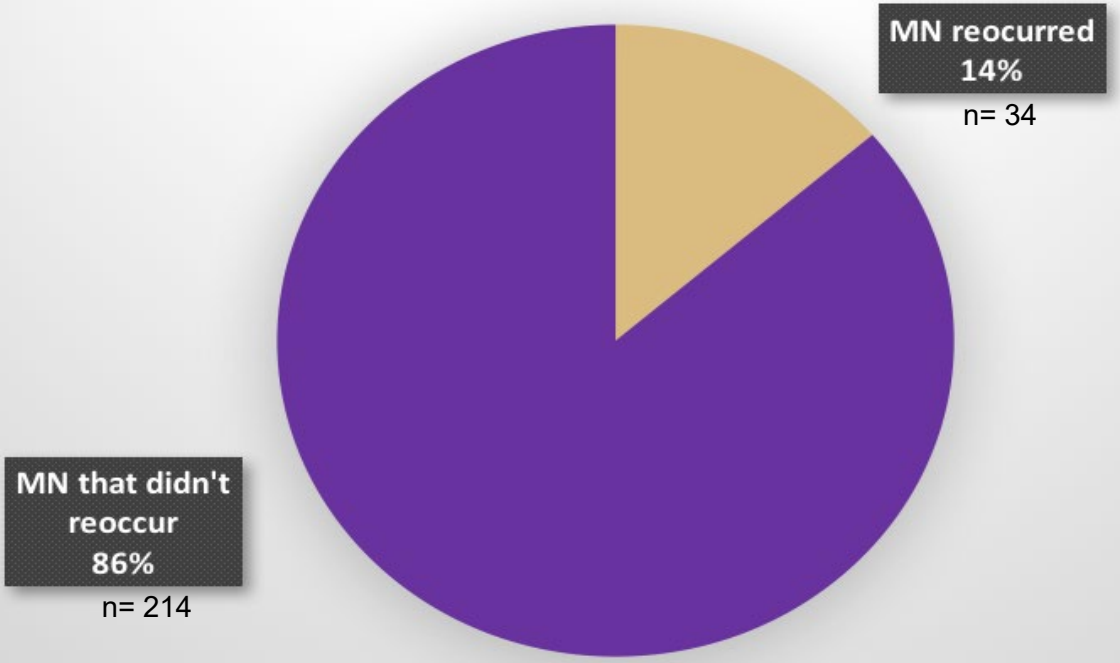
Table 1 WHO 2007 tumor grade

Grade I (benign)	Grade II (atypical)	Grade III (anaplastic/malignant)
Any major variant other than clear cell, chordoid, papillary, or rhabdoid	Frequent mitoses (≥4 per 10 hpf[a]) **or** Three or more of the following: Sheeting architecture Hypercellularity (focal or diffuse) Prominent nucleoli Small cells with high nuclear cytoplasmic ratio Foci of spontaneous necrosis **or**	Excessive mitotic index (≥20 per 10 hpf)[a] **or** Frank anaplasia defined as focal or diffuse loss of meningothelial differentiation resembling: Sarcoma Carcinoma or melanoma **or**
Does not fulfill criteria for grades II or III	Additional subtypes/features: Chordoid meningioma Clear cell meningioma Brain invasion	Additional subtypes/ features: Papillary meningioma Rhabdoid meningioma

Grade 1.5 Meningiomas

- Grade one non-recurring and grade one recurring are indistinguishable to pathologist.
- Our lab proposes a 1.5 grade level for recurrent grade one meningiomas
- Pathologists want to find diagnostic criteria to distinguish tumors that recur
- Genetic mutations may indicate recurrence

Recurrence/Progression of Grade I Meningiomas (MN) n=248



Genomic Analysis of Non-*NF2* Meningiomas Reveals Mutations in *TRAF7*, *KLF4*, *AKT1*, and *SMO*

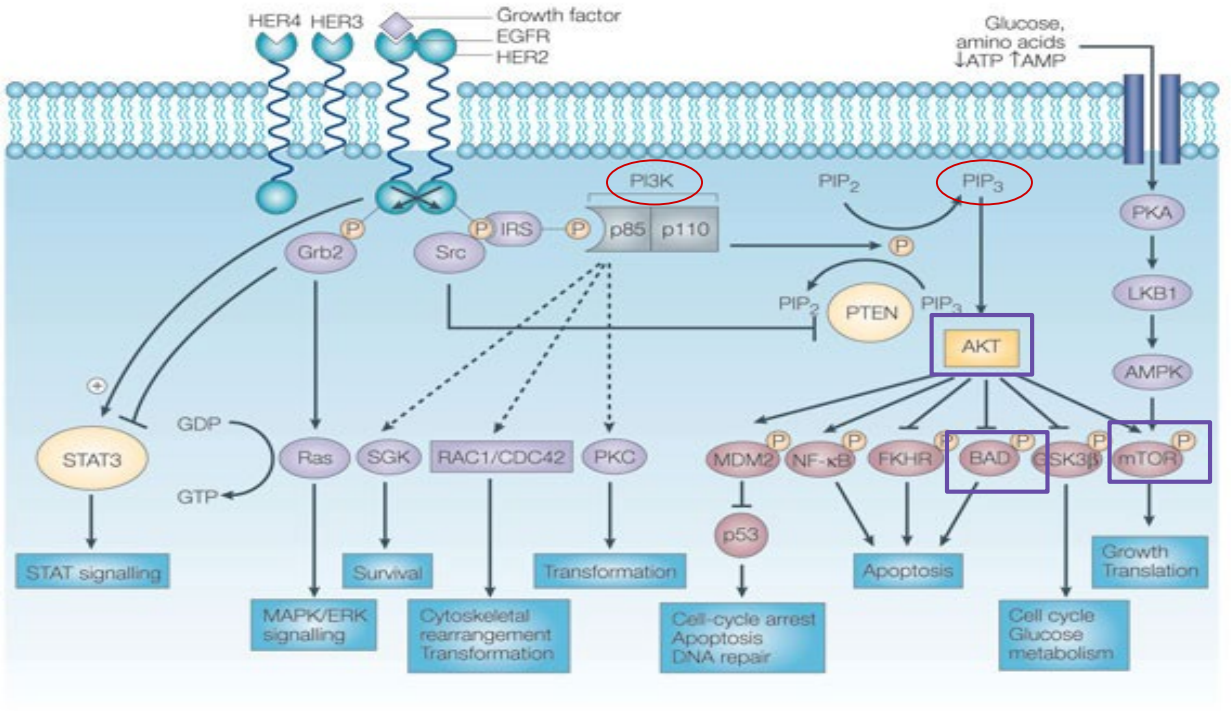
Victoria E. Clark,[1] E. Zeynep Erson-Omay,[1] Akdes Serin,[1] Jun Yin,[2] Justin Cotney,[2] Koray Özduman,[3] Timuçin Avşar,[4] Jie Li,[5] Phillip B. Murray,[1] Octavian Henegariu,[1] Saliha Yilmaz,[1] Jennifer Moliterno Günel,[6] Geneive Carrión-Grant,[1] Baran Yılmaz,[7] Conor Grady,[1] Bahattin Tanrıkulu,[7] Mehmet Bakırcıoğlu,[1] Hande Kaymakçalan,[8] Ahmet Okay Caglayan,[1] Leman Sencar,[1] Emre Ceyhun,[1] A. Fatih Atik,[7] Yaşar Bayri,[7] Hanwen Bai,[1] Luis E. Kolb,[1] Ryan M. Hebert,[1] S. Bulent Omay,[1] Ketu Mishra-Gorur,[1] Murim Choi,[2] John D. Overton,[9] Eric C. Holland,[10] Shrikant Mane,[2,9] Matthew W. State,[11] Kaya Bilgüvar,[1] Joachim M. Baehring,[12] Philip H. Gutin,[6] Joseph M. Piepmeier,[13] Alexander Vortmeyer,[5] Cameron W. Brennan,[14] M. Necmettin Pamir,[3] Türker Kılıç,[15] Richard P. Lifton,[2,16] James P. Noonan,[2,17] Katsuhito Yasuno,[1] Murat Günel[1,18]*

We report genomic analysis of 300 meningiomas, the most common primary brain tumors, leading to the discovery of mutations in *TRAF7*, a proapoptotic E3 ubiquitin ligase, in nearly one-fourth of all meningiomas. Mutations in *TRAF7* commonly occurred with a recurrent mutation (K409Q) in *KLF4*, a transcription factor known for its role in inducing pluripotency, or with $AKT1^{E17K}$, a mutation known to activate the PI3K pathway. *SMO* mutations, which activate Hedgehog signaling, were identified in ~5% of non-*NF2* mutant meningiomas. These non-*NF2* meningiomas were clinically distinctive—nearly always benign, with chromosomal stability, and originating from the medial skull base. In contrast, meningiomas with mutant *NF2* and/or chromosome 22 loss were more likely to be atypical, showing genomic instability, and localizing to the cerebral and cerebellar hemispheres. Collectively, these findings identify distinct meningioma subtypes, suggesting avenues for targeted therapeutics.

AKT

- Critical regulator of cell survival and proliferation
- AKT1E17K mutation has been shown to be activated by the PI3K enzyme
- AKT1 E17K is a mutation that has been identified in cancers such as breast cancer
- Understanding Akt and its pathways is important for the creation of better therapies to treat cancer and tumor cells.There are 14 types of Grade I tumors that have not been subcategorized and there are no known systemic therapies for the meningiomas. AKT1 is well characterized and AKT1 drugs are available.

AKT/PRKB



DNA Extraction from tissue

- There are many uses for DNA ranging from fundamental research to routine diagnostics and therapeutic decisions.
- We extracted DNA from the tumors that were removed from the patients at Harborview and the UW Medical Center
- Our lab used it for DNA sequencing and finding out what genes get expressed and locate mutations in signaling pathways such as PDGF pathway and AKT

Experiment

Day 1

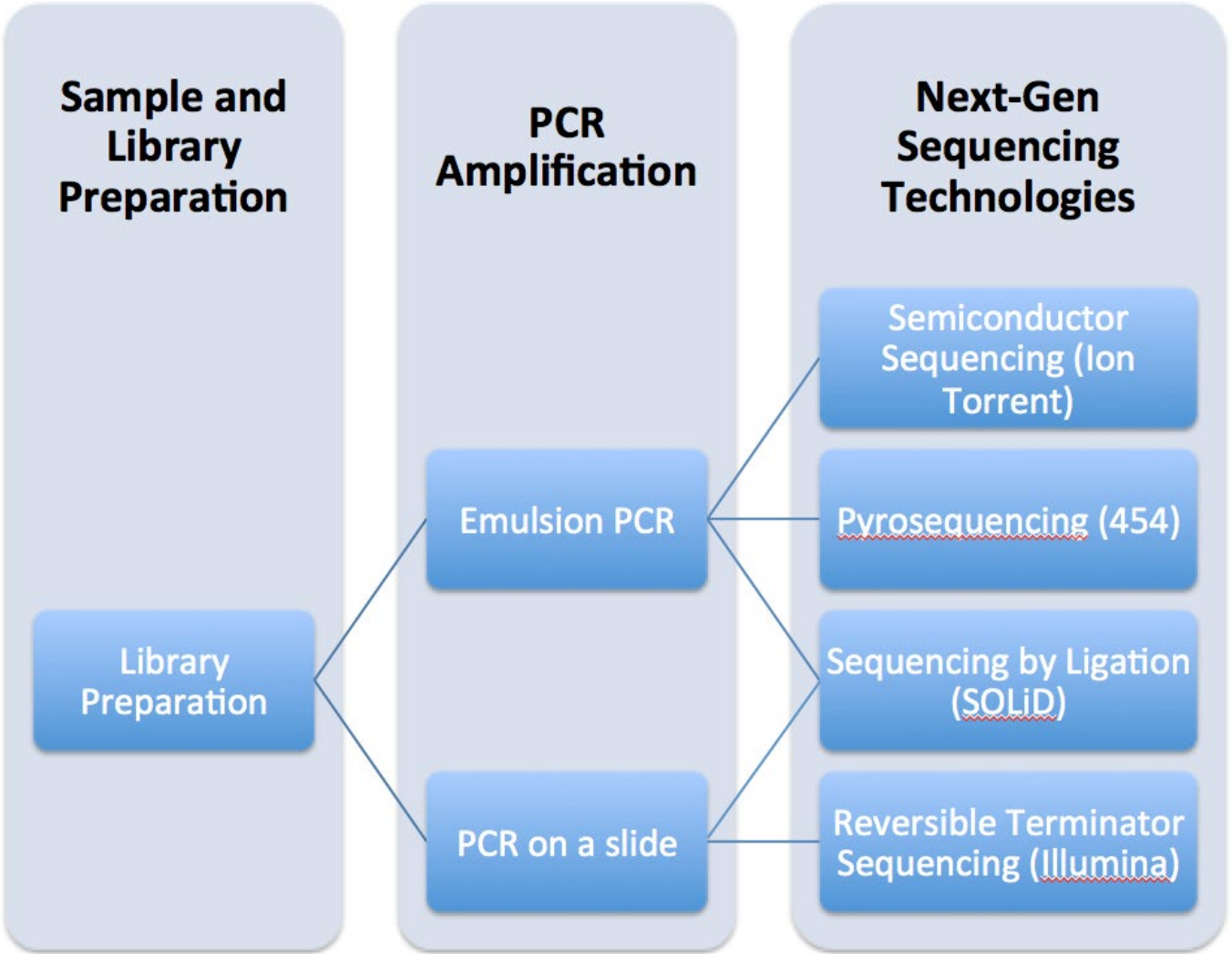
- Locate the Meningioma sample you want DNA from
  - The protocol calls for 25 mg of tissue
- Lysis buffer
  - Lysis buffer will break down all the cell membranes
- Proteinase
  - This buffer will denature the proteins
- Incubate at 56 degrees celsius overnight

Day 2

- Collect the sample for the incubator
- Set heat block to 70 degrees celsius
- You add a series of buffers
  - RNAse
  - AL buffer
  - Ethanol
- Place the reaction into a silica-based column that will bind the DNA to the silica membrane
- You perform series of washes
  - Changing the collection tube with every wash
- Elute DNA with nuclease-free water

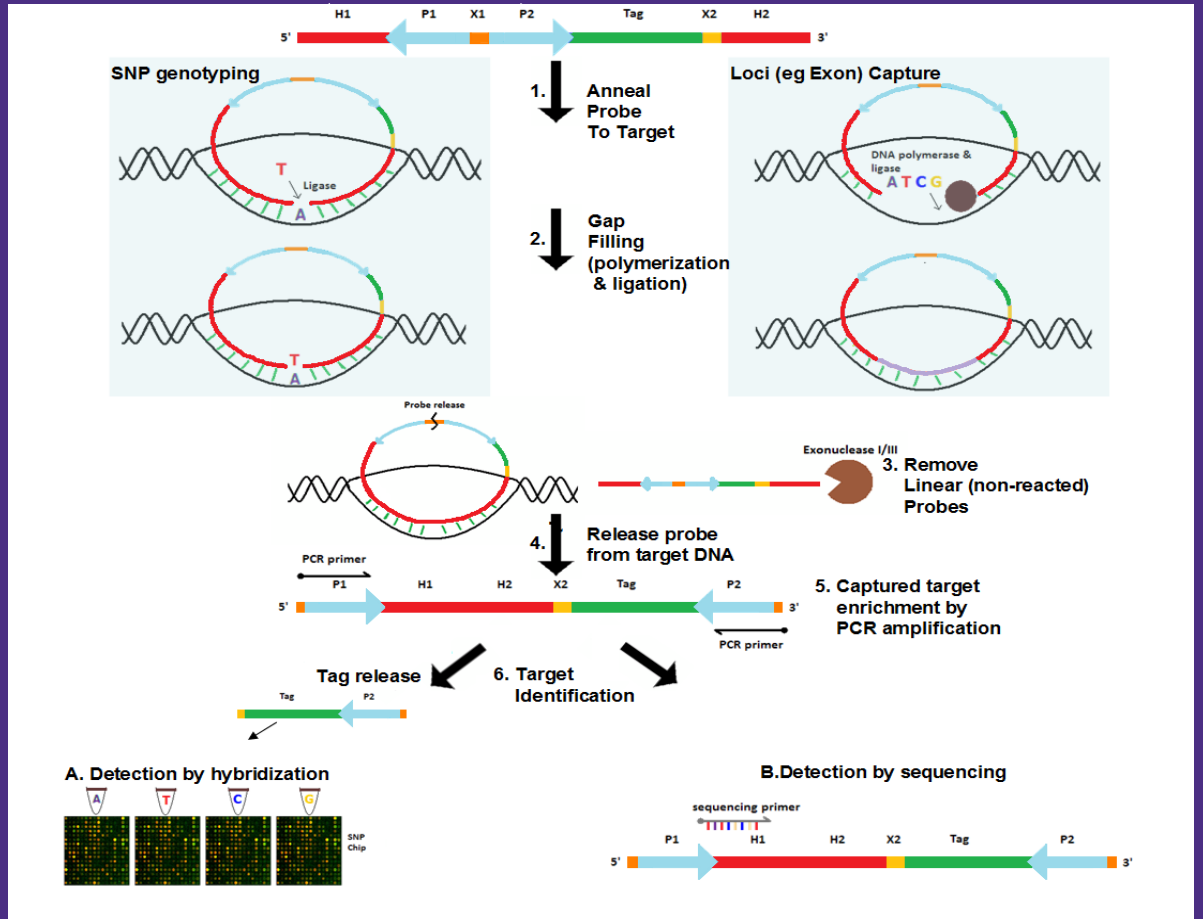
Next Generation sequencing

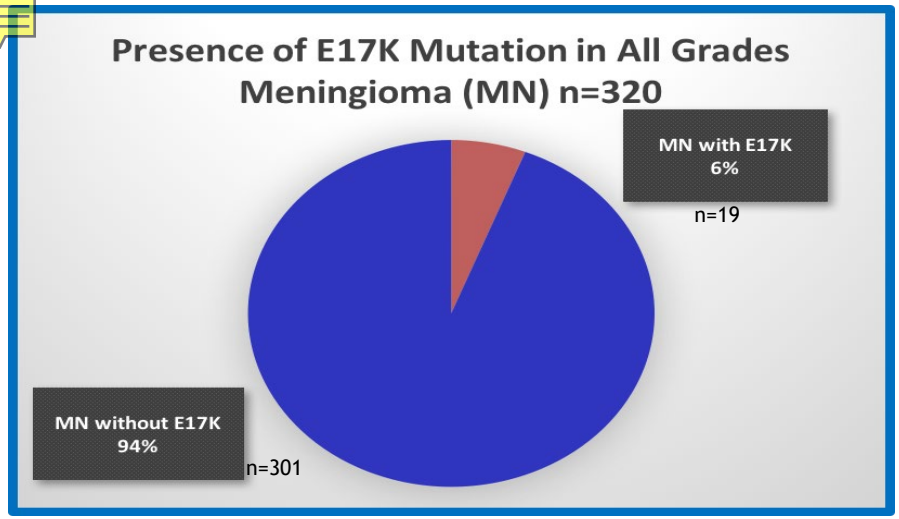
- It uses the standard dye-terminator methods.
- The purpose is to find the order of the four bases—adenine, guanine, cytosine, and thymine—in a strand of DNA.
- Next-generation sequencing applies to genome sequencing, genome resequencing, transcriptome profiling, DNA-protein interactions, and epigenome characterization.

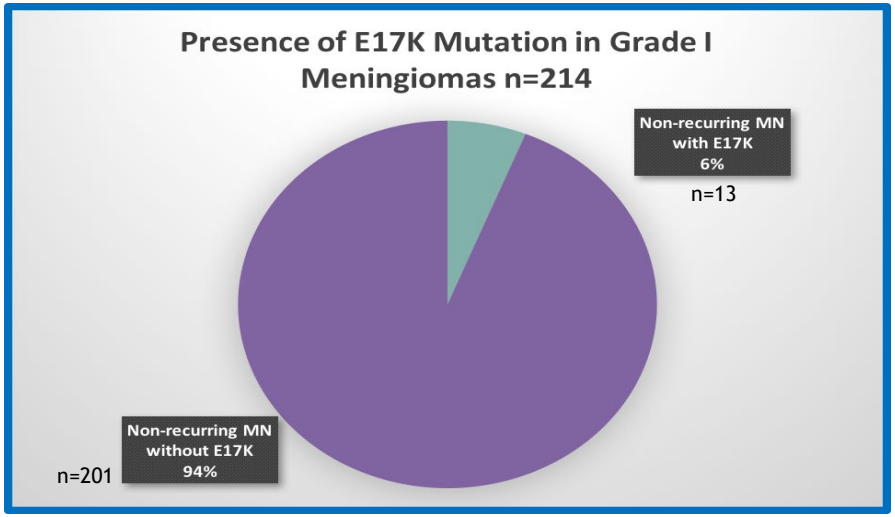

Sample and Library Preparation
PCR Amplification
Next-Gen Sequencing Technologies
Library Preparation
Emulsion PCR
PCR on a slide
Semiconductor Sequencing (Ion Torrent)
Pyrosequencing (454)
Sequencing by Ligation (SOLiD)
Reversible Terminator Sequencing (Illumina)

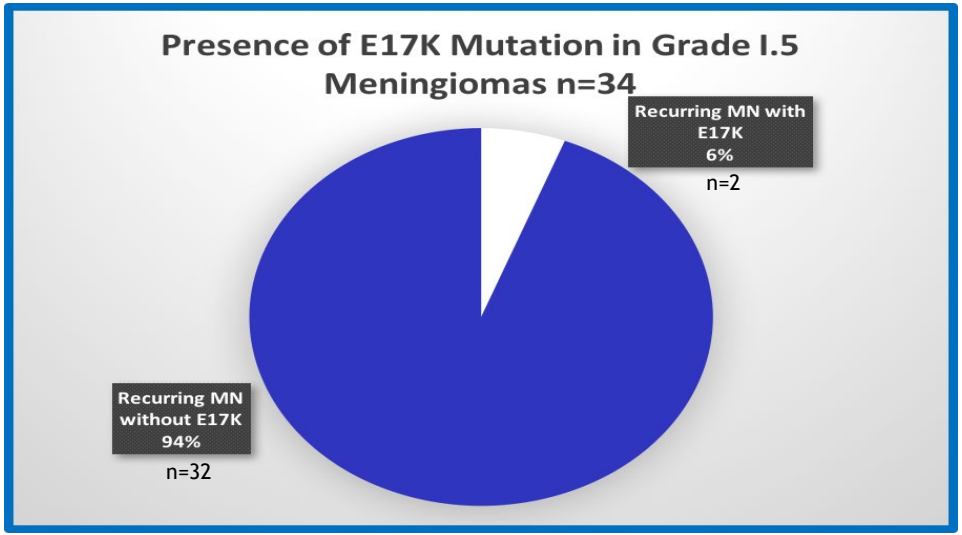
MOLECULAR INVERSION PROBE

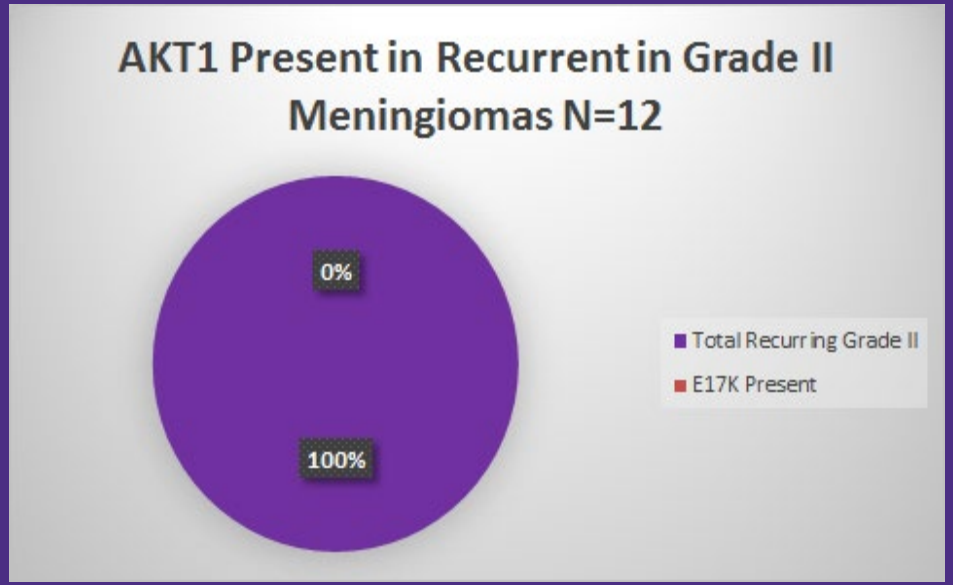
- MIP is used for SNP genotyping (measurement of genetic variations), identify biomarkers, and studying gene copy alterations.
- Used for genomic partitioning, a technique used for enriching specific regions of the genome.

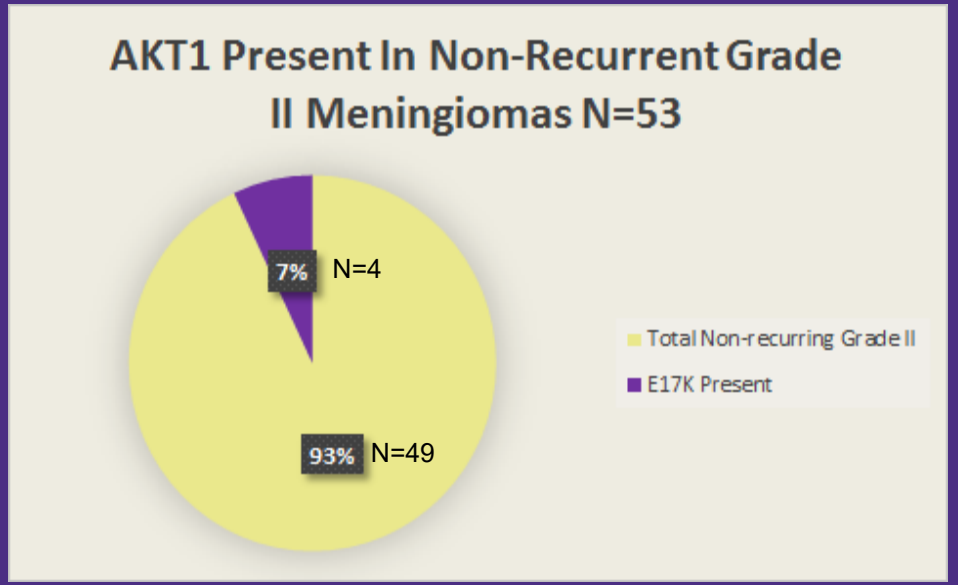



Presence of E17K Mutation in All Grades Meningioma (MN) n=320
MN with E17K 6%
n=19
MN without E17K 94%
n=301


Presence of E17K Mutation in Grade I Meningiomas n=214
Non-recurring MN with E17K 6%
n=13
Non-recurring MN without E17K 94%
n=201


Presence of E17K Mutation in Grade I.5 Meningiomas n=34
Recurring MN with E17K 6%
n=2
Recurring MN without E17K 94%
n=32


AKT1 Present in Recurrent in Grade II Meningiomas N=12
0%
100%
Total Recurring Grade II
E17K Present


AKT1 Present In Non-Recurrent Grade II Meningiomas N=53
7%
N=4
93%
N=49
Total Non-recurring Grade II
E17K Present

CONCLUSION

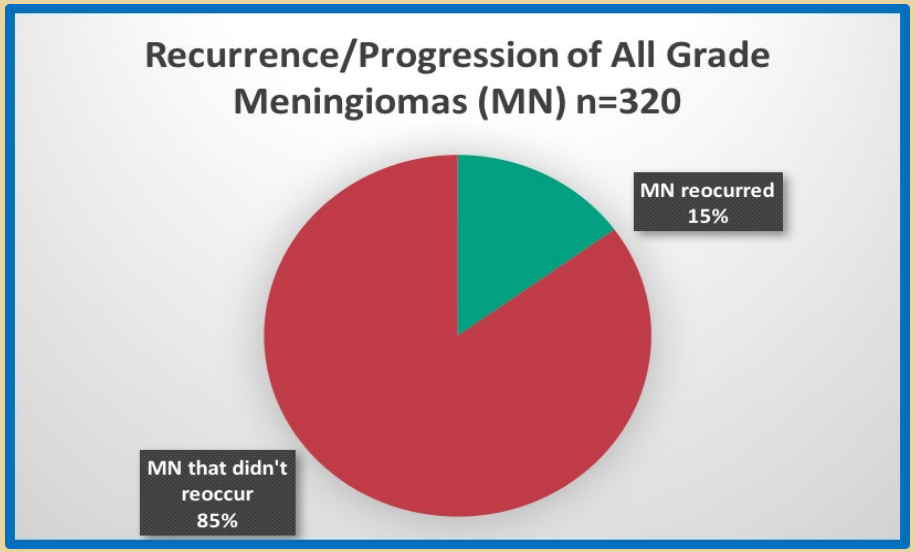
- The results validate the paper by confirming the modest presence of AKT1 E17K mutations in meningiomas by sequencing the gene in a larger sample size

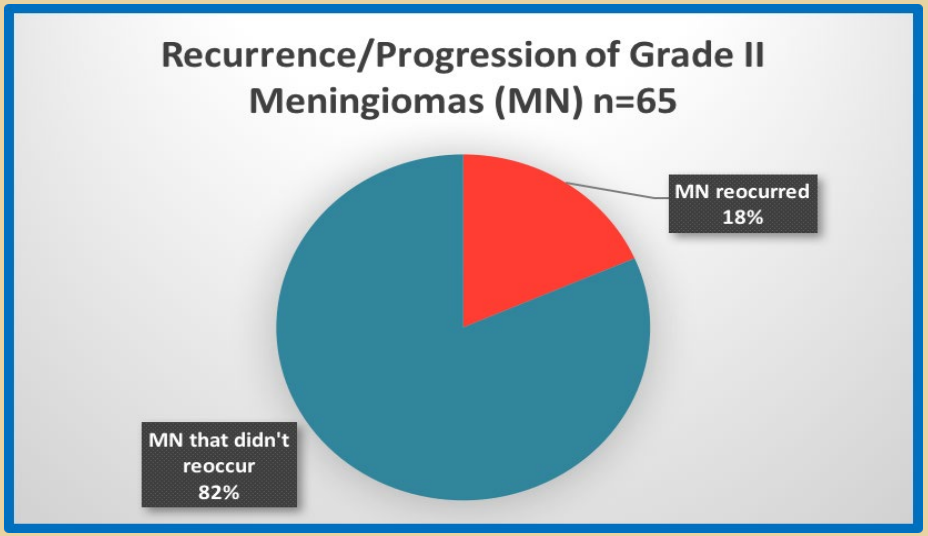
- Some AKT1 inhibitors are promising: Perifosone is being investigated to be used in colorectal cancer in combination with capecitabine & MK-2206, also under investigation, demonstrated synergistic activity when combined with lapatinib in breast cancer cell lines

- It is not efficient to treat meningiomas with AKT1 inhibitors because only a small percent of meningiomas express the mutation
  - AKT1 E17K mutation is not found in a specific subgroup of meningiomas
  - AKT1 E17K not a good biomarker

Thank you

- Dr. Ellenbogen
- Mrs. Ellenbogen
- NIH Summer Research Experience in Translational Neuroscience and Neurological Surgery
- Dr. Hevener
- Mrs. Hevener
- University of Washington
- Dr. Primrose
- UWMC, Seattle Children's, Harborview
- Jim Pridgeon
- Christina Buckman
- Dr. Ferreira
- Tina Busald
- Dr. Carolina Parada
- Wydia Adidharma
- Dr. Rapport
- Yigit Karasozen


Recurrence/Progression of All Grade Meningiomas (MN) n=320
MN reocurred 15%
MN that didn't reoccur 85%


Recurrence/Progression of Grade II Meningiomas (MN) n=65
MN reocurred 18%
MN that didn't reoccur 82%