BRIDGE

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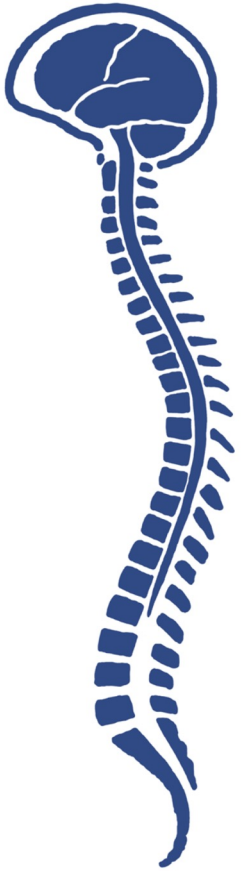
Region Syddanmark



Department of Neurosurgery

Odense University Hospital

University of Southern Denmark

Danish 3R-Center

RRR

# Replacement of meningioma animal models with a meningioma ex vivo/organoid model to test pharmacological advances in meningioma treatment

Mikkel Schou Andersen

MD, PhD-student

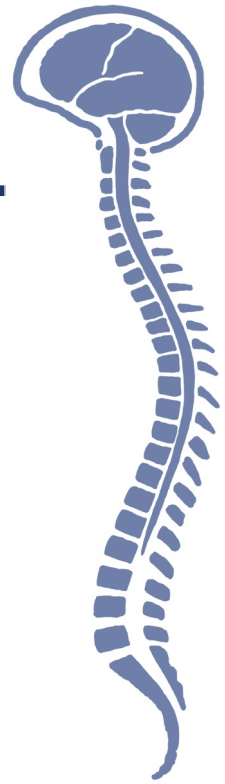
# Aims

1) **To establish a meningioma organoid model** based on patient-derived tumors

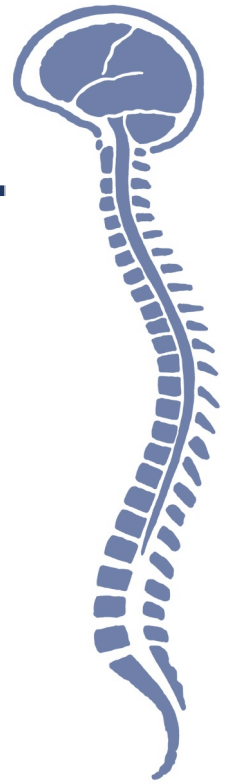
2) To Investigate if organoids are reliable models for **drug testing in vitro**

1) Establish and characterize meningioma organoid models and compare the histopathology, DNA methylation classification and protein expression of the organoids with the parent tumor.

2) Test various already FDA-approved drugs – with focus on selective progesterone receptor modulators(SPRM) but also other previous tested drugs in meningiomas such as but not limited to e.g mifepristone, octreotide.



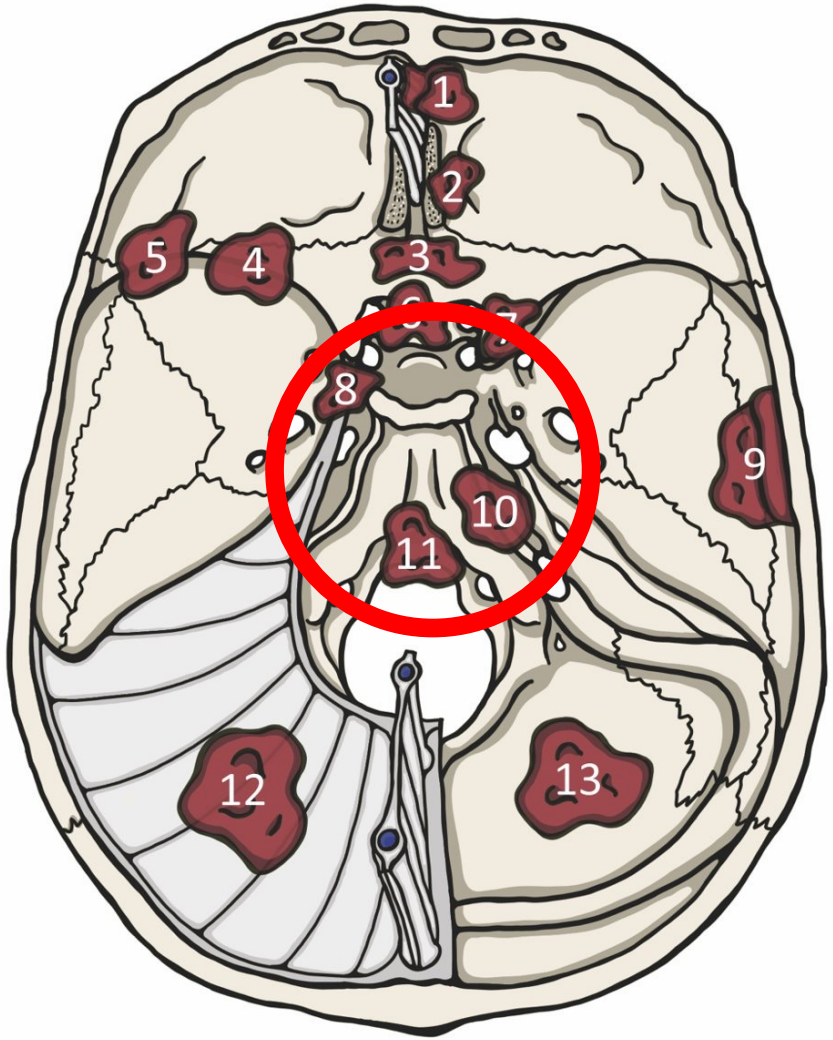
# The Meningioma



- Meningiomas originate from meningeal cap cells and grows where dura resides – Receive external blood supply (no BBB)
- Mostly **benign** connective tissue tumor (80-90%) - Slow growing tumor
- The estimated prevalence in the US population is **50.4-70.7/100.000**
- Incidence is approximately 1.9 for men and 4.5 for women **(≈1:3)** per 100.000 in a combined epidemiological study from Scandinavia
- *Estimated 1/100 with a meningioma-like tumor on MRI-scans*

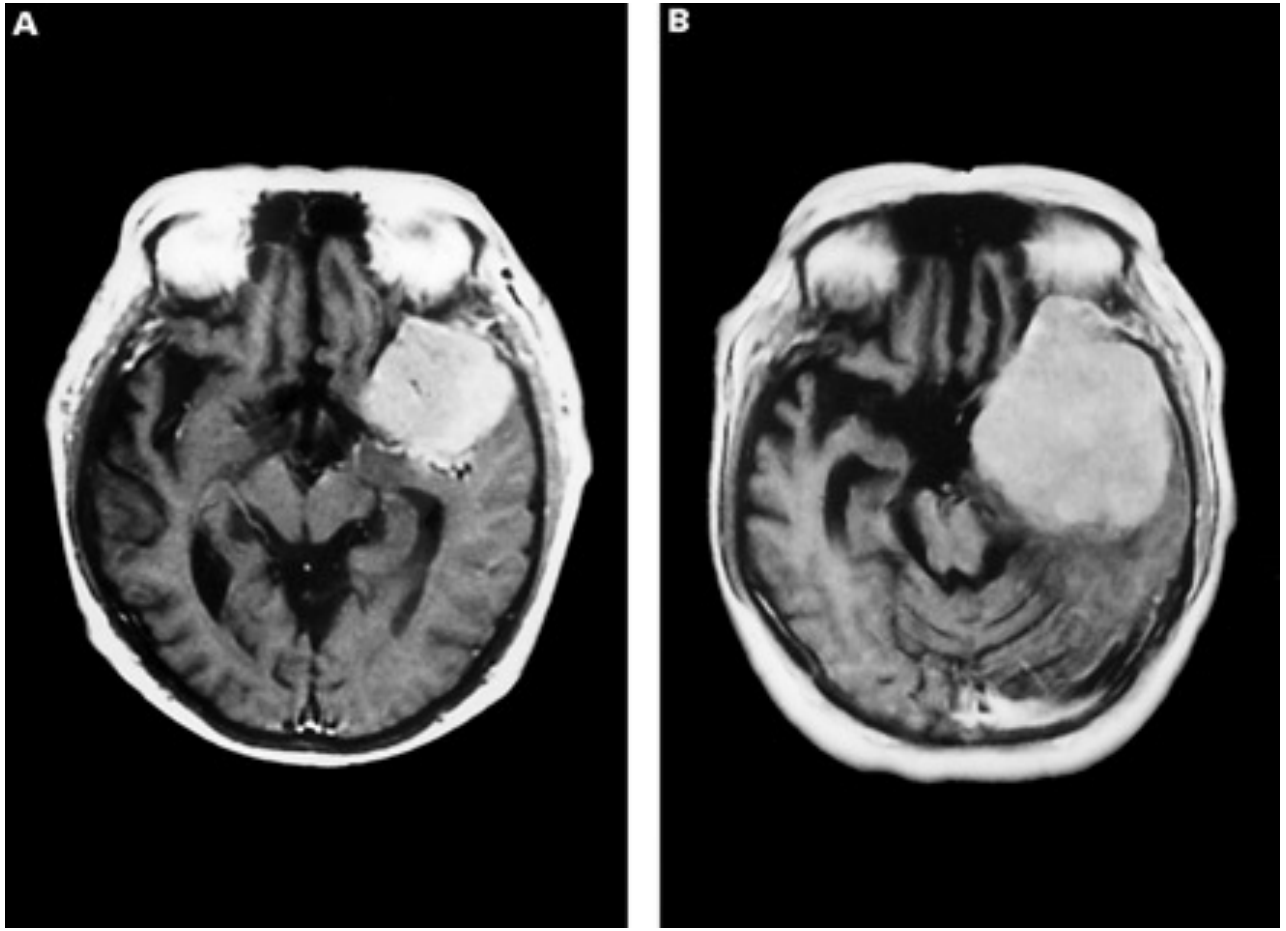
# Location, Location and size'n'symptoms

- In regards to treatment – Location is key – Skull base (Red circle) = Difficult to acces surgically
- Surgery remains first line treatment with radiation as backup in specific cases




Andersen MS, Pedersen CB, Mathiesen T, Poulsgaard L, Kristensen BW, Halle B, Poulsen FR. [Intracranial meningiomas]. Ugeskr Laeger. 2019 Jan 21;181(4):V07180489. Danish. PMID: 30722833.

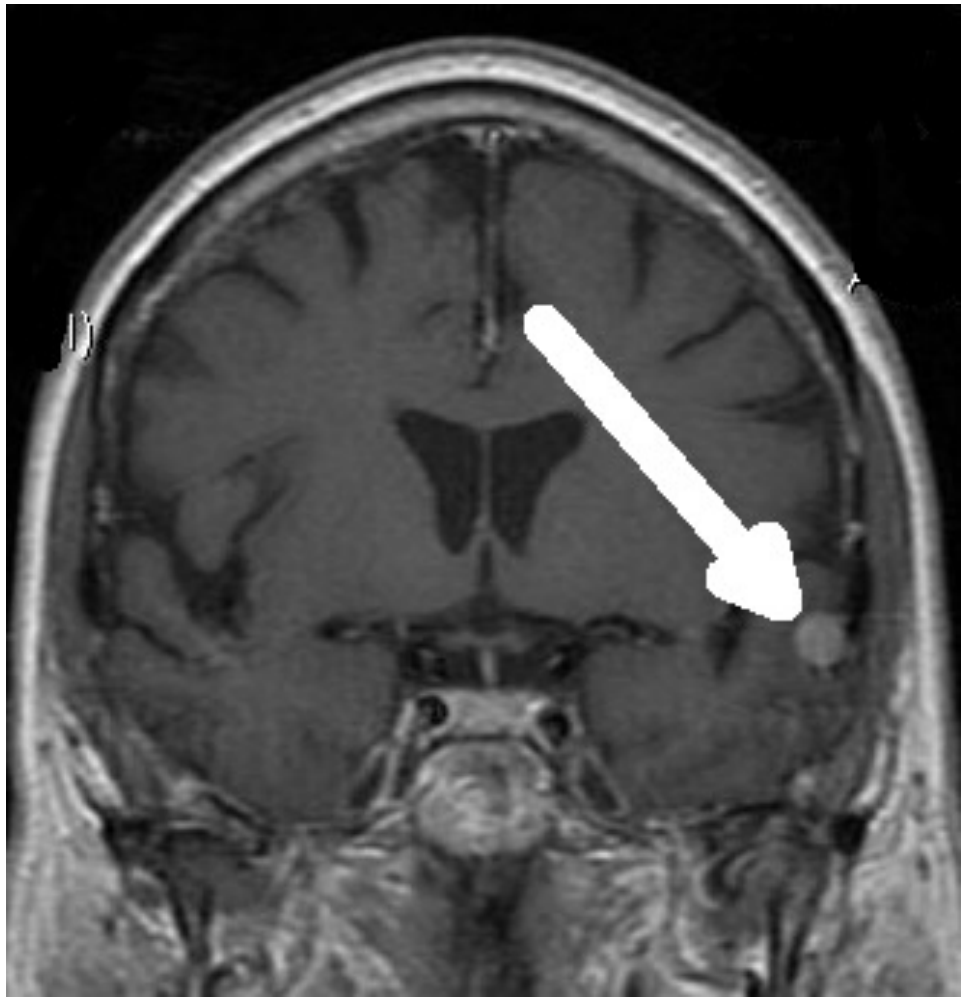
# They can be big causing mass effect




Niiro M, Yatsushiro K, Nakamura K*, et al* Natural history of elderly patients with asymptomatic meningiomas
*Journal of Neurology, Neurosurgery & Psychiatry* 2000;**68:**25-28.

# And they can be small

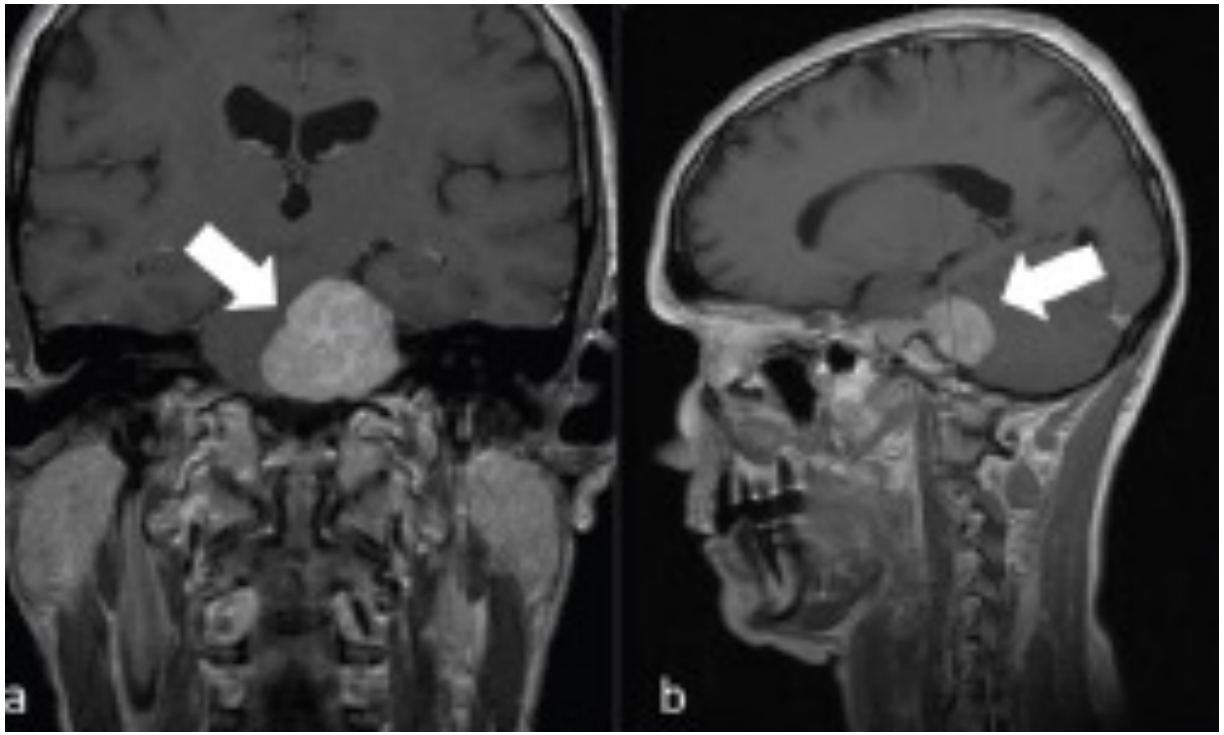
- Not requiring treatment, unless symptomatic



https://dizziness-and-balance.com/disorders/tumors/meningioma.htm

# And they can be difficult to remove

- Only 63% of all skull base meningiomas can achieve gross total through surgery




Nicosia Luca, Di Pietro Salvatore, Catapano Michele, Spadarella Gaia, Sammut Lara, Cannataci Christine, Resta Federico, Reganati Paolo (2019) **Petroclival meningiomas: radiological features essential for surgeons** *ecancer* **13** 907

# Intrakraniale meningeomer

Mikkel Schou Andersen[1], Christian Bonde Pedersen[1], Tiit Mathiesen[2], Lars Poulsgaard[2], Bjarne Winther Kristensen[3], Bo Halle[1] & Frantz Rom Poulsen[1]

- High risk of recurrence = new surgical procedure
- Need for alternative treatments

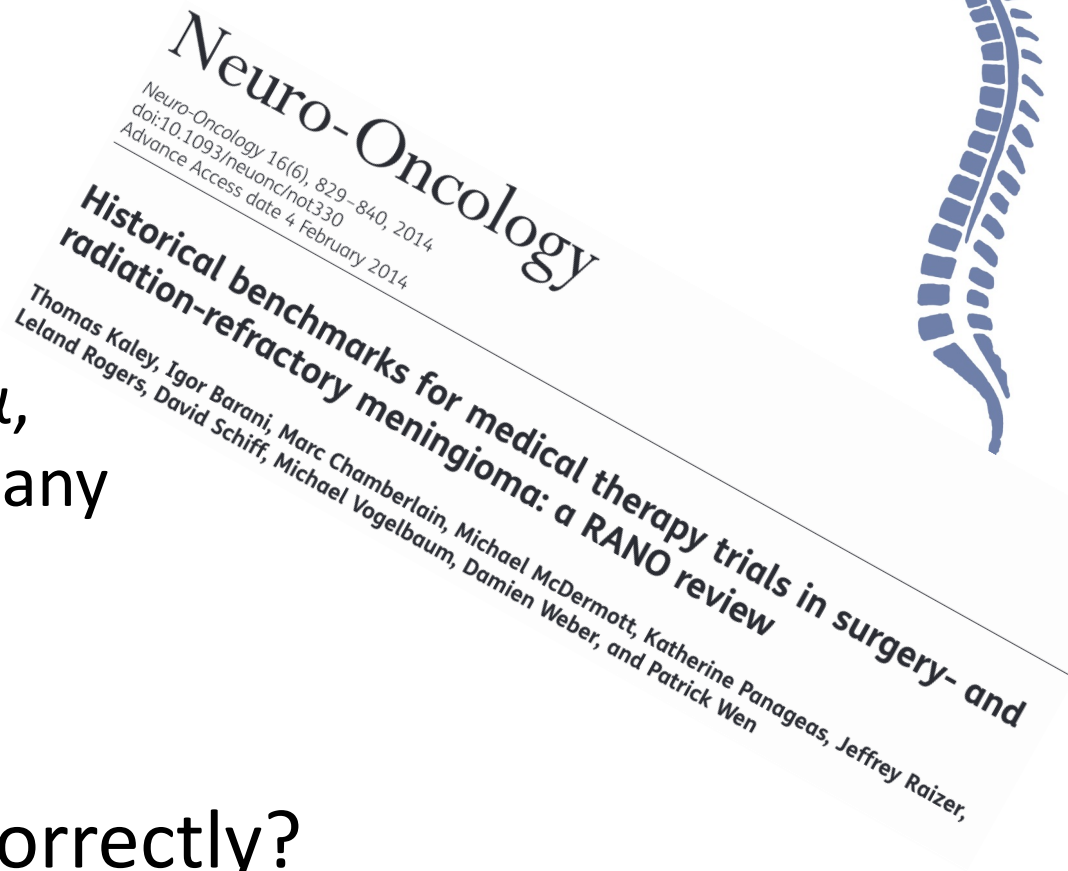
**Simpsongraderingssystemet til recidivbestemmelse ti år postoperativt. Radikal fjernelsee (GTR) bestemmes som grad 13, og subtotal resektion (STR) bestemmes som grad 4 [7].**

| Resektionsgrad | Definition | Recidiv, % |
|---|---|---|
| 1 | GTR af tumor, durale vedhæng og abnormal knogle | 9 |
| 2 | GTR af tumor, koagulation af durale vedhæng | 19 |
| 3 | GTR af tumor, med/uden resektion eller koagulation af durale vedhæng eller ekstradurale udvidelser: invaderet/hyperostotisk knogle | 29 |
| 4 | STR af tumor | 44 |
| 5 | Simpel dekompression: biopsi | –[a] |

a) Grad 5-recidiver kan ikke bestemmes, da tumor ikke reseceres.

# Pharmacological treatment?

- As of now, medical treatment for meningiomas has shown limited efficacy
- Hydroxyurea, temozolomide, interferon-α, mifepristone, ocreotide-analogues and many more without significant effect - *RANO review 2014*

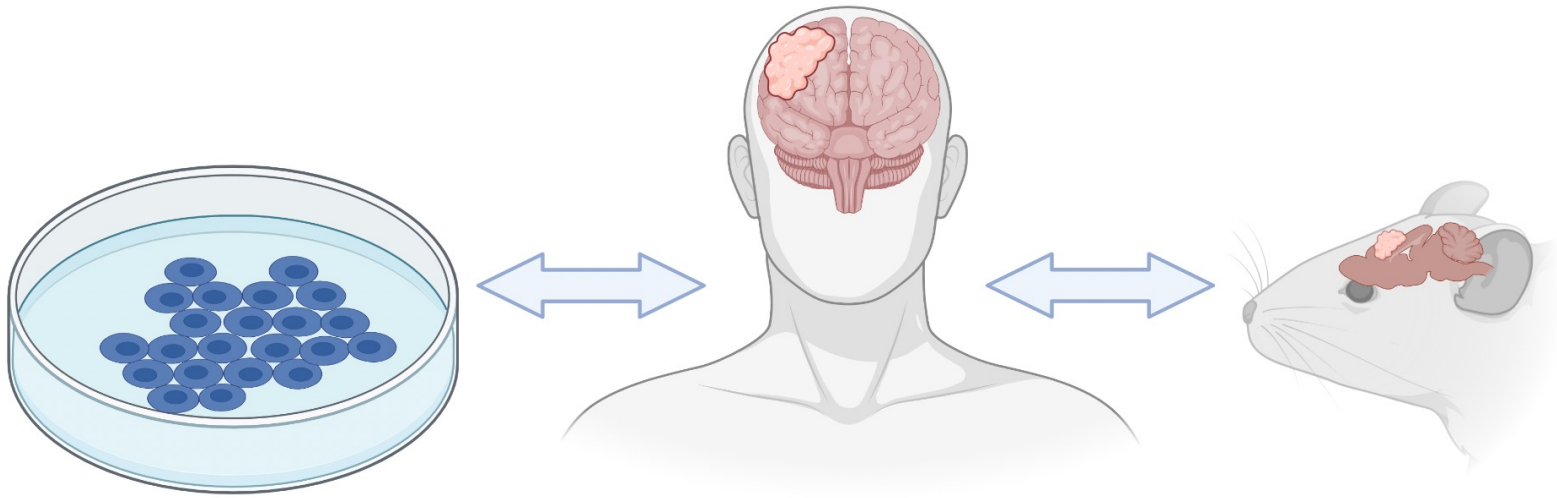



- Maybe we assess the tumor type incorrectly?

# Meningiomas – *a Heterogenous Tumor*

- WHO Classification 2021 identifies 15 subtypes of meningiomas
- Various genomic markers: NF2, TRAF7, AKT1, KLF4 etc.
- Different epigenomic profiles (i.e. DNA-Methylation)

Models should reflect the heterogeneity



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Am J Transl Res 2014;6(2):114-118
www.ajtr.org /ISSN:1943-8141/AJTR1312010

*Review Article*

# Lost in translation: animal models and clinical trials in cancer treatment

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Received December 20, 2013; Accepted December 5, 2013; Epub January 15, 2014; Published January 30, 2014

**Abstract:** Due to practical and ethical concerns associated with human experimentation, animal models have been essential in cancer research. However, the average rate of successful translation from animal models to clinical cancer trials is less than 8%. Animal models are limited in their ability to mimic the extremely complex process of human carcinogenesis, physiology and progression. Therefore the safety and efficacy identified in animal studies is generally not translated to human trials. Animal models can serve as an important source of *in vivo* information, but alternative translational approaches have emerged that may eventually replace the link between *in vitro* studies and clinical applications. This review summarizes the current state of animal model translation to clinical practice, and offers some explanations for the general lack of success in this process. In addition, some alternative strategies to the classic *in vivo* approach are discussed.

# Example of a promising drug *in vivo* - ***Hydroxyurea***

- In vivo tumors significantly smaller (40-80%) with a decrease of 10% in MIB-1 ratio and 75 % decrease in vascularity. Calcium antagonists increase effectiveness of RU486 and **Hydroxyurea**

EXPERIMENTAL STUDIES

## Calcium Channel Antagonists Augment Hydroxyurea- and RU486-induced Inhibition of Meningioma Growth In Vivo and In Vitro

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**David L. Gillespie, Ph.D.**
Huntsman Cancer Institute, Salt Lake City, Utah

**Vitaly Kushnir, M.D.**
Huntsman Cancer Institute, Salt Lake City, Utah

**Nika Polevaya, B.S.**
Huntsman Cancer Institute, Salt Lake City, Utah

**David Kelly, B.S.**
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Email: randy.jensen@hsc.utah.edu

**OBJECTIVE:** Although the chemotherapy drug hydroxyurea (HU) and the antiprogesterone mifepristone (RU486) have been used to treat meningiomas for which surgical and radiation therapies have failed, results have been disappointing. The addition of calcium channel antagonists (CCAs) to chemotherapeutic drugs enhances tumor growth inhibition in other tumor types, and the authors demonstrated that CCAs can block meningioma growth in vitro and in vivo. The purpose of this study was to test the effects of the addition of a CCA to HU or RU486 on meningioma growth.

**METHODS:** Primary and malignant (IOMM-Lee) meningioma cell lines were treated with HU, RU486, or either of these plus diltiazem or verapamil. Assays for cell growth, apoptosis, and fluorescent-activated cell sorting were performed on in vitro cultures. Similar cell lines were implanted into nude mice and were treated with HU or RU486, in combination with a CCA. Tumors were analyzed by light microscopy, MIB-1, and factor VIII immunohistochemical staining studies.

**RESULTS:** The addition of diltiazem or verapamil to HU or RU486 augmented meningioma growth inhibition by 20 to 60% in vitro. In vivo, tumors treated with combination drugs were smaller; and immunohistochemical analysis of the IOMM-Lee tumors showed a 10% decrease in the MIB-1 ratio (from 0.41 to 0.30) and an approximate 75% decrease in microvascular density.

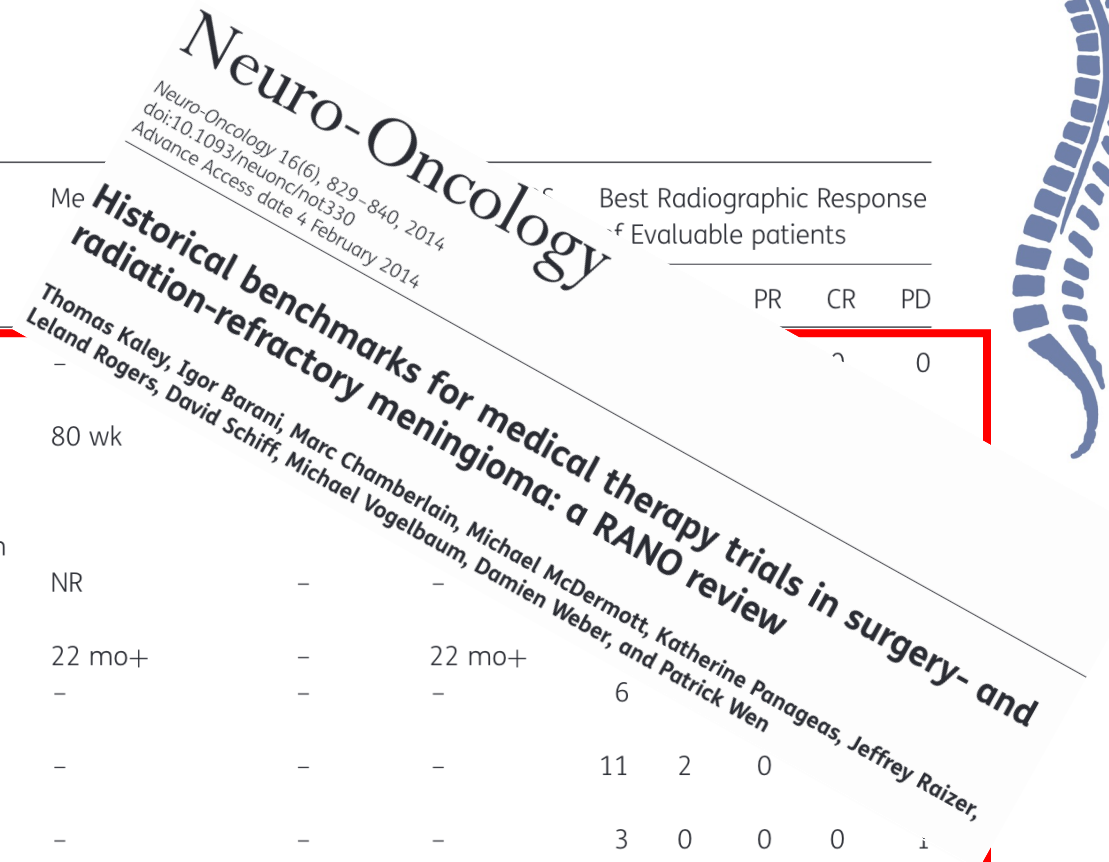
**CONCLUSION:** The addition of diltiazem or verapamil to HU or RU486 augments meningioma growth inhibition in vitro by inducing apoptosis and $G_1$ cell-cycle arrest. The combination of HU and diltiazem inhibited the growth of meningiomas in vivo by decreasing proliferation and microvascular density. These results suggest a possible role for these drugs as an additional adjuvant therapy for recurrent or unresectable meningiomas.

**KEY WORDS:** Calcium channel antagonists, Diltiazem, Hydroxyurea, Meningioma, Mifepristone, Verapamil

*Neurosurgery 59:1109-1121 2006* DOI: 10.1227/01.NEU.0000245597.46581.FB www.neurosurgery-online.com

# Human study counterparts

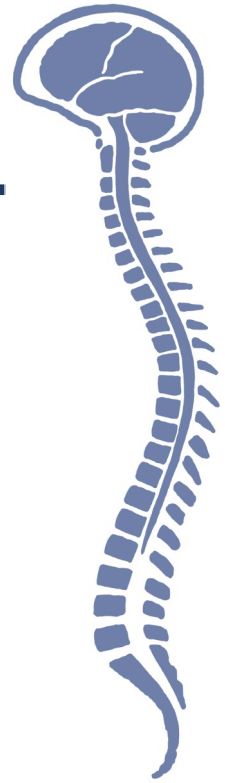
| Agent/Regimen | Author | Year | WHO Grade | | Prior Therapy | Me | | | Best Radiographic Response of Evaluable patients | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | n/a | I | | | | | | PR | CR | PD |
| Hydroxyurea | Schrell | 1997[42] | – | 3 | 3 prior surgery<br>2 prior RT | – | | | | | | 0 |
| Hydroxyurea | Newton | 2000[32] | 4 | 13 | 13 prior surgery<br>7 prior RT<br>9 with POD pre-tx<br>1 atypical due to brain invasion | 80 wk | | | | | | |
| Hydroxyurea | Mason | 2002[29] | – | 16 | 16 prior surgery<br>4 prior RT | NR | – | – | | | | |
| Hydroxyurea | Paus | 2003[35] | – | 1 | None | 22 mo+ | – | 22 mo+ | | | | |
| Hydroxyurea | Loven | 2004[27] | – | 8 | All prior surgery<br>6 prior RT | – | – | – | 6 | | | |
| Hydroxyurea (with RT) | Hahn | 2005[19] | – | 13 | All surgery<br>None prior RT | – | – | – | 11 | 2 | 0 | |
| Hydroxyurea | Weston | 2006[46] | 1 | 5 | Not documented | – | – | – | 3 | 0 | 0 | 0 |
| Hydroxyurea | Swinnen | 2009[25] | – | 28 | Not documented | 27 mo | – | NR | 20 | 0 | 0 | 0 |
| Hydroxyurea | Chamberlain | 2011[7] | – | 60 | All prior surgery (29 >1 op)<br>All prior RT | 4 mo | 10% | – | 21 | 0 | 0 | 0 |



# Possible reasons for ‘lost in translation’

1. Xenograft placement:
   - **Heterotopic models:** flank models does not mimic intracranial microenvironment
   - **Orthotopic models:** Brain size – animal size, but what are the alternatives?
   - Also xenograft models lack (part) immunesystem
2. Use of immortalized cell lines
   - Does not mimic heterogenous composition of cells in patient-derived samples and inter-patient tumor heterogeneity
     **What works for one tumor might not work for another**

**Bonus:** Benign xenograft models based on patient-derived samples require long incubation time and have lower induction rates – **More costly and less feasible as a drug testing model**



# *In Vivo* Models in Meningiomas

Closer to a true model,
but issues…

Cheaper, more accessible alternatives should be considered

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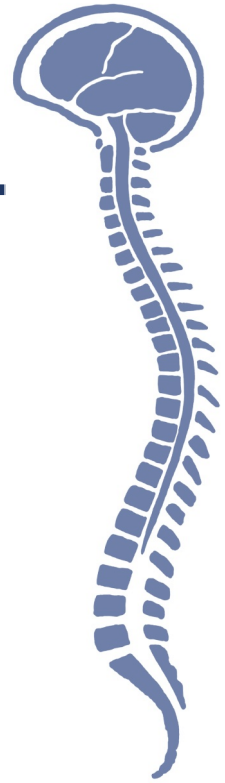
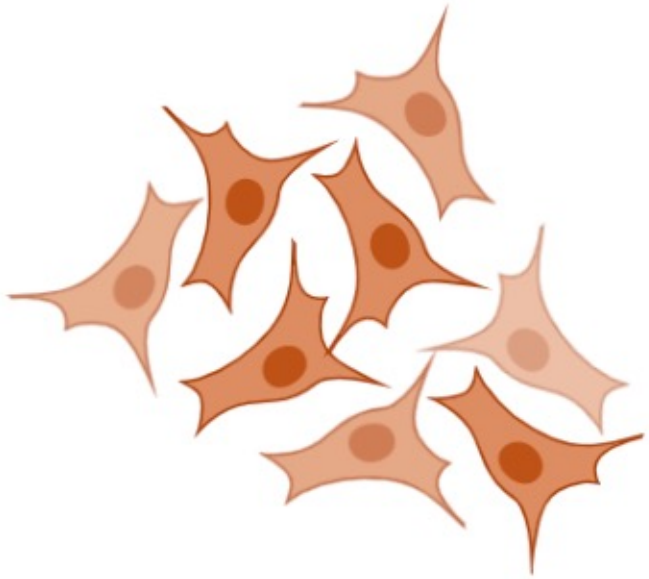
# A paradigm shift in assessing treatment options in meningiomas is needed

*Focus on heterogeneity and 'personalized' treatment*

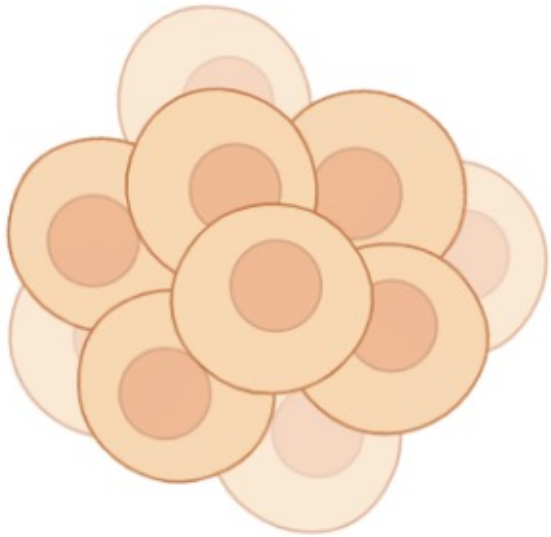
*Test of pharmaceuticals already approved for human use in vitro instead of in vivo*



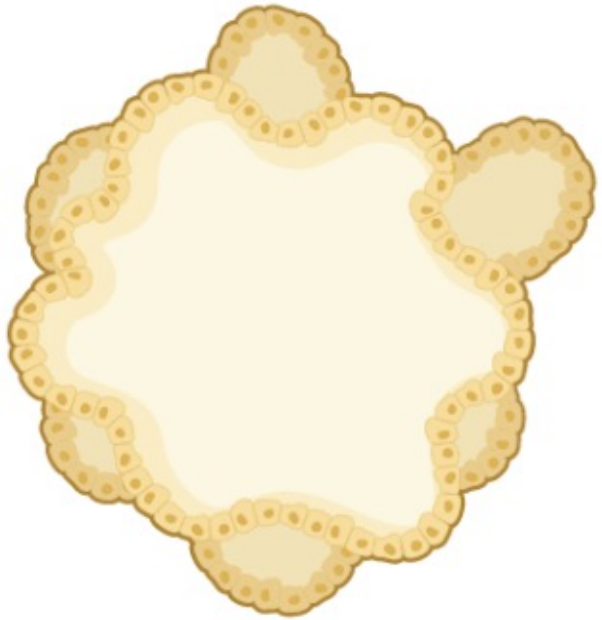
# *In Vitro* Models in Meningiomas



2D Cells



3D Spheroids



Organoids

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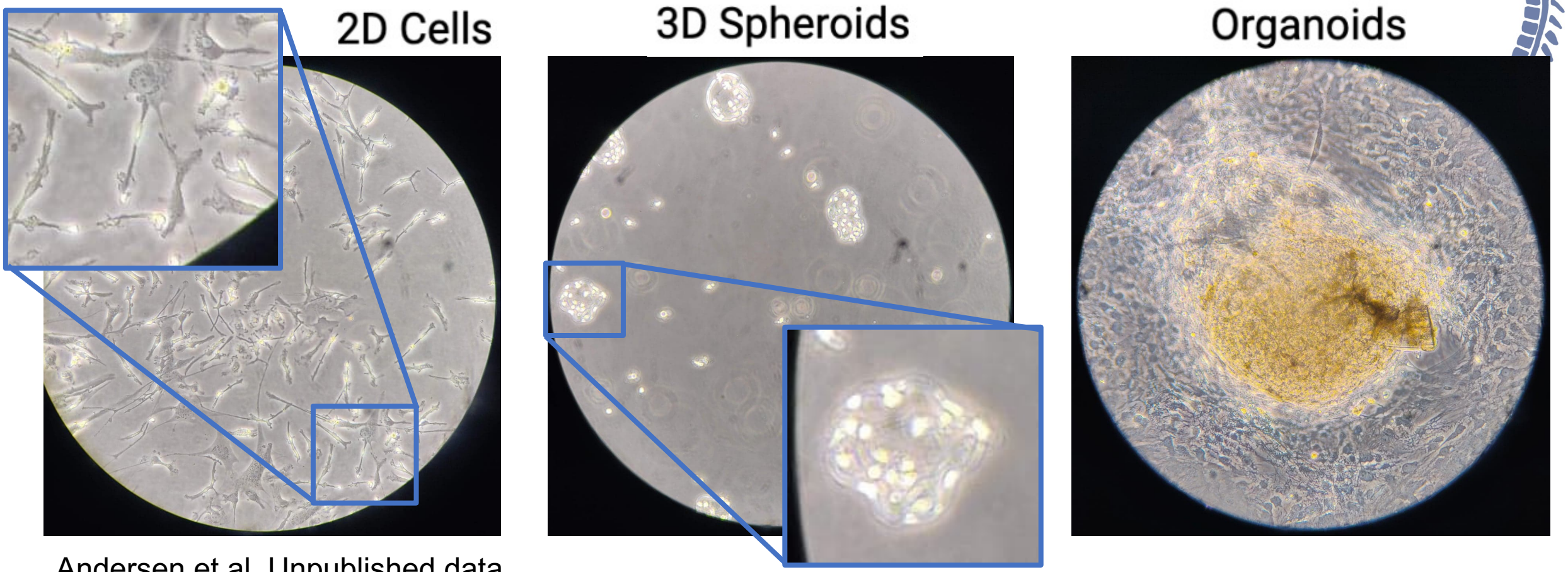
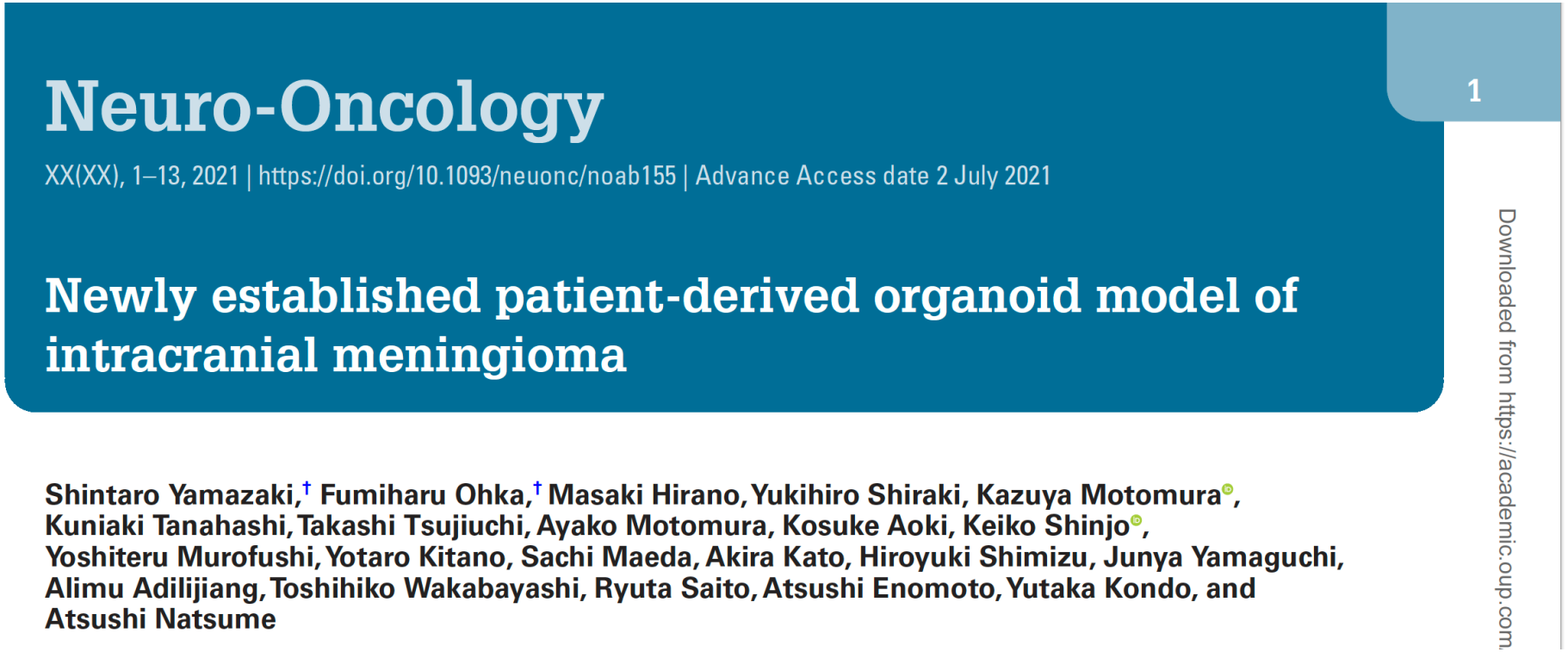
# *In Vitro* Models in Meningiomas




Andersen et al. Unpublished data

# New emerging modelling in Meningiomas



Neuro-Oncology

XX(XX), 1–13, 2021 | https://doi.org/10.1093/neuonc/noab155 | Advance Access date 2 July 2021

1

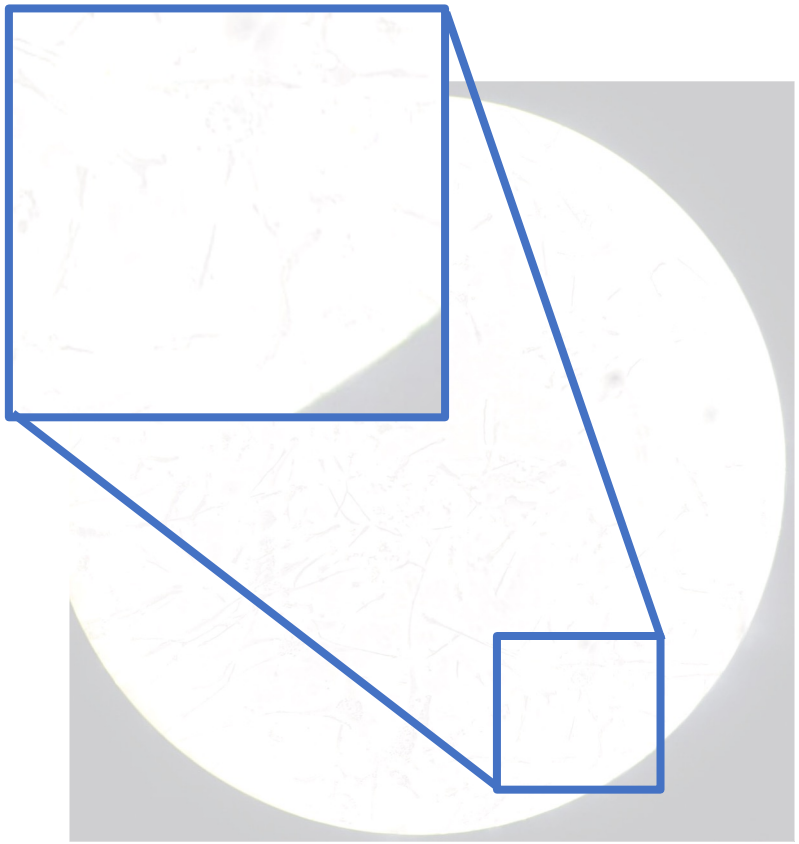


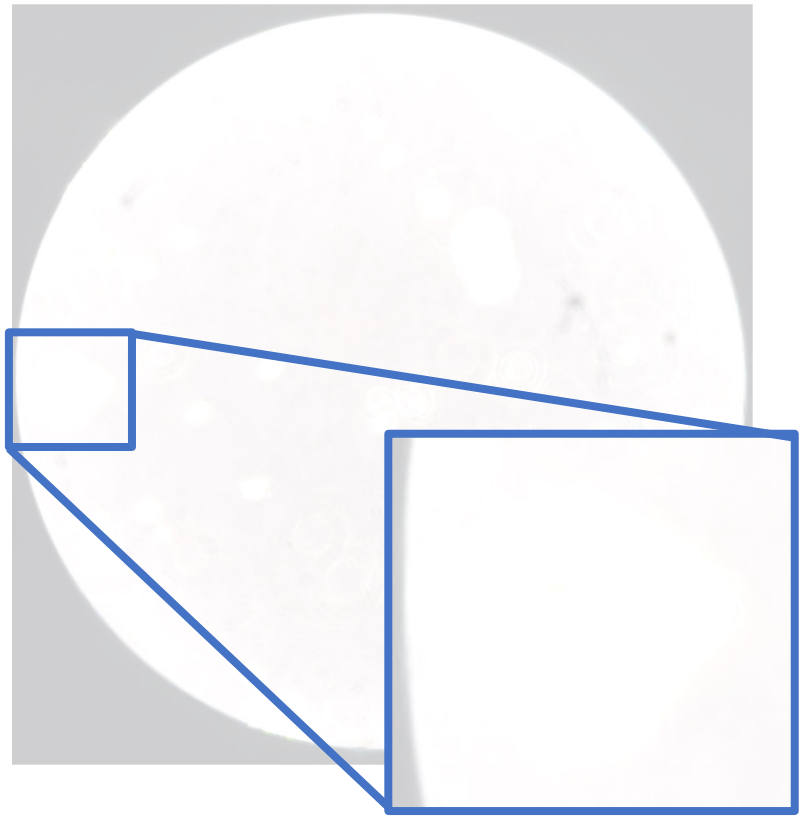
**Newly established patient-derived organoid model of intracranial meningioma**

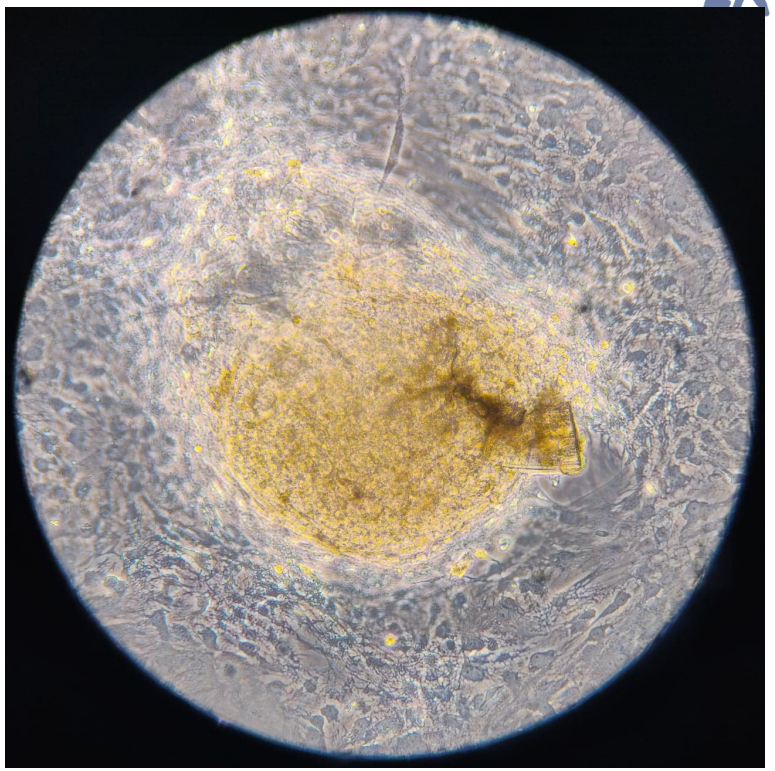
Shintaro Yamazaki,† Fumiharu Ohka,† Masaki Hirano, Yukihiro Shiraki, Kazuya Motomura, Kuniaki Tanahashi, Takashi Tsujiuchi, Ayako Motomura, Kosuke Aoki, Keiko Shinjo, Yoshiteru Murofushi, Yotaro Kitano, Sachi Maeda, Akira Kato, Hiroyuki Shimizu, Junya Yamaguchi, Alimu Adilijiang, Toshihiko Wakabayashi, Ryuta Saito, Atsushi Enomoto, Yutaka Kondo, and Atsushi Natsume

A new way to studying treatment and tumor traits in meningiomas

# Meningioma organoids



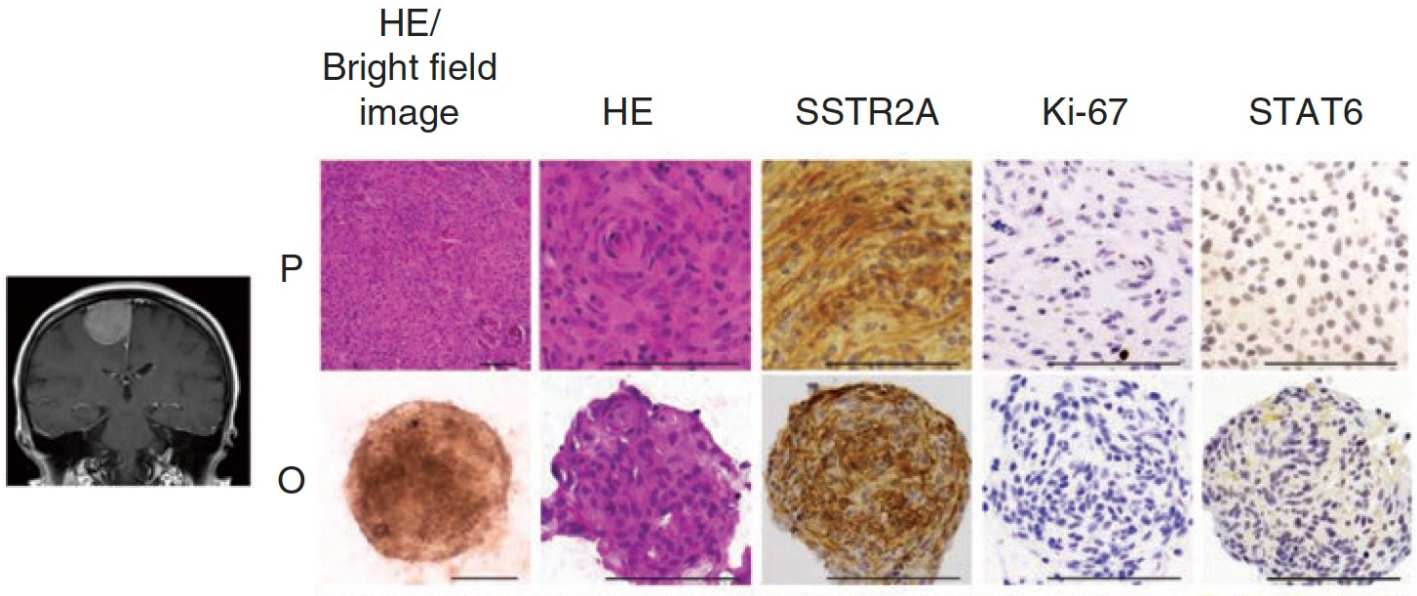





Andersen et al. Unpublished data

# 'Close(r)' *In Vitro* resemblance to human tumors

- Most accurate *In Vitro* presentation
  - Morphology
  - Surface and nucleus markers
- Similar epigenetic profiles



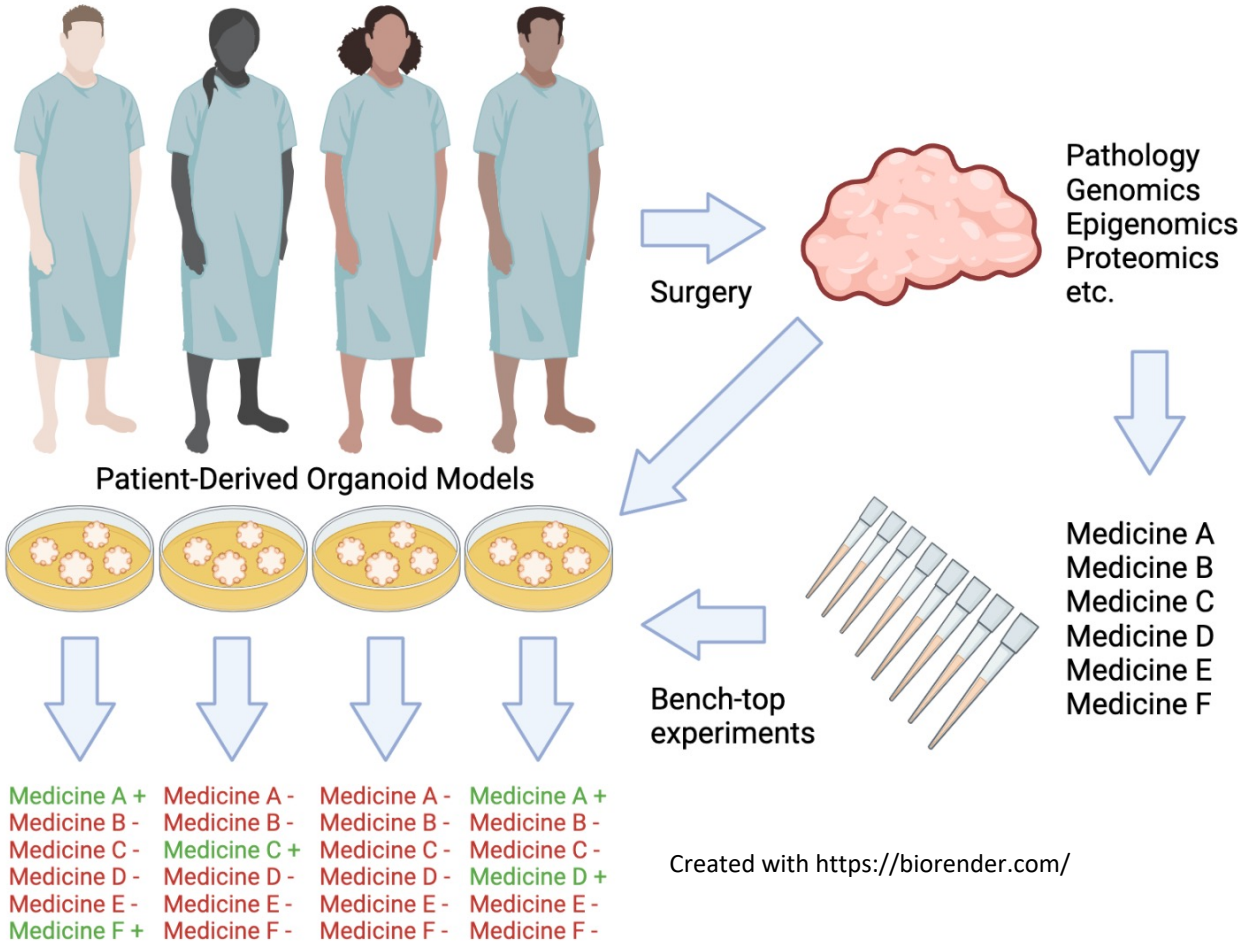

Yamazaki S et al. Newly established patient-derived organoid model of intracranial meningioma. Neuro Oncol. 2021 Nov 2;23(11):1936-1948. doi: 10.1093/neuonc/noab155. PMID: 34214169; PMCID: PMC8563327.

# The Age of Personalized Medicine

- Heterogenous tumors = need for targeted-individual therapy

Personalized organoids could be used for

- … meningiomas undergoing subtotal resection in surgical inaccesible areas
- … tumors displaying atypical or malignant features

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# If we are succesful!

- Perhaps many previous human trials have failed due to heterogeneity – *In vitro/in vivo studies primarily test few samples or established cell lines*

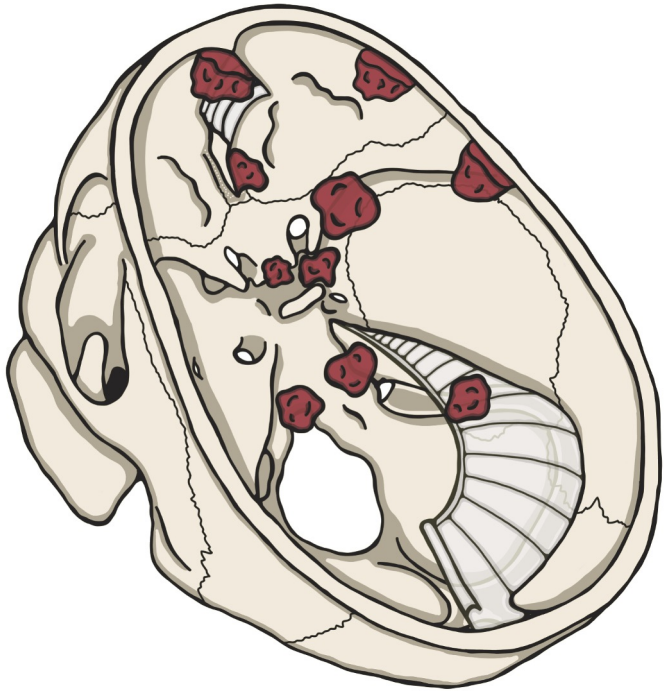
- **We aim and hope we can establish a clinical-to-bench-top pathway in a day-to-day clinical setting to the benefit of the individual patient**

**This study is to test this**

# Thank you – Questions?

## Research team and collaborators

- Department of Neurosurgery (OUH, RH)
  - Frantz Rom Poulsen, Bo Halle, Christian Bonde, Tiit Mathiesen
- Department of Pathology (SVS, OUH, RH)
  - Martin Wirenfeldt Nielsen, Jeanette Krogh Pedersen, Bjarne Winther Kristensen
- Cell laboratories (RUH, OUH)
  - Birgitte Brinkmann Olsen, Åsa Fex Svenningsen



Illustrator: Mikkel Schou Andersen, 2019