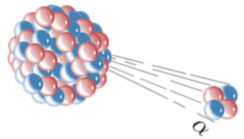
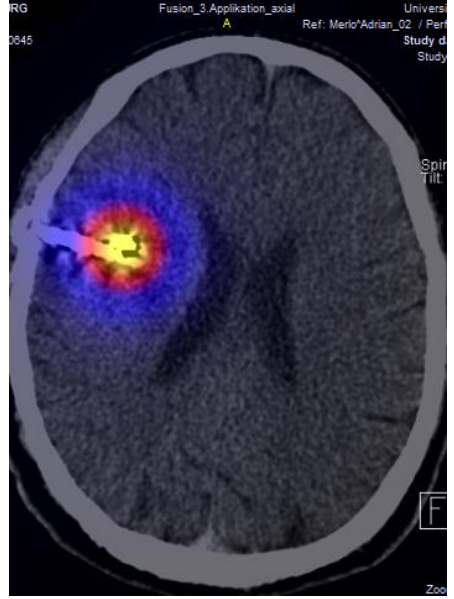




Targeted alpha Therapy

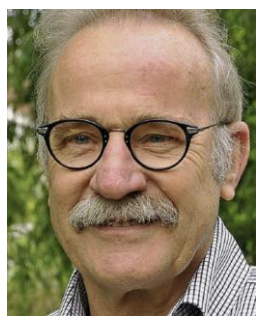
For the Treatment of

Malignant Gliomas WHO Grades II-IV

Adrian Merlo, Dominik, Cordier, Helmut Mäcke, Jan Müller, Otmar Gratzl
Neurosurgery and Nuclear Medicine, U of Basel, Switzerland

Frank Bruchertseifer, Alfred Morgenstern
European Commission, Joint Research Centre, Karlsruhe, Germany

Leszek Krolicki, Jolanta Kunikowska
Institute of Nuclear Medicine, U of Warsaw, Poland



Preclinical and clinical development: 25 year of research

Topics

- **How do we understand cancer today?**
- **Specific factors of malignant brain tumors (gliomas, glioblastomas)?**
- **Why do most solid tumors resist therapeutic efforts?**
- **How can radioactivity be used to treat cancer?**
- **How to apply a radiopharmaceutical to treat malignant gliomas**
- **How to produce therapeutic radionuclides, e.g. Actinium-225?**
- **How to apply targeted alpha therapy in malignant gliomas?**
- **How to overcome to impasse of clinical development in orphan disease?**

How do we understand cancer today?

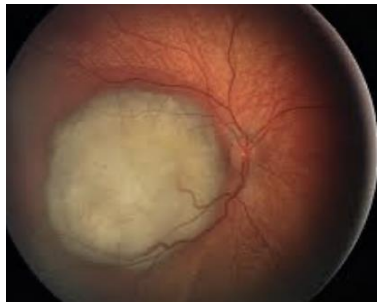
- Cancer a genetic disease
- Gap between immense knowledge gain and therapeutic application

Cancer = genetic disease

Sporadic Cancers = acquired mutations

Syndromatic = Hereditary Cancers

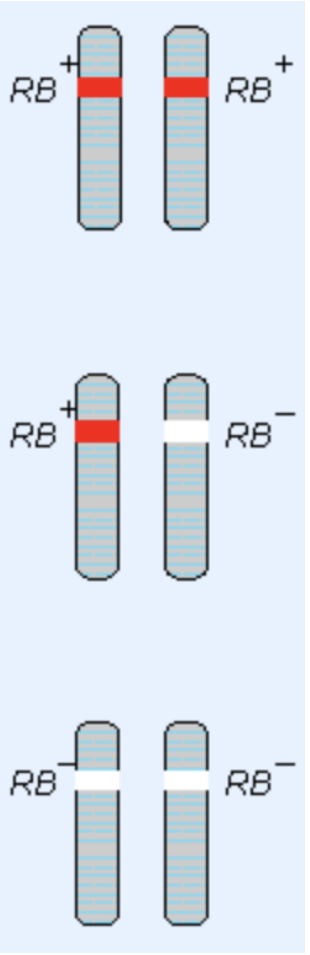
Sporadic : hereditary ≈ 90% : 10%



Tumor suppressor gene,

loss of function mutation

- e.g. retinoblastoma gene

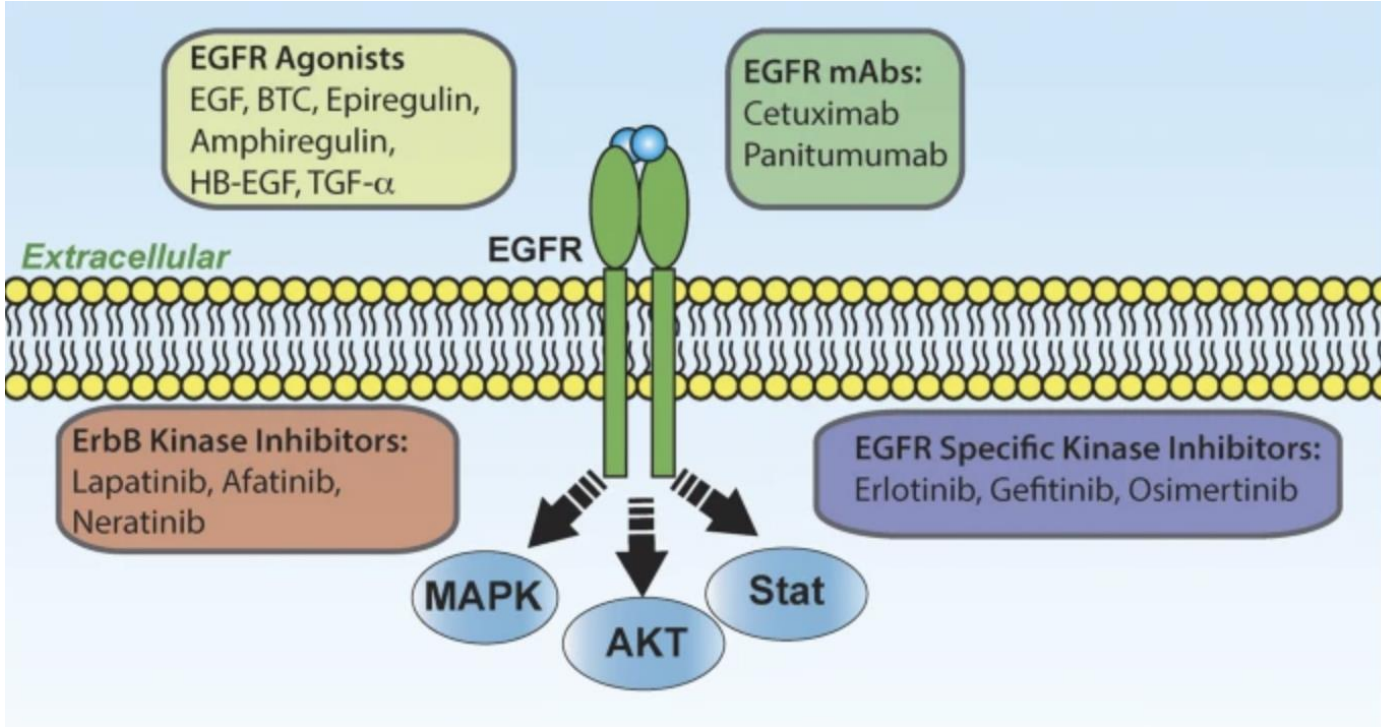


Cancer = genetic disease

Oncogene

Gene activating mutations

e.g. Ras, EGFR

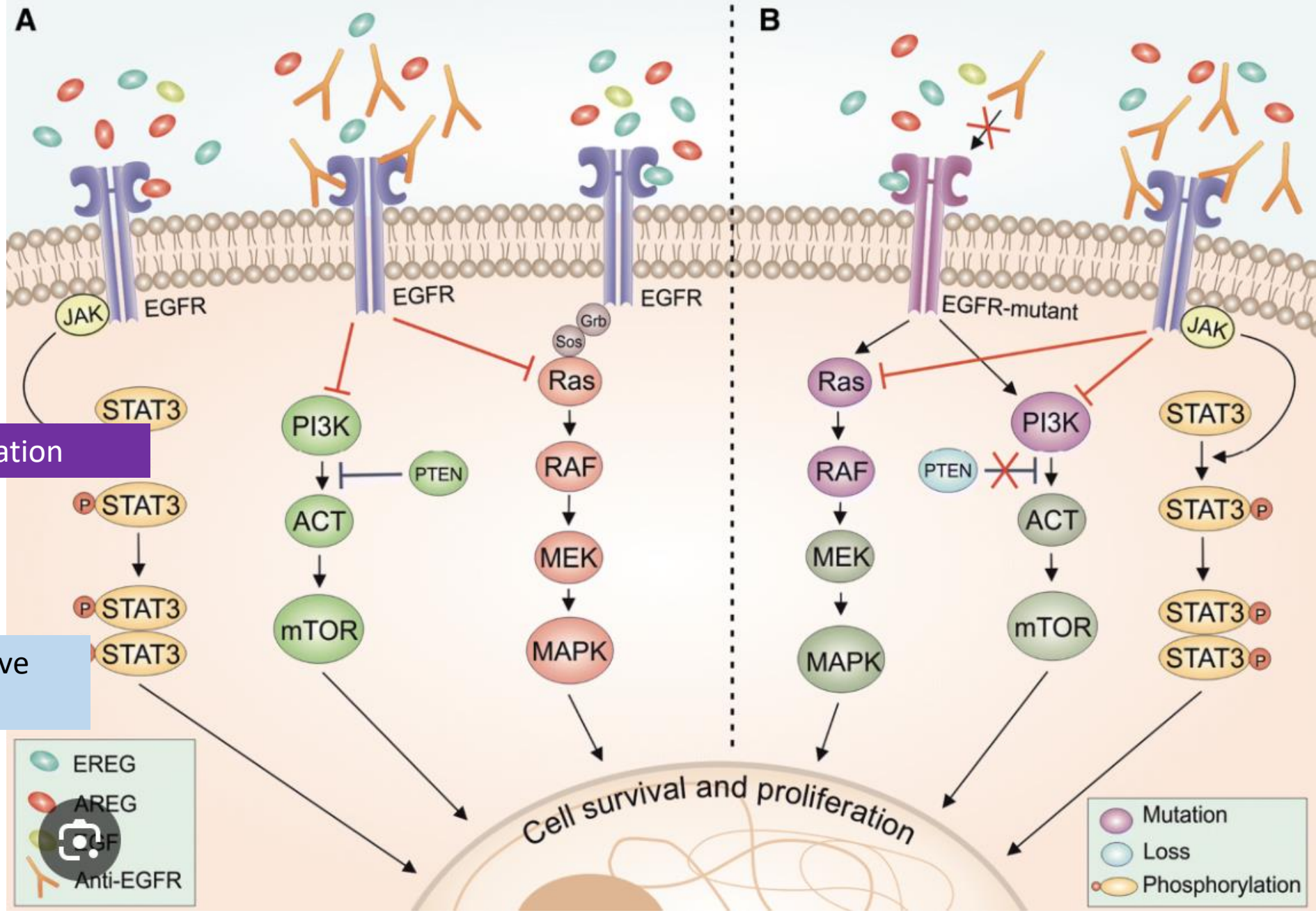


Oncogenes and TSGs change signal transduction pathways

2 classes of cancer mutations

Tumor Oncogene: activating mutation

Tumor suppressor genes: recessive
(2 hits for gene inactivation)



wild-type

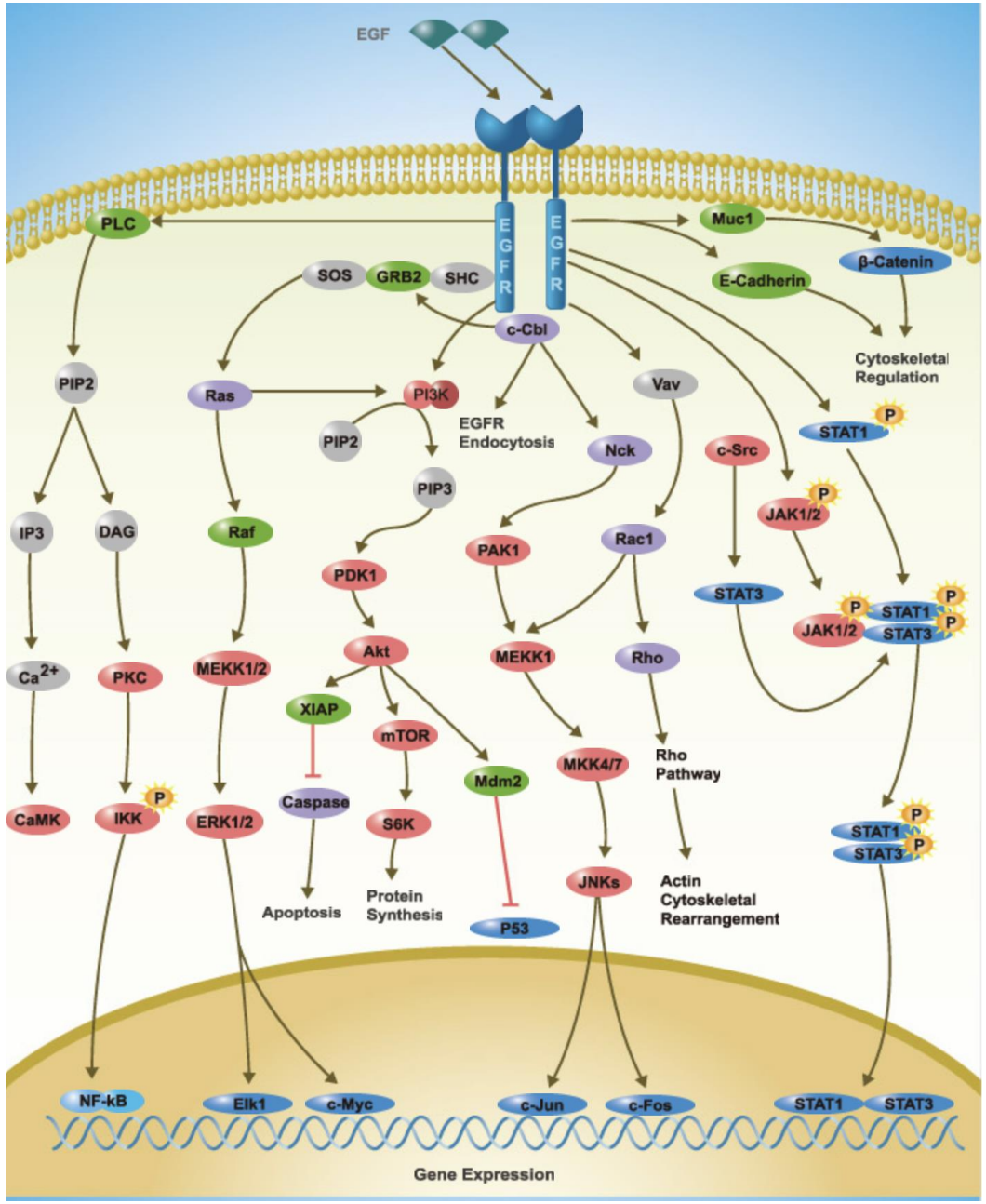
mutant

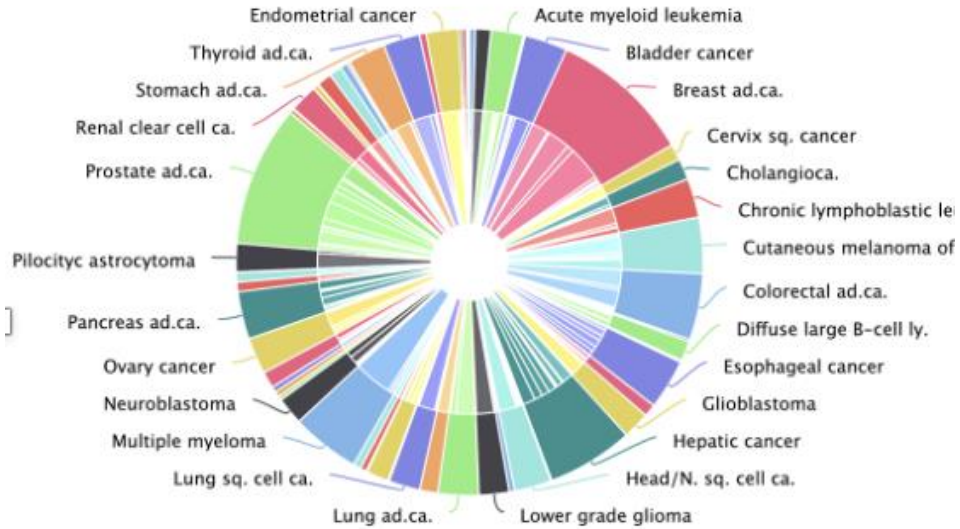
Functional Order in Mutant Cancer Pathways

Pathways	**Genes/Mechanisms**
Proliferation	RTKs/Oncogenes: EGFR, VEGFR etc
Apoptosis	p53/MDM/BCL-2 etc
Cell Cycle Regulation	CycD/CDKN2a/Rb etc
Migration/Invasion	FAK, CD44, PTEN etc
Angiogenesis	VEGFR, Ang2, STAT3 etc
Metabolism	Glycolysis, *Isocitratdehydrogenase*

Epigenetics / Histone-DNA Methylation /
non-coding RNAs: micro, longer, circular

Regulation of gene expression with altered cell signalling…





28,076 Tumors · 221 cohorts · 66 Cancer Types · 203,003,747 Mutations

568 Cancer Genes across 66 Cancer Types

- Human genome: 3 Mia base pairs
- On average 7230 mutations per tumor
- About 10 cancer genes per cancer type
- Most mutations the result of genetic instability!
- Dysfunctional DNA repair

Limitation:

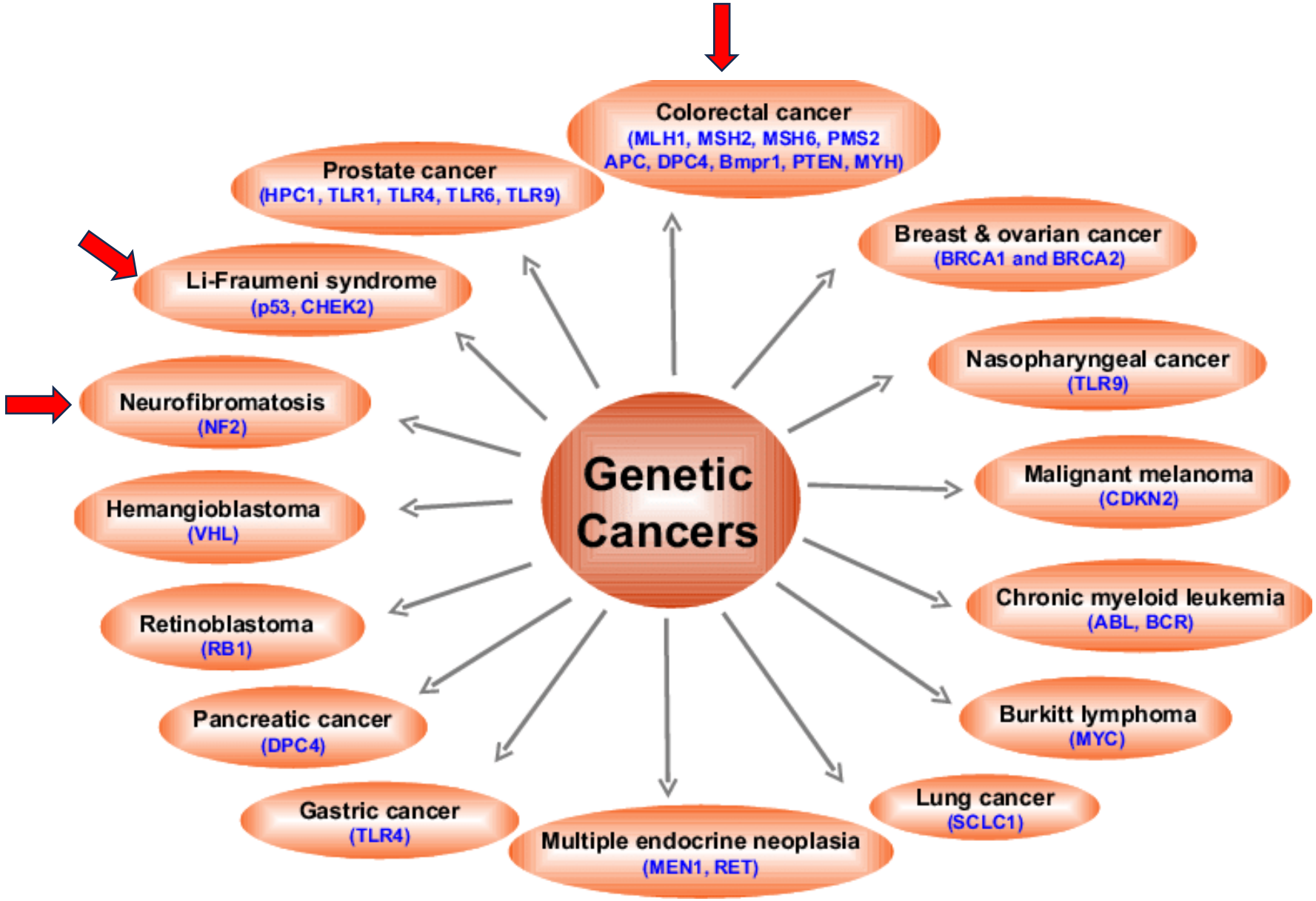
A few therapeutically relevant mutations known at present

Bcr-abl: Glivec

B-raf mutations

etc.

Nature Reviews Cancer 2020, 10, 1038



Hereditary cancer syndromes: occurence of malignant gliomas

Specific factors of malignant brain tumors (gliomas, glioblastomas)?

- Classification of glioma
- Glioma genetics: prognostic, but not therapeutic implications

Brain Tumorigenesis

Glial restricted precursor cell

Acquired mutations

Genetic instability

↓

Genotype change

↓

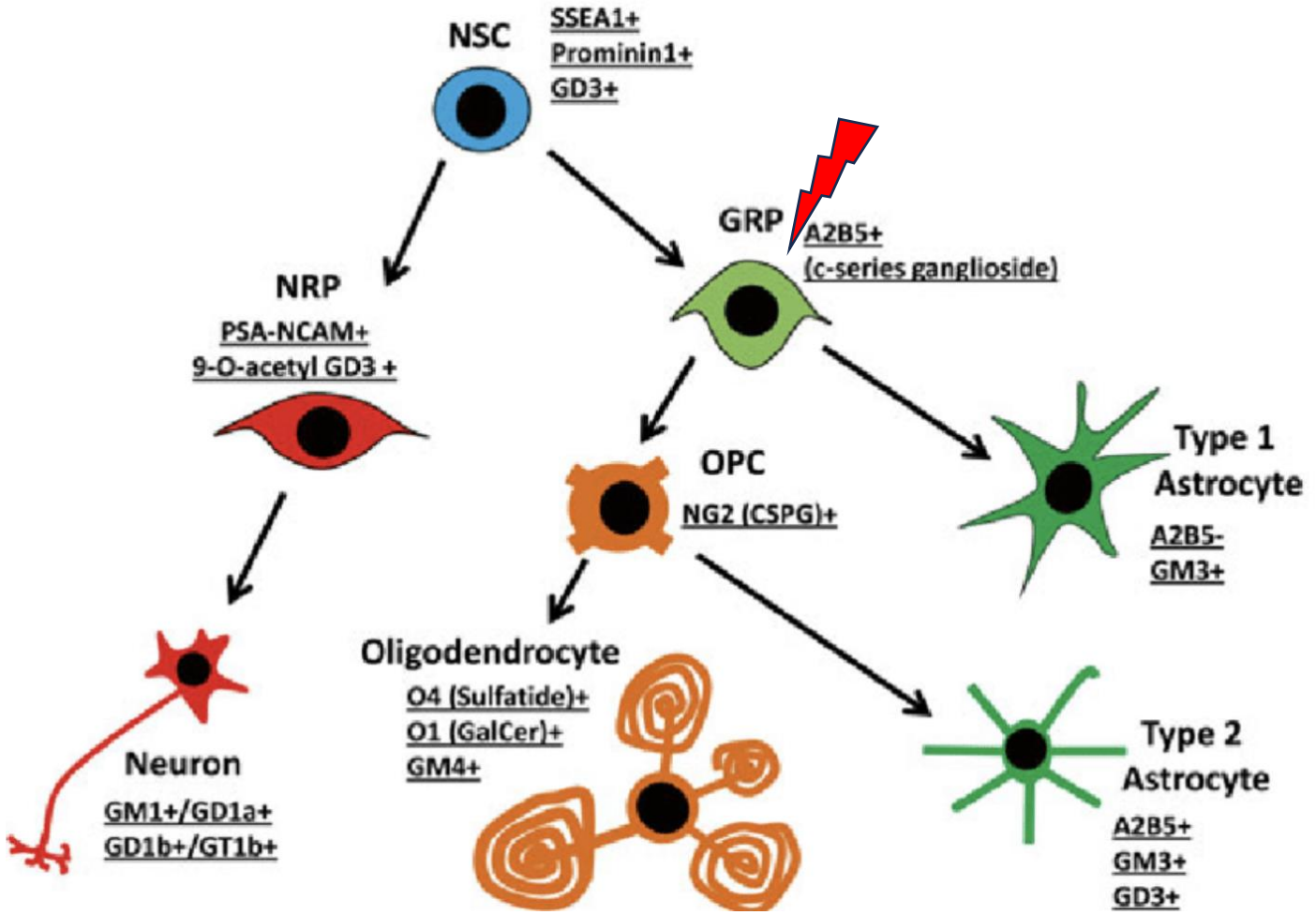
Phenotype change

cell division

cell migration

ECM change

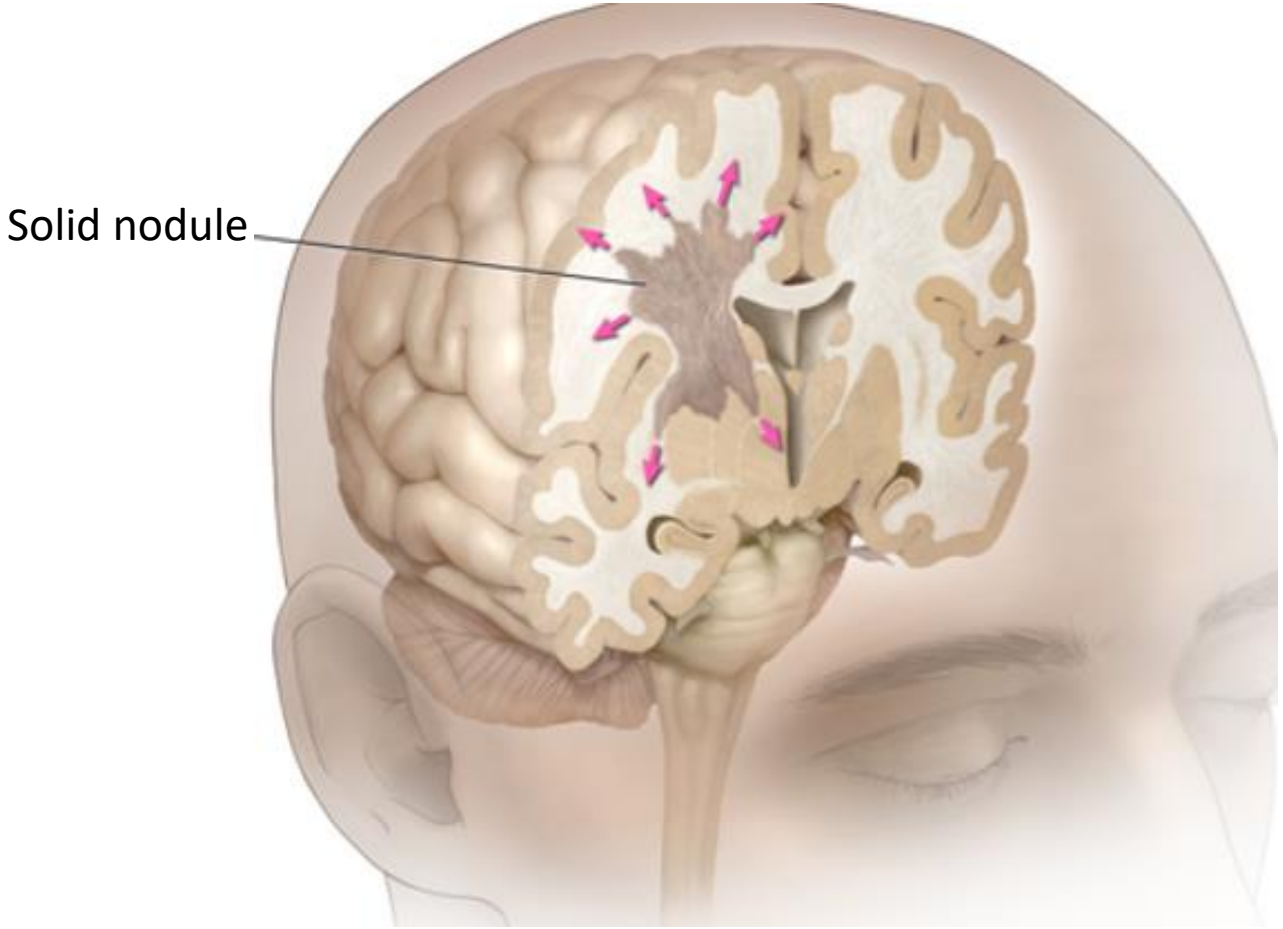
Normal brain development



Malignant Glioma WHO II-IV

= 2 component disease

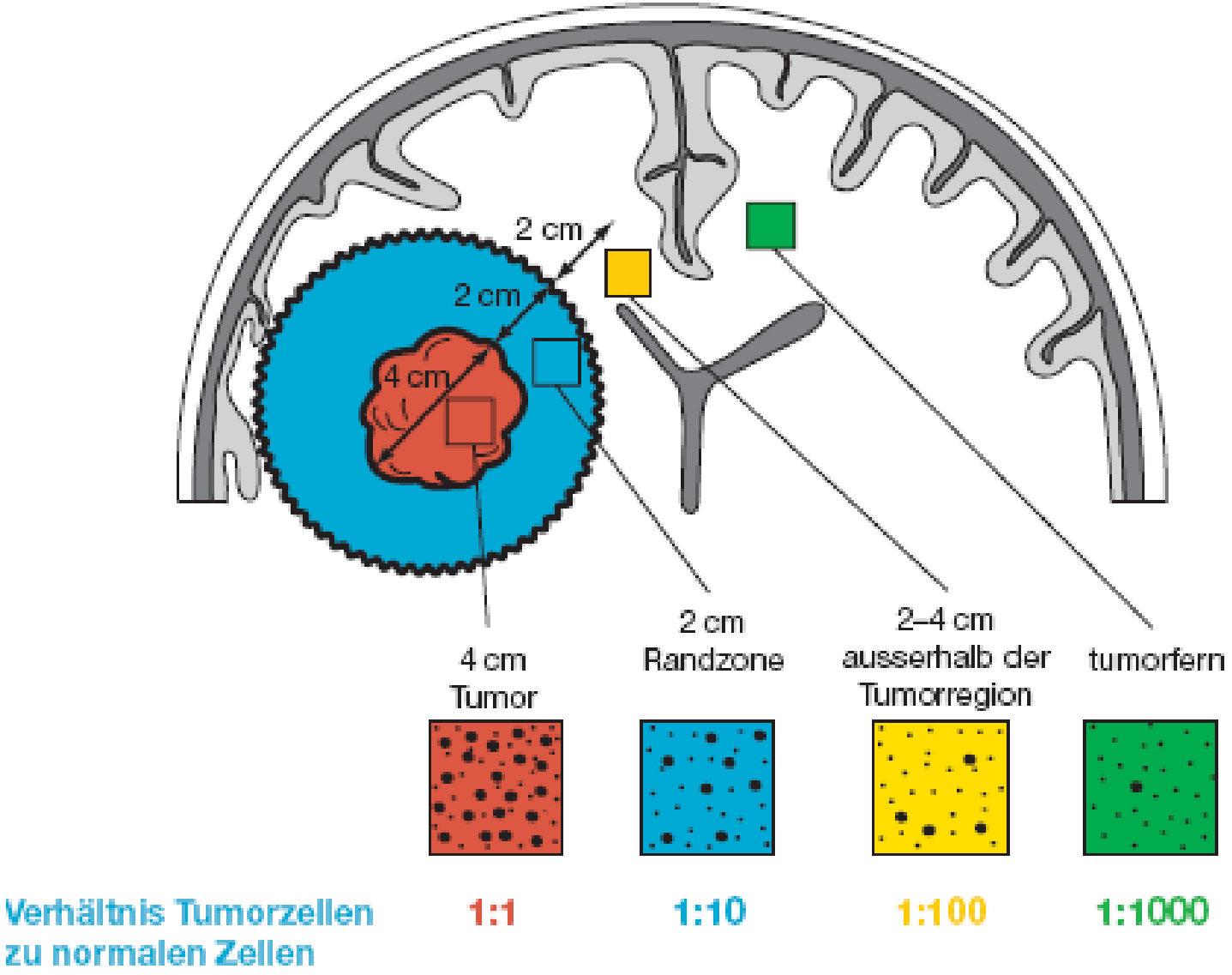
- **Tumor nodule** (surgery?)
- **Invasive tumor cells** (?)



Peter Burger
Pathologist Duke/JHH
Sabbatical USZ

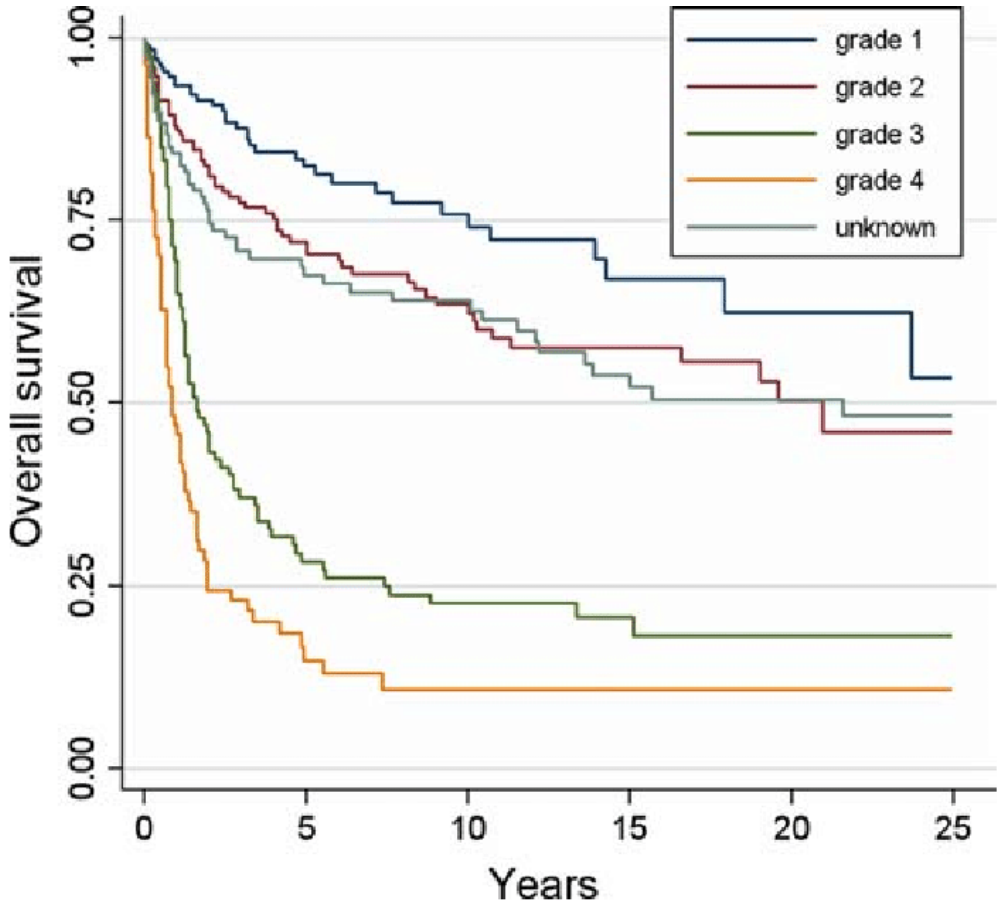
Whole Brain Cuts of GBM patients
Microscopic Analysis
Visualization of Invasive Cells

Glioma = Whole Brain Disease!



Burger PC. Pathology of Tumours of the Nervous System *Jama: the Journal of the American Medical Association*. 239: 973.

Malignant Gliomas: Brain Intrinsic Tumors: Orphan Disease 3-4 cases/100’000/y

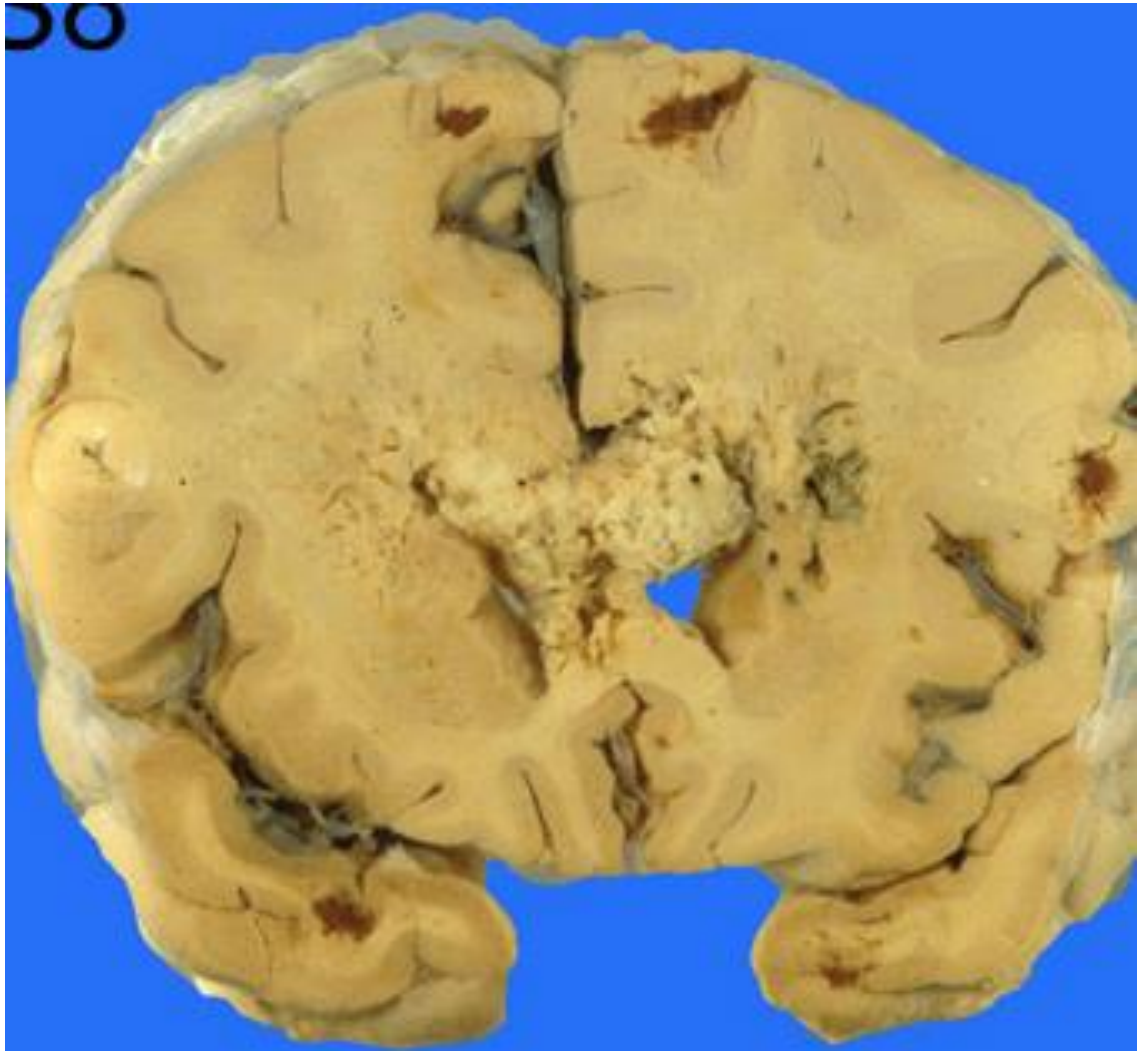


Kaplan-Meier survival curve

Grade 4 Gliomas

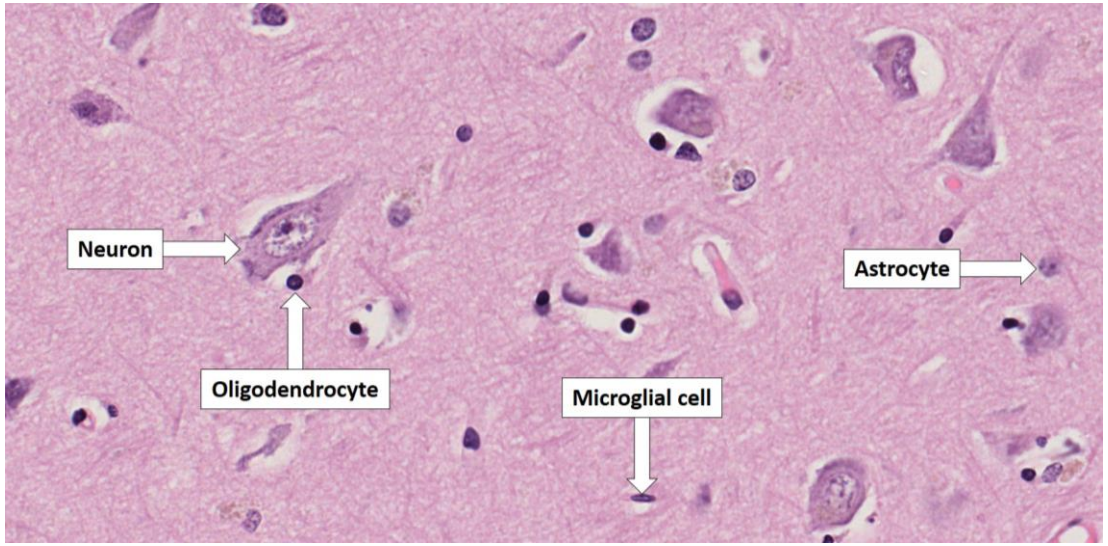
Median Survival Time 12-15 months

about 50% of patients die within 1 year

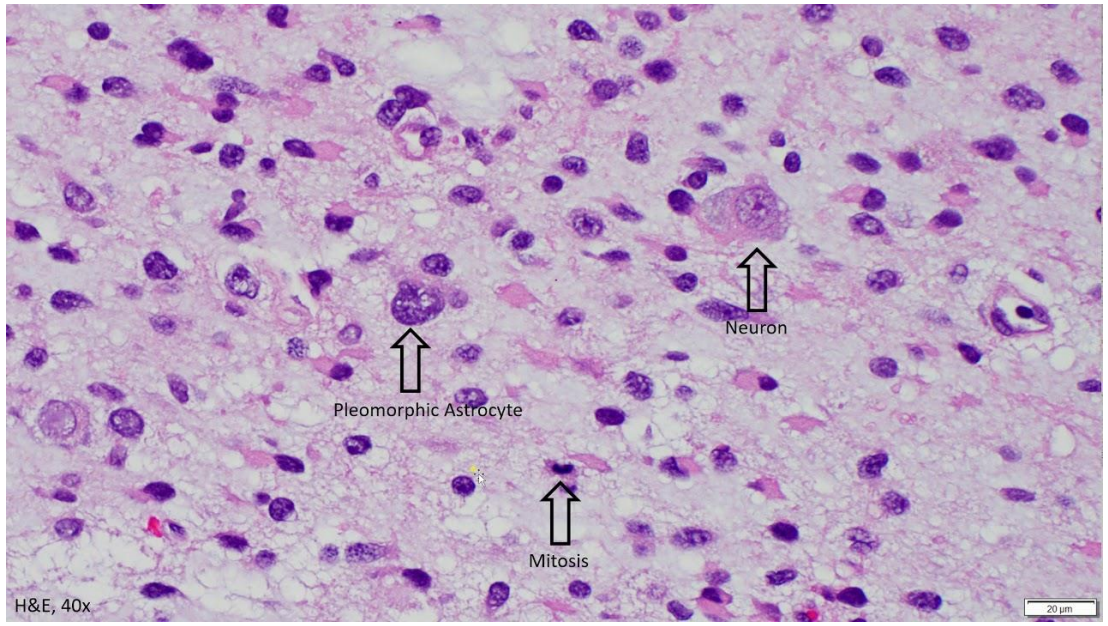


IDH-mutant astrocytoma grade 2

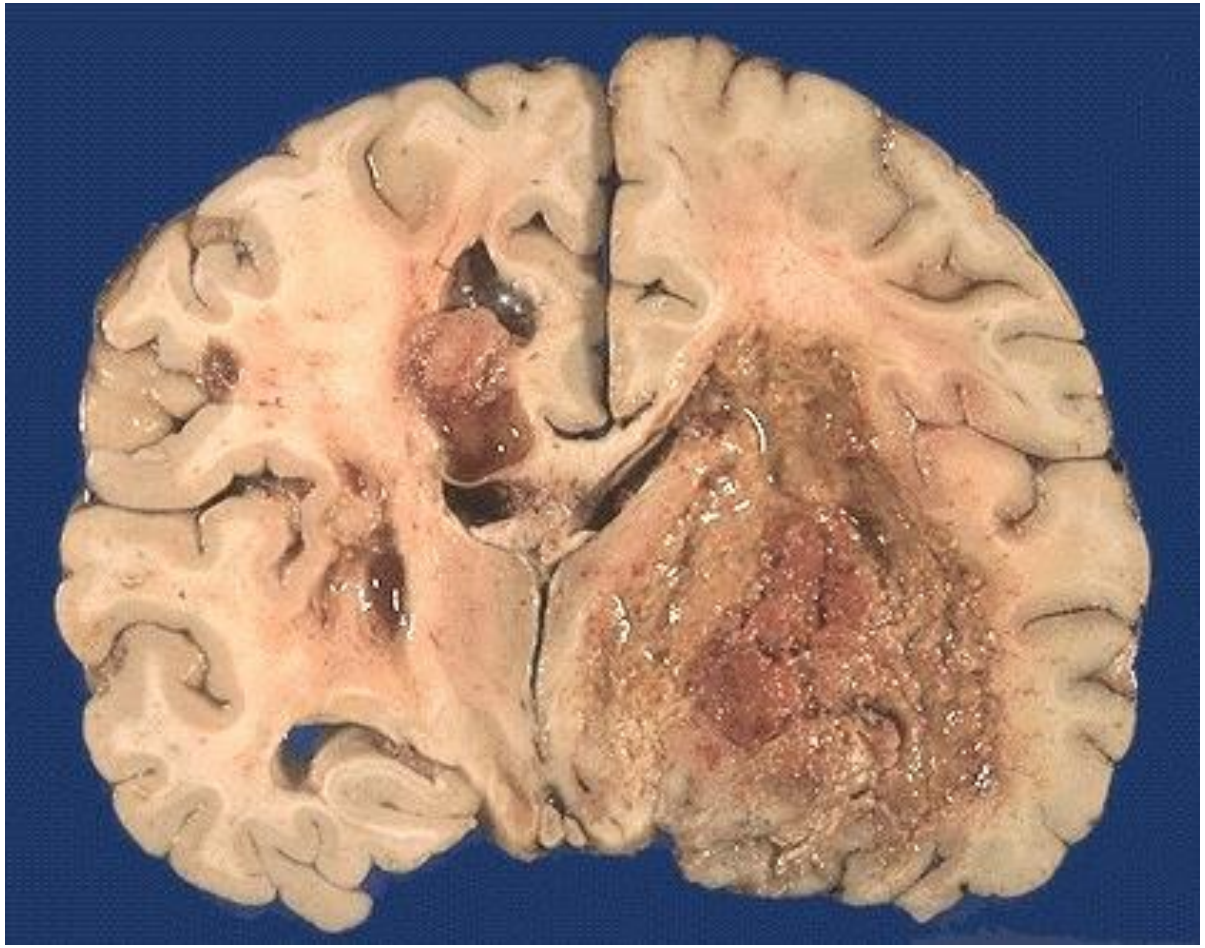
«better» survival time: median about 5 years



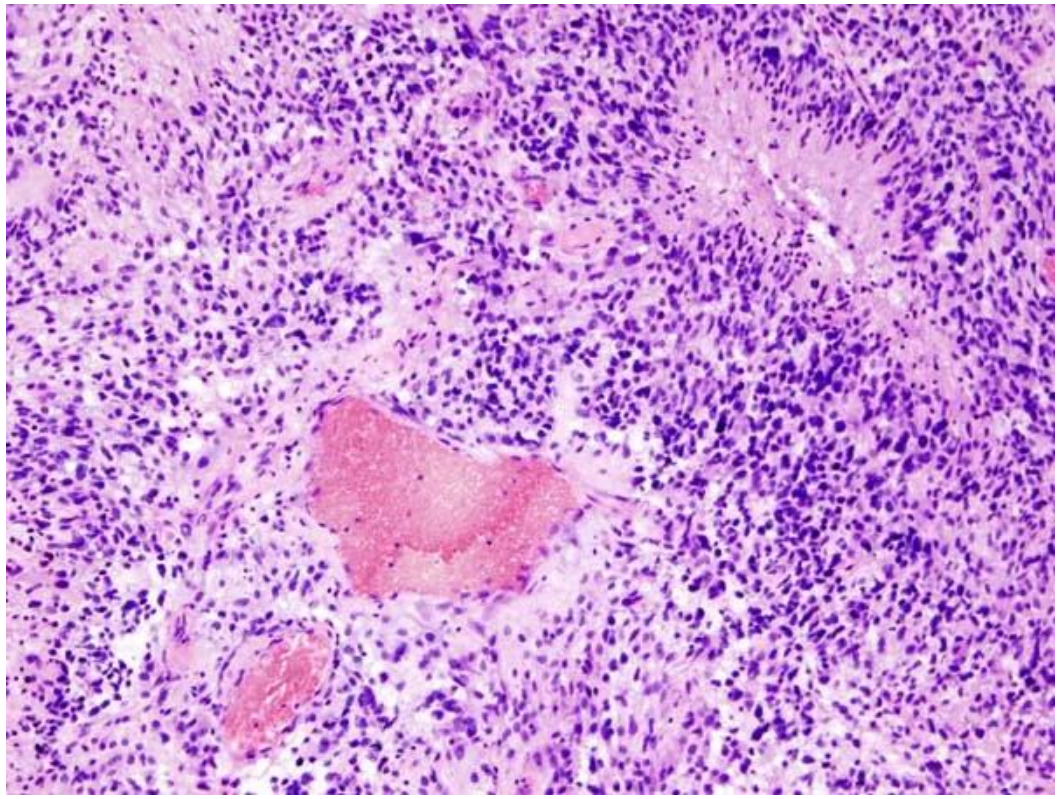
Normal brain



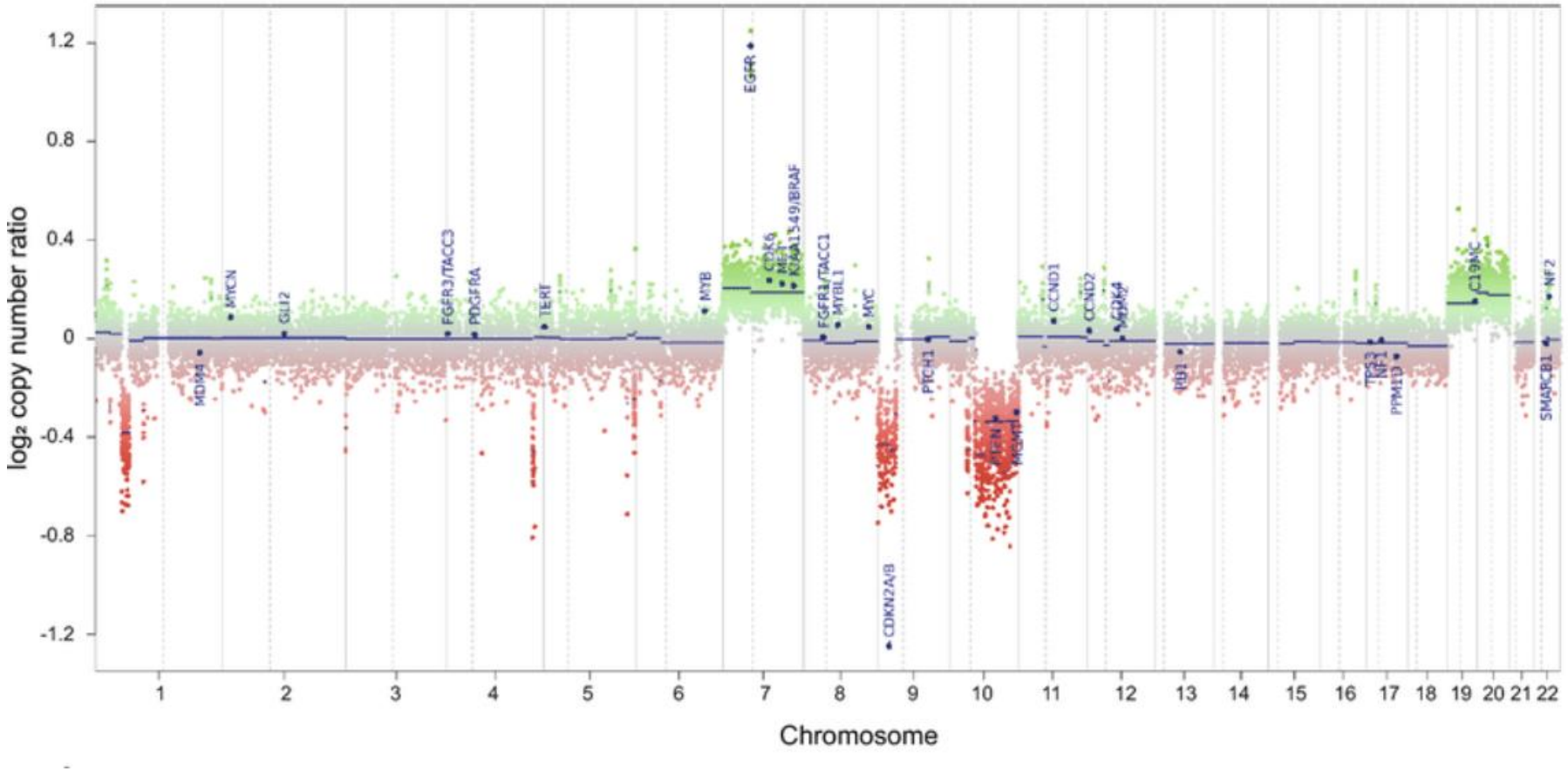
Astrocytoma grade 2 (mild hypercellularity)



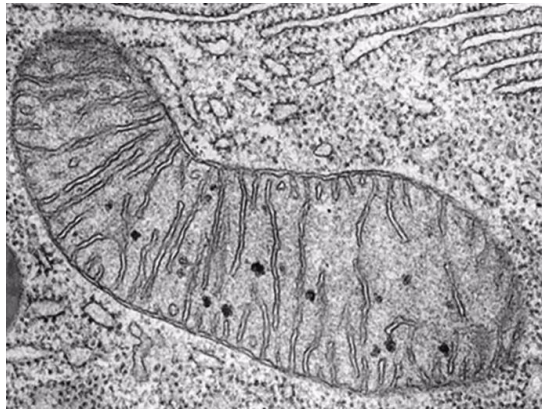
Malignant astrocytoma grade 4 (glioblastoma)



Necrosis, vascular proliferation, hypercellularity



Glioblastoma Allelotype: Trisomy 7, Loss of Chromosome 10



Hans Adolf Krebs
German biochemist
1937 discovery
1954 Nobel prize

IDH 1,2,3 (isocitrate-dehydrogenase) associated with astrocytoma II, secondary GBM

mitochondrial enegery production (Krebs-Cycle)

Normal function: oxidative decarboxylation of isocitrat to α-ketoglutarate

Mutant IDH: production of 2-hydroxyglutarate =
= oncometabolite: changes methylation of histone and DNA structure, **modifies gene expression patterns**

IDH1 mutations: R132H (Arg-His), most frequent

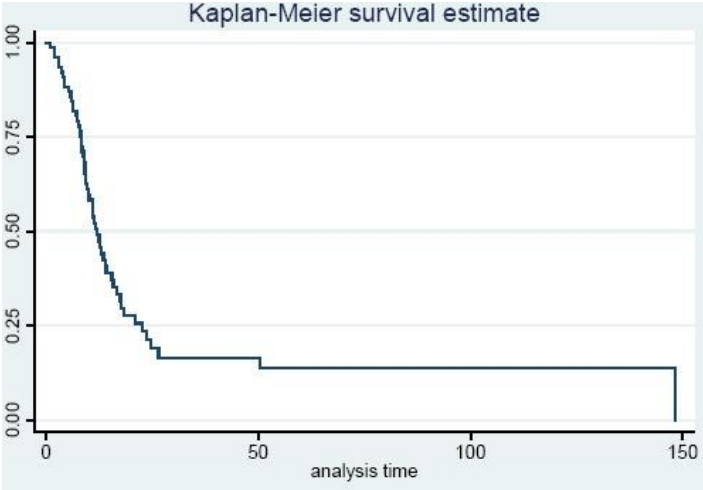
Therapeutic obstacles...

Why do most solid tumors resist therapeutic efforts?

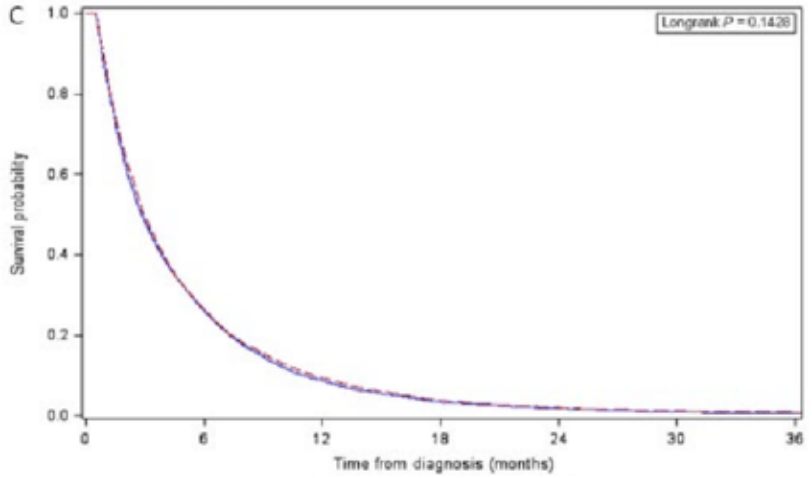
Biophysical factors, tissue penetration of drugs

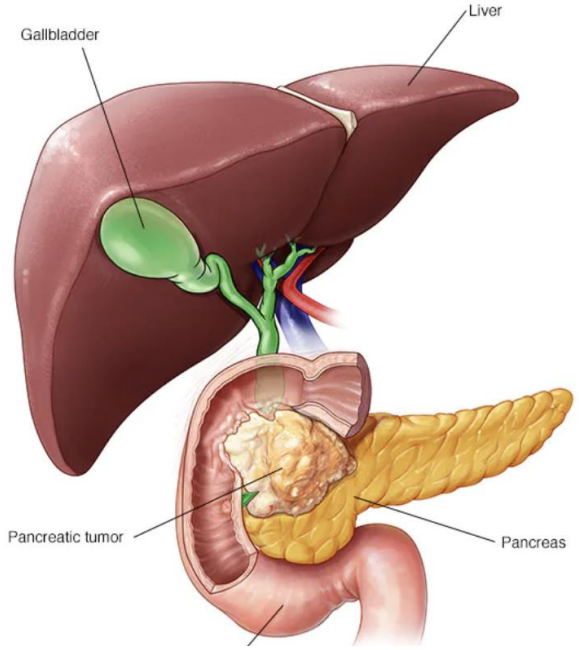
Two of the most malignant human malignant neoplasms

GBM



Pancreas-CA

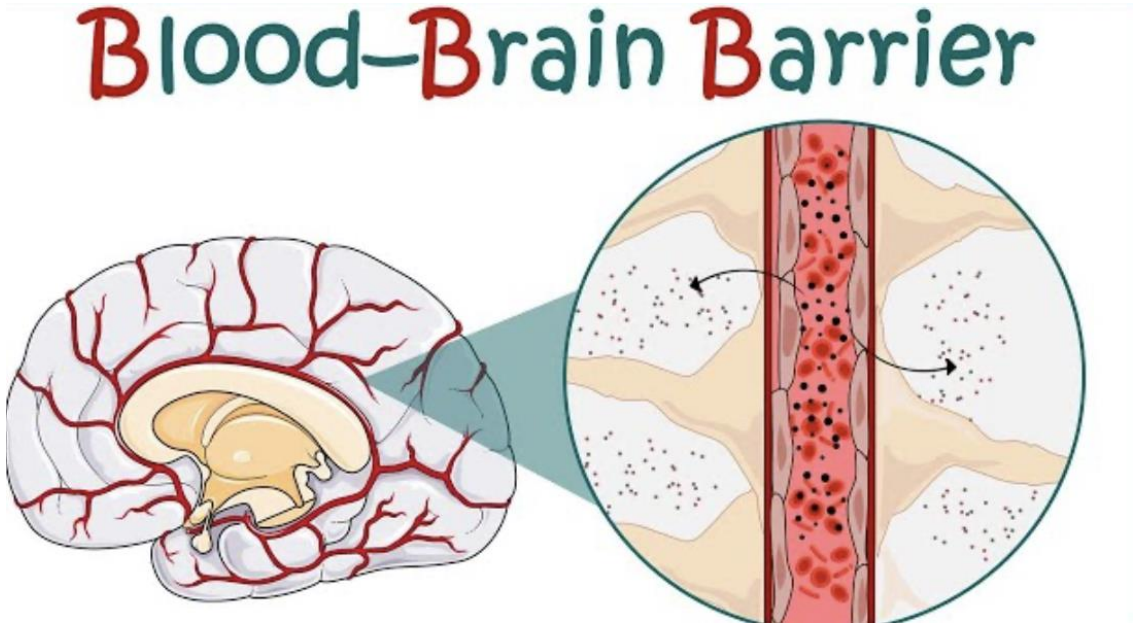




Solid tumors in general? GBM, pancreas CA, malignant melanoma, ovarian CA etc

Genetic, anatomical and biophysical factors of therapeutic resistance?

Anatomical factor of therapeutic resistance in the brain

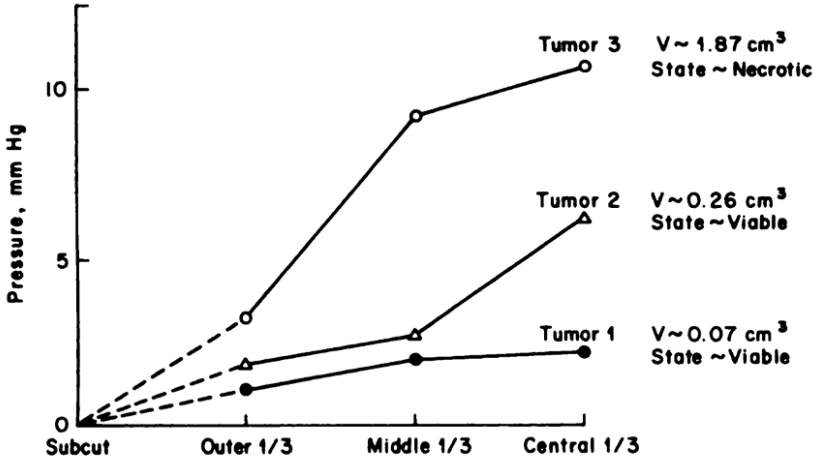


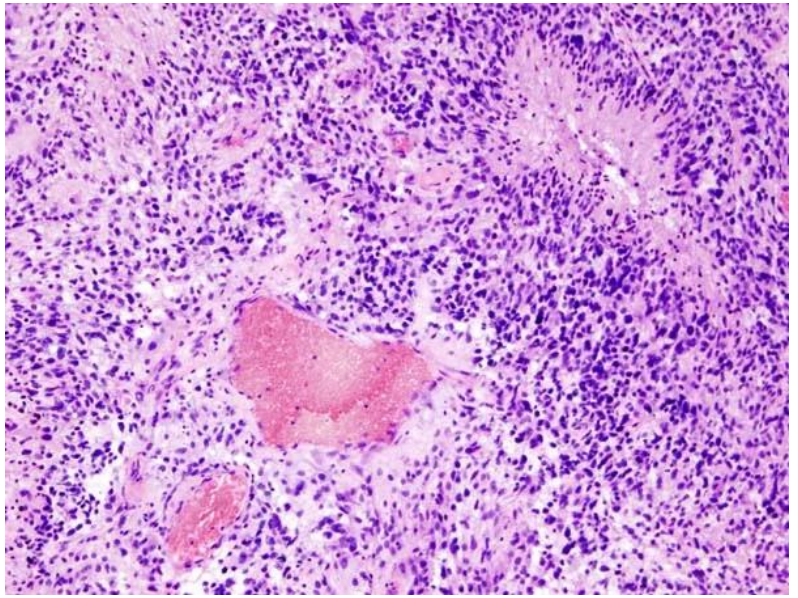
Grade 2: BBB closed!

Grades 3-4: BBB partially open

BBB: active transport, lipiphilic compounds

Biophysical factors of therapeutic resistance





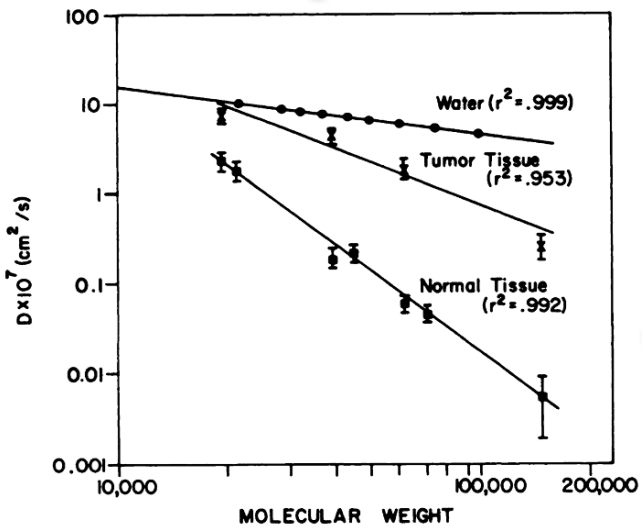
The larger the tumor grows,
the higher the interstitial pressure

Intratumoral pressure > interstititial pressure (normal: 5-15 mmHg / 7.5-20 cm H_2O)

capillary and venulary collapse (decreased accessability of drugs?)

R Jain. Barriers to drug delivery in solid tumors. Scientific American 271 (1), 58-65
R Jain, T Stylianopoulos. Delivering nanomedicines to solid tumors. Nature reviews Clinical oncology 7 (11), 653-664

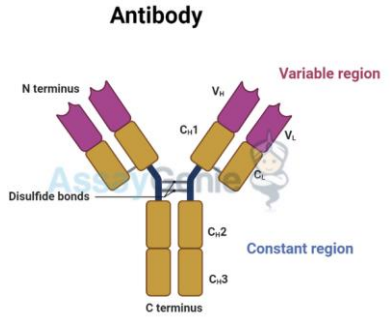
Biophysical factors of therapeutic resistance



The larger the compound (dextrane), the worse the diffusion

Vector size

Monoclonal antibody: 155‘000 Daltons



Peptide vector: 1800 Daltons

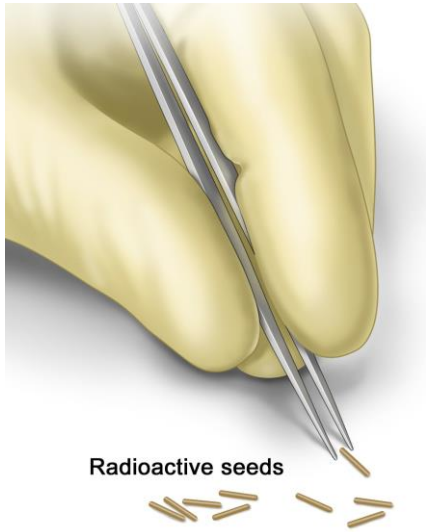
R Jain. Barriers to drug delivery in solid tumors. Scientific American 271 (1), 58-65
R Jain, T Stylianopoulos. Delivering nanomedicines to solid tumors. Nature reviews Clinical oncology 7 (11), 653-664

How can radioactivity be used to treat cancer?

- The Marie Curie experience
- How to make the static seed approach dynamic
- Bifunctional molecules for tumor targeting
- Targeting gliomas (defining the appropriate vector)

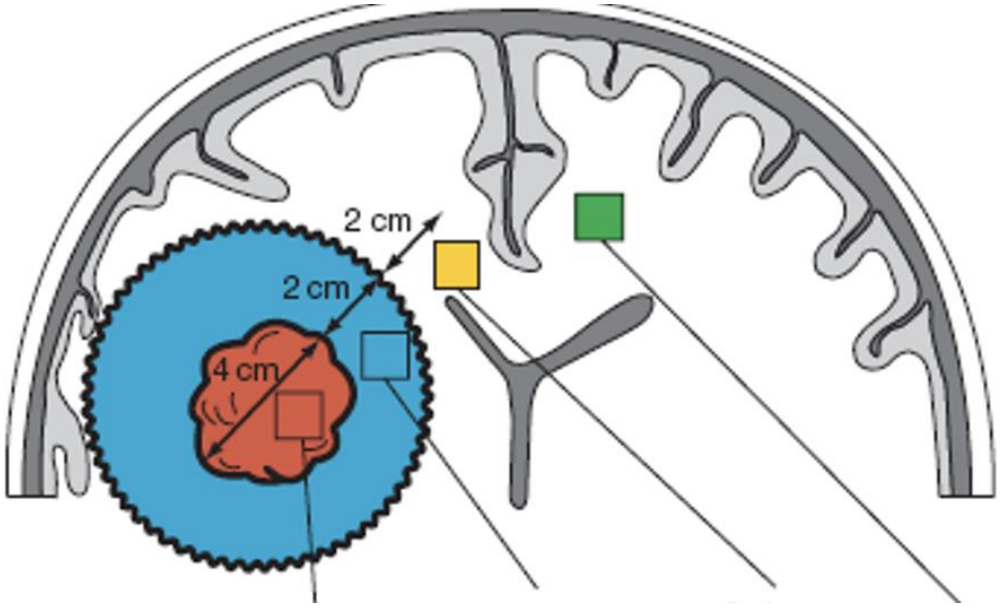
Marie (1867-1934) and Pierre (1859-1906) Curie

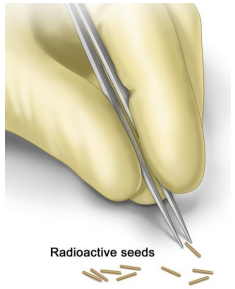
1898 (Dec 26) detection of radioactivity separating uranium from uraninite



Radon-222
Iodine-125
Aureum-198
Iridium-192
etc

Breast CA, Prostate CA, Brain CA etc.

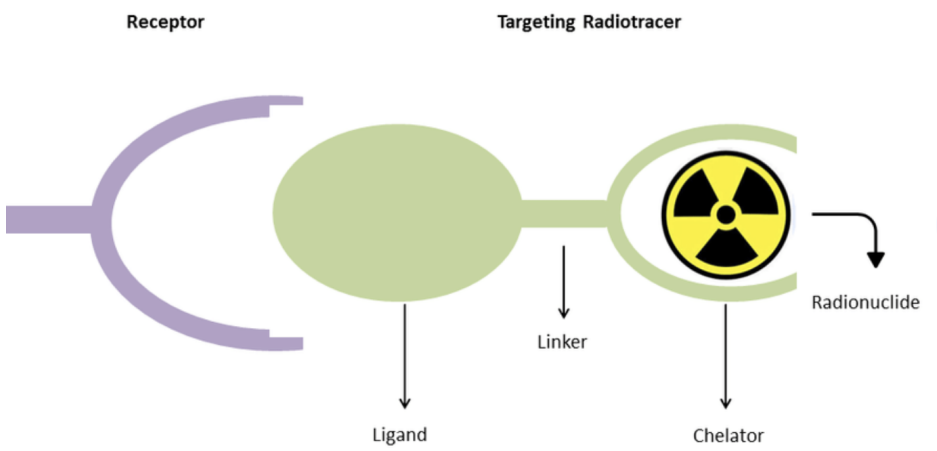




Seeds very **limited** to target invasive tumor cells

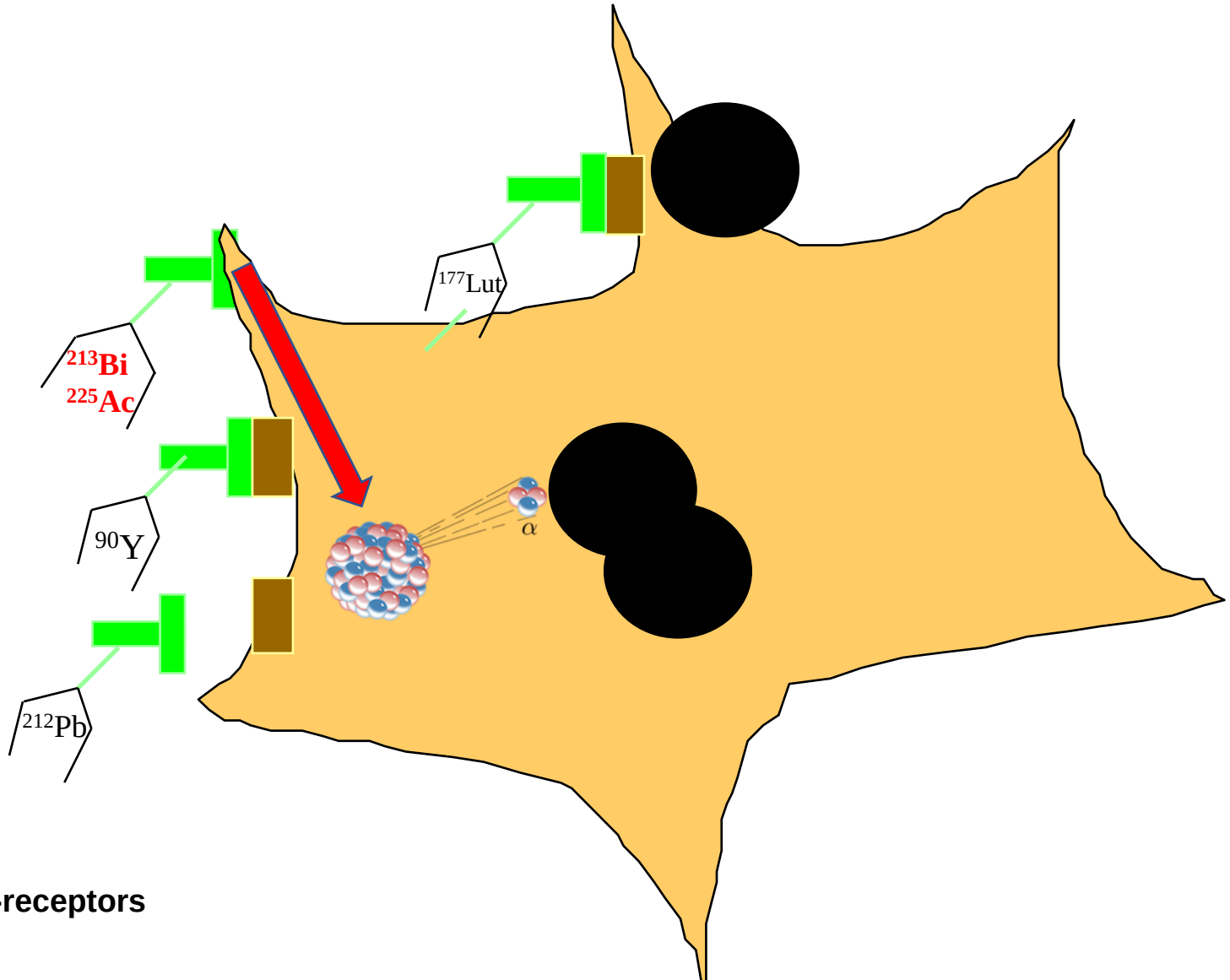
Dose range mm

Static seeds



Dynamic peptide vector

Targeted DOTA-Radionuclide Peptide Radiotherapy



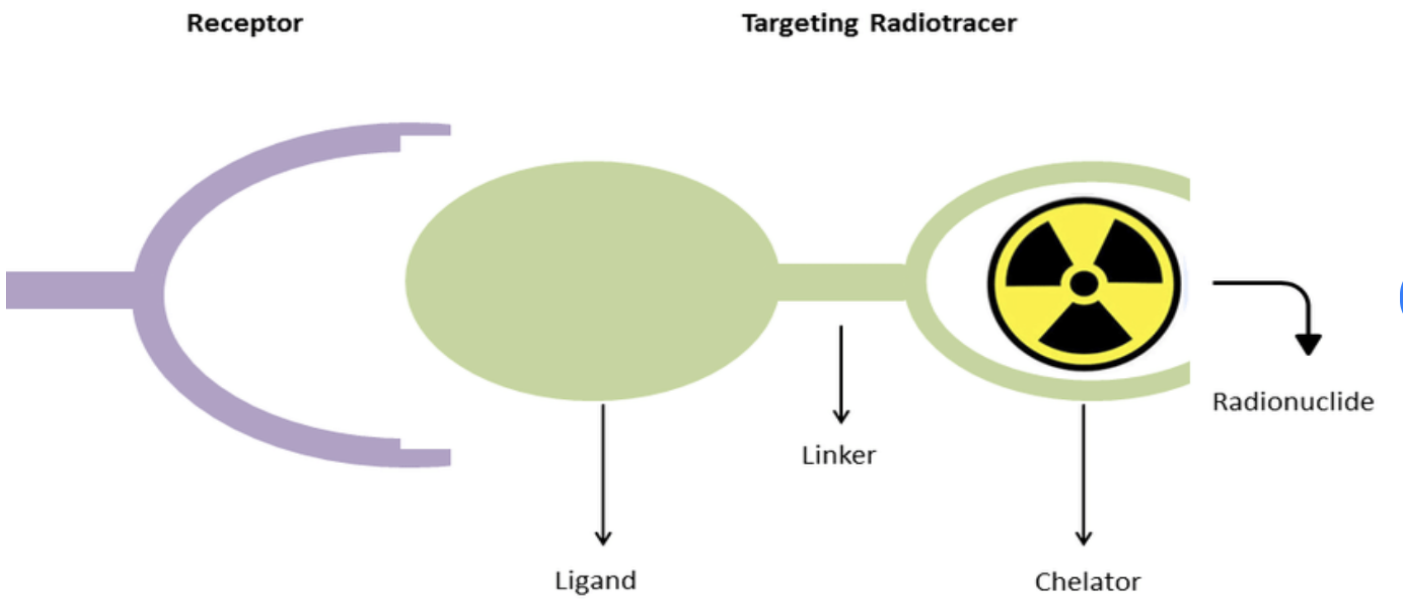
^{177}Lut **DOTA / DOTAM-chelators**

peptide vector

neurokinin type 1 / somatostatin type 2-receptors

Bifunctional molecules

- Receptor binding domain (tumor cell binding)
- Effector domain (therapeutic radionuclide)
- ***Platform technology***



Clinical development steps

- **Preclinical**: definition of drug, drug target, biochemical assays, animal studies
- **Phase 1** trial: toxicity, safety
- **Phase 2** trial: dose finding, evtl efficacy
- **Phase 3** trial: comparison to state of the art (randomized trial)
- **Phase 4:** clinical application after market authorization
- **Phase 2b/3**: oncology, dose finding, efficacy, market authorization (orphan)

Selection of an optimal targeting vector

Result of 4 clinical phase-1 trials over a period of 10 years!

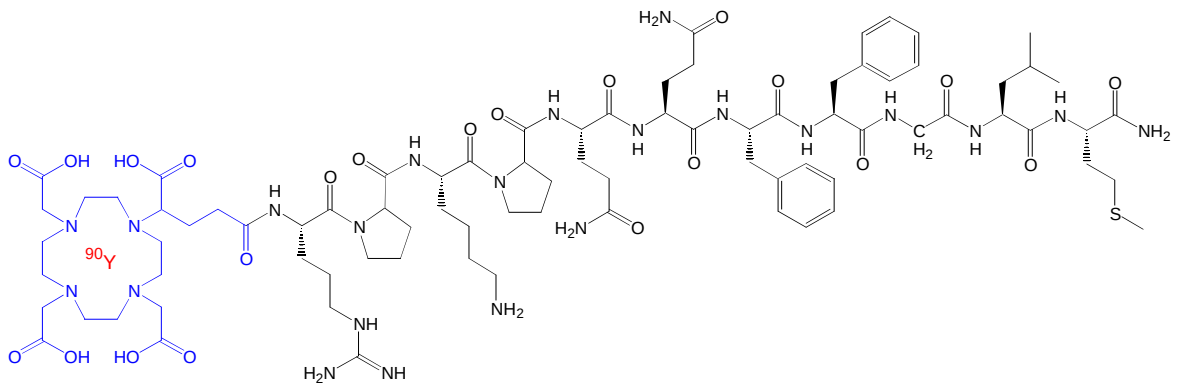
- Anti-BC-**mAB**: 155‘000 D**: too large, poor diffusion**
- **DOTATOC**: 1300 D, excellent size, but **not specific (**neuropil), **kidney:** tubular re-uptake
- **Substance P**: specific in brain (expression in tumors, inflammation, trauma, hemorrhage)
- **Modified Substance P**: limited blood passage, no kidney uptake!

Size: rapid diffusibility

Specific target binding: compartment

DOTA-modified Substance P

$^{213}Bi/^{225}Ac$

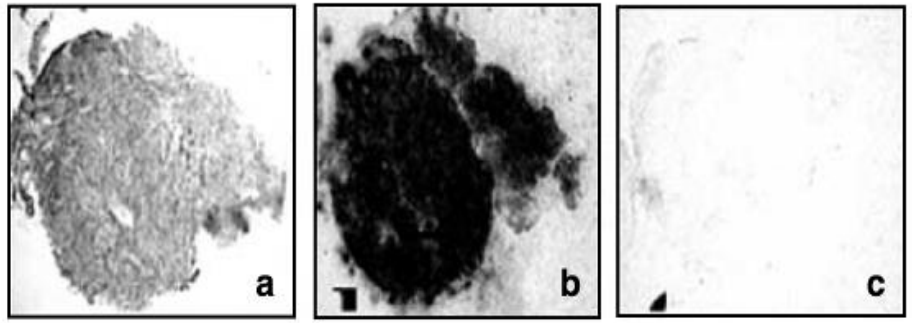


1800 Daltons

[X] - DOTA - modified Substance P

Radionuclide *Chelator* *Peptidic Vector*

Substance-P (NK-1) receptor autoradiography

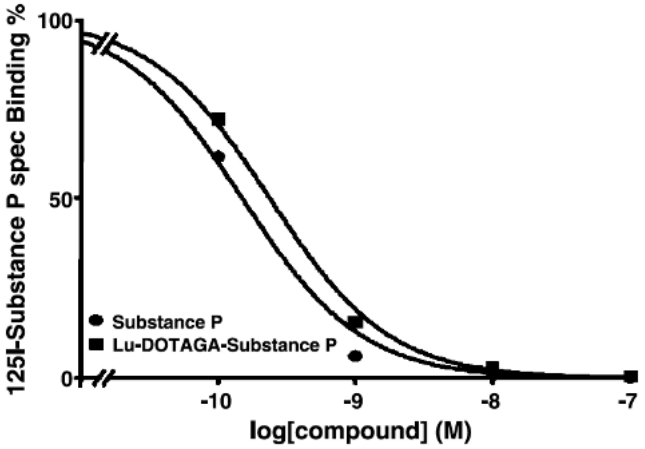


H&E Substance P control

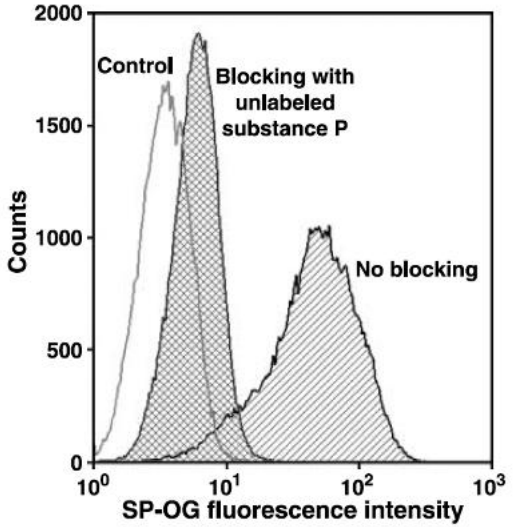
10‘000-50‘000 NK-1 receptors / glioma cell (grades 2-4)

Jean-Claude Reubi, Pathology, Unibe in Kneifel et al. Eur. J. Nucl. Med. 2006

Preclinical testing



Binding in low-nanomolar range



Competitive binding assay

How to apply a radiopharmaceutical to treat malignant gliomas

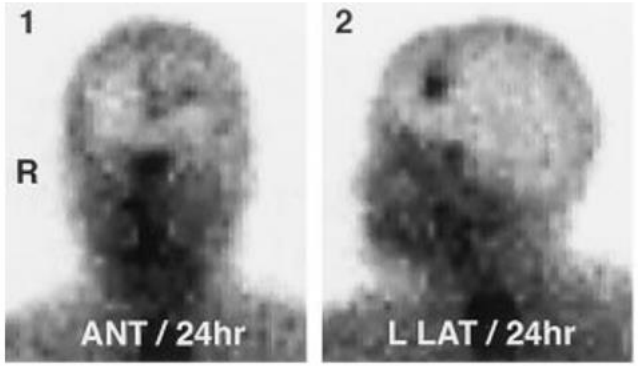
- **Local application versus systemic (i.v., i.a.) injection / diffusibility**
- Compartmental specificity to limit toxicity
- Synthesis and size of the targeting vector / receptor affinity

Brain Tumor Targeting:
The most efficient mode of drug application?

Intravenous – intraarterial – intratumoral (interstitial)?

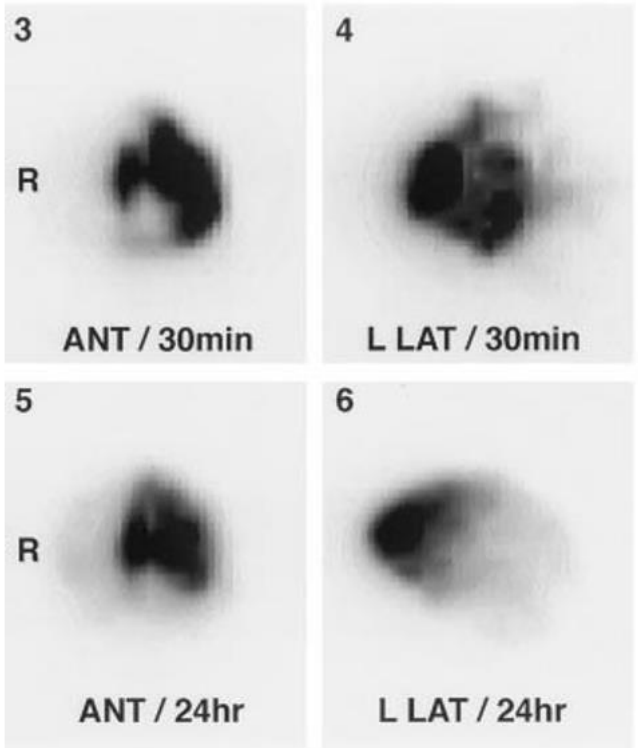
Testing circular vector DOTATOC (8AA, 1300 D)

i.v. ≈ i.a.



Systemic = intravenous or intraarterial injection:
< 5% of injected activity reaches the tumor (systemic radio-toxicity)

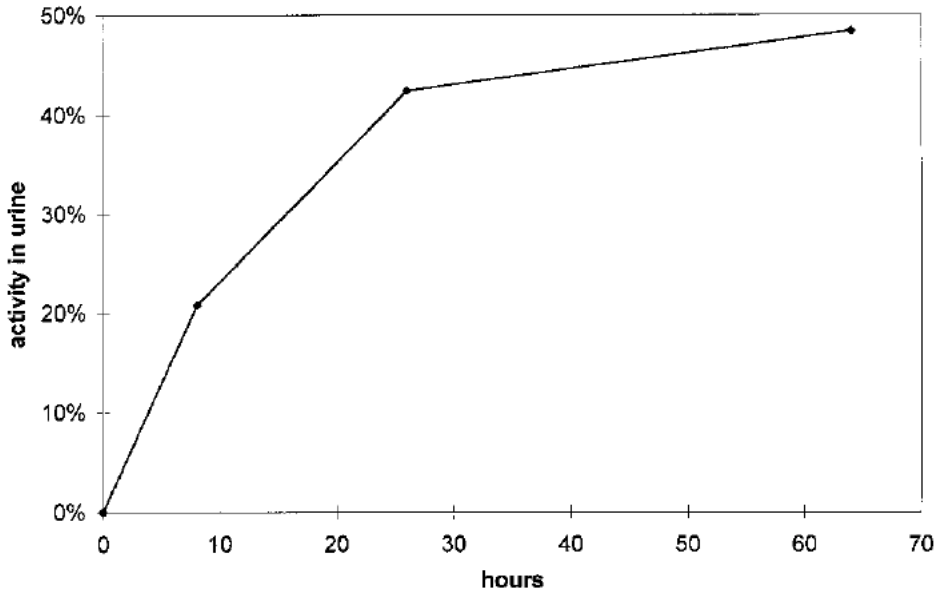
Intratumoral=interstitial



Injection	K/T^b	T/B_{head}	T/B_{abd}	K/B_{abd}
i.v.	29.40	0.65	0.43	12.60
i.a.	26.41	0.80	0.55	14.66
i.t.	0.03	18.31	61.25	1.58

[a] 100–190 MBq.
[b] K, kidney; T, tumor; B_{head}, cephalic background; B_{abd}, abdominal background.

A.Merlo et al. Clinical Cancer Research. 1025–1033, May 1999



Astrocytoma grade 1-2:

Intracystic injection of In-111 DOTATOC

40% loss of injected activity /24hrs

A.Merlo et al. Clinical Cancer Research. 1025–1033, May 1999

^{111}In-DOTATOC
1300 Daltons

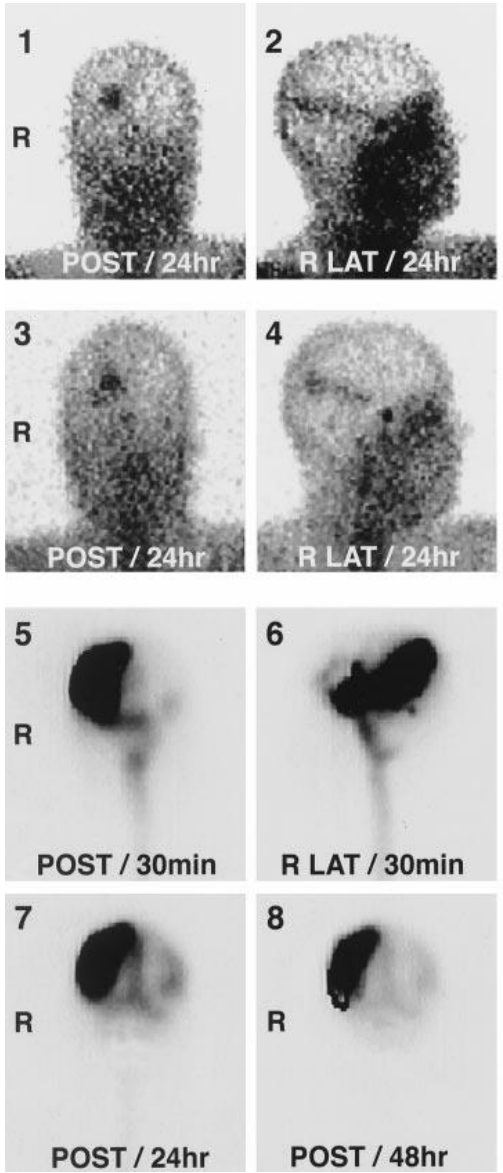
i.v.

i.a.

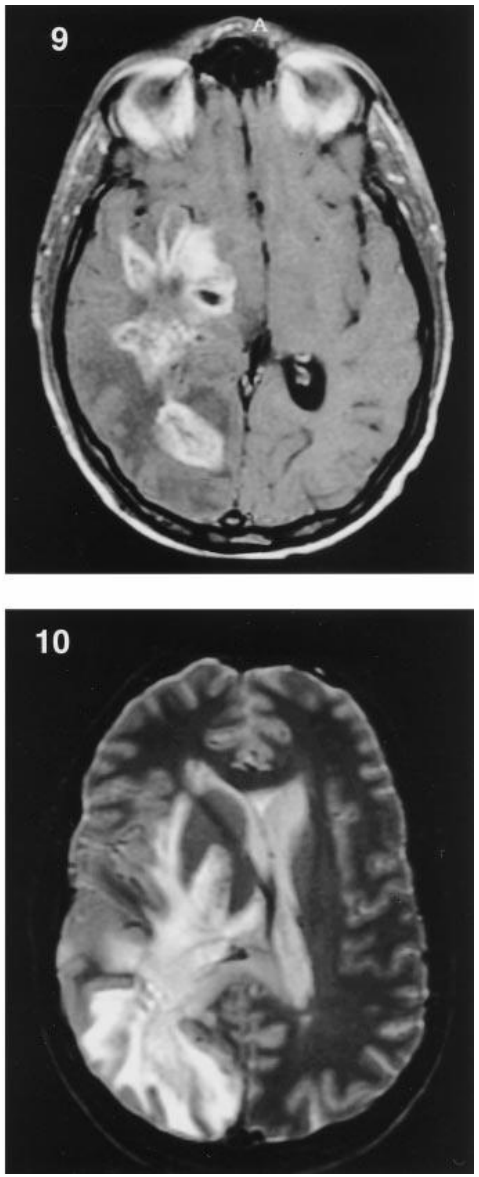
i.t.
30min

injection volume
2ml

i.t.
24/48hr

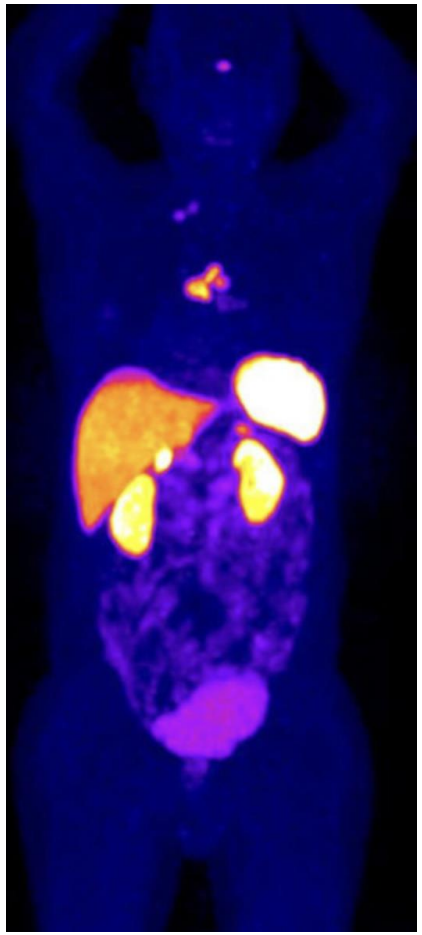


2D SPECT



MRI

intravenous



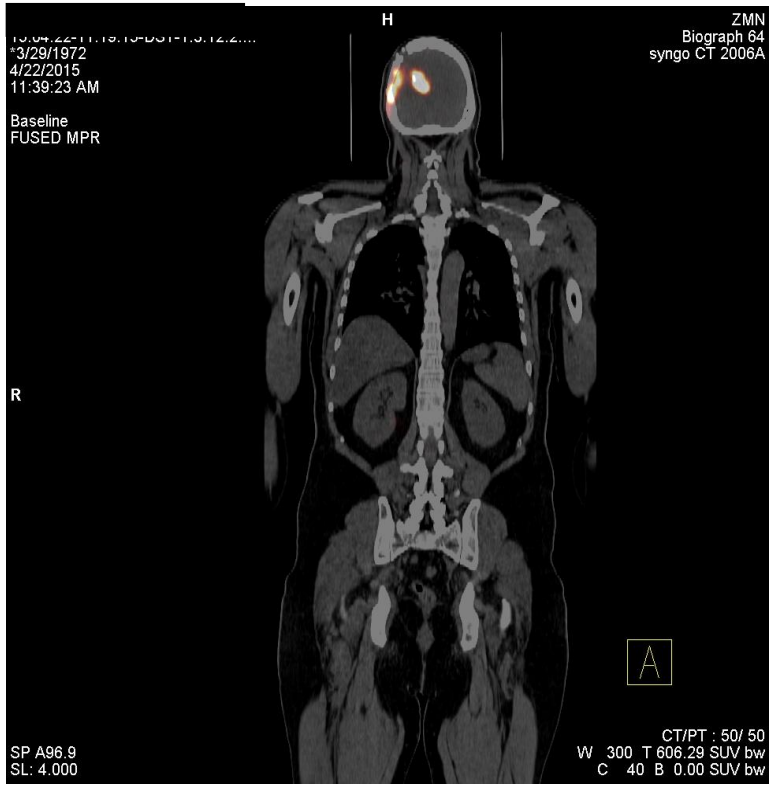
Kidney uptake:
DOTATOC-PET

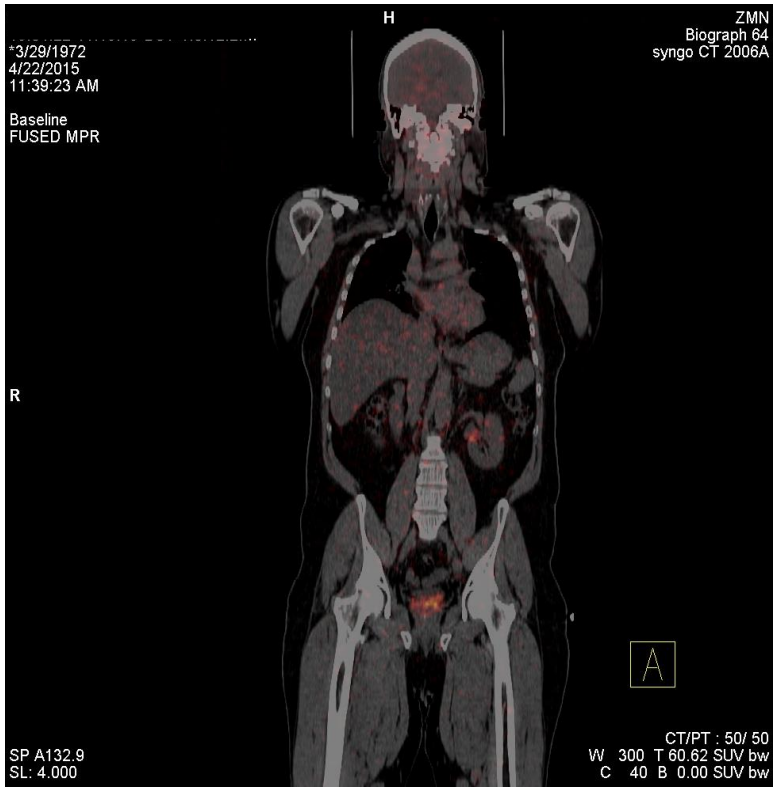
Kidney, the dose-limiting organ

in octreotide-based systemic approaches

Gühne et al. Diagnostics. 2021;11(7):1216

Intratumoral (intracerebral) injection



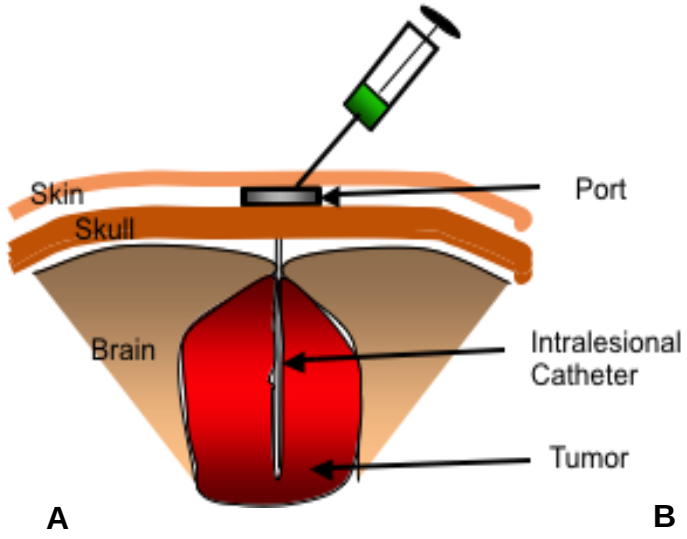


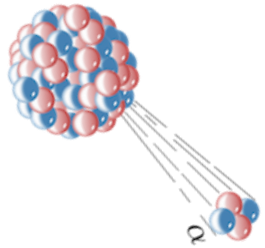
No kidney uptake: only slight bladder signal

Modified substance P

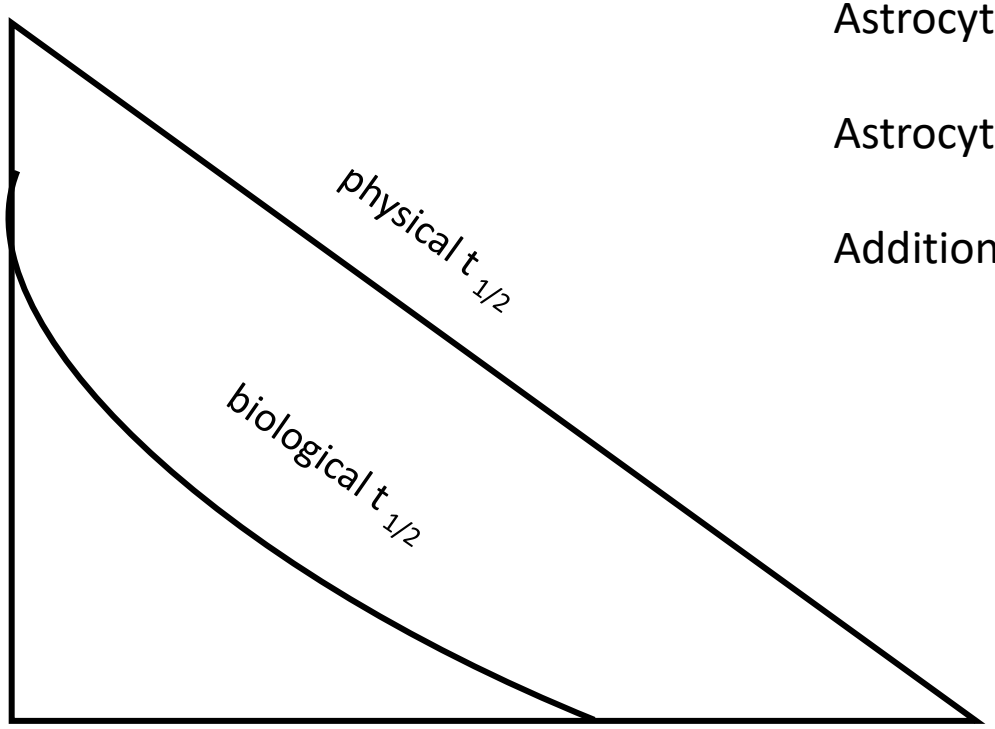
Linear peptide 11 AA, cleaved by serum **peptidases**,
modified (prolonged half-life 4x), only peptide fragments in systemic circulation,
Rapid clearence into bladder

Local application of the radiopharmaceutical the way to go!





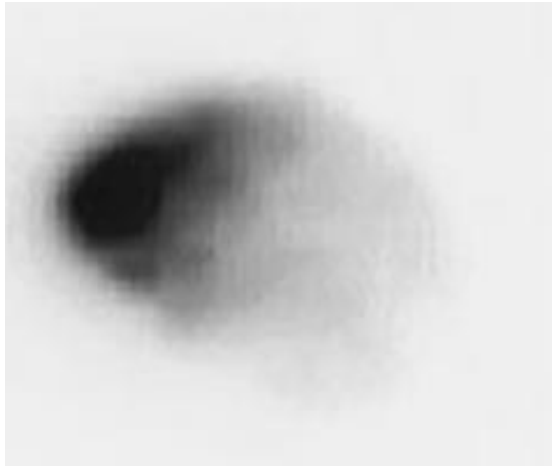
Injection volume about 2ml



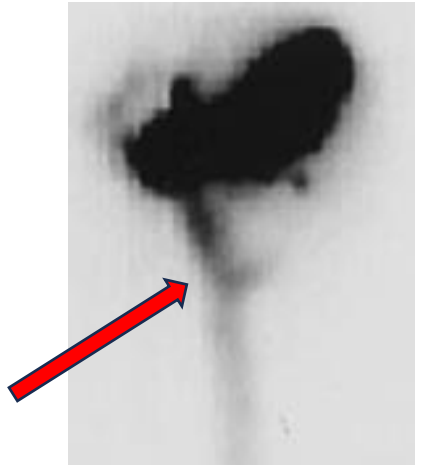
Astrocytoma Grade 2: **BBB closed, limited leakage**

Astrocytoma Grade 3-4: **BBB partially open, variable leakage**

Additional factors: open CSF spaces, post-RT



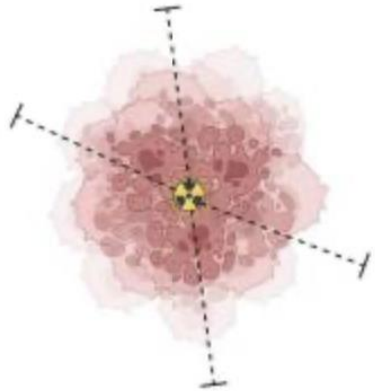
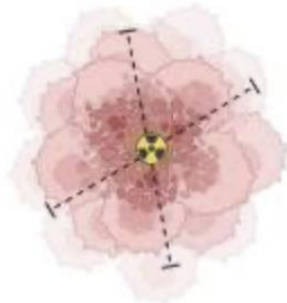
Grade 2



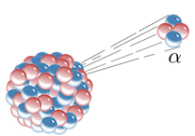
Grade 4

Selection of the appropriate medical radionuclide

- Beta-emitters
- Alpha-emitters

	β-particle	α-particle	Auger electron
			
Mean energy	50-2300 keV	5-9 MeV	eV-keV
Path-length	Tumors, tissues (≤ 12 mm)	< 10 cells (50–100 μm)	< 1 μm
LET	< 1 keV/μm	~ 80 keV/μm	4-26 keV/μm
Examples of radionuclides	^{161}Tb, ^{177}Lu, ^{90}Y	^{149}Tb,^{225}Ac, ^{223}Ra	^{123}I, ^{111}In

Dose Range, Dose Decay Curve, Tumor Cell Size 20-60 μm



±0.08 mm alpha-particles / **5.84/8.5 MeV**

Beta: 1 mm Lutetium-177 / **0.13 MeV**

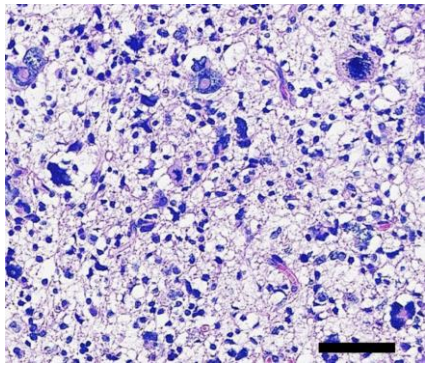
Beta: 5 mm Yttrium-90 **/ 2.1 MeV**

10 mm GammaKnife

10-20 mm Photons

Tissue range (alpha:beta) 1:10 (Lut-177) to 1: 50 (Y-90)

Toxicity profile!

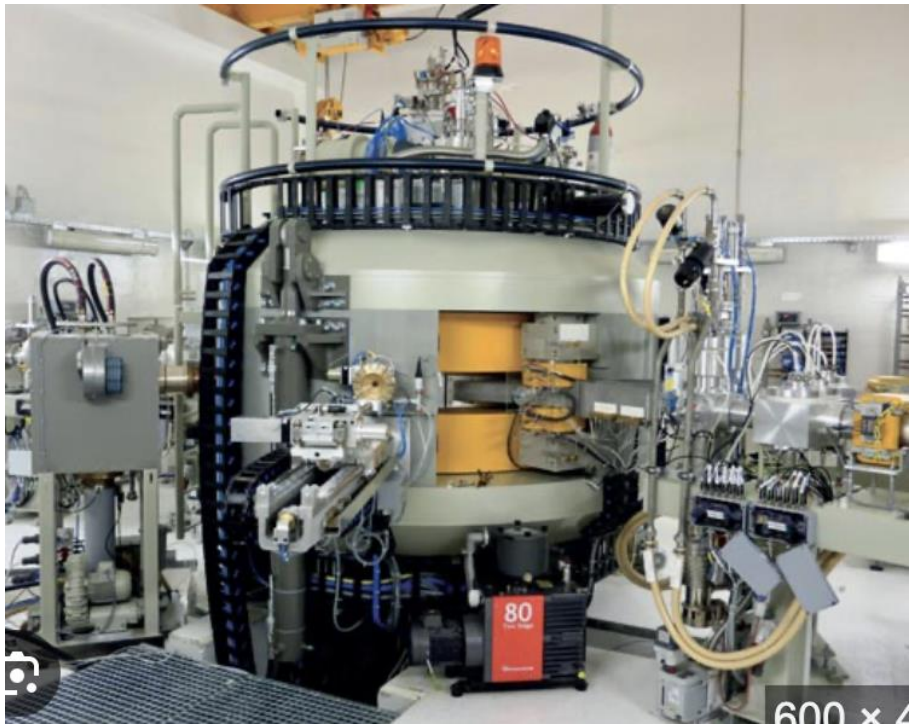


bar 50 μm

alpha-emitters (0.1mm)

beta-emitter Lutetium-177 (1mm)

Can we perform a large clinical trial with targeted alpha therapy?



The bottleneck for alpha therapy

Insufficient supply with alpha emitters world-wide

2018 Vienna Conference IAEA / ITU:

consensus to develop Ac-225 for clinical trials

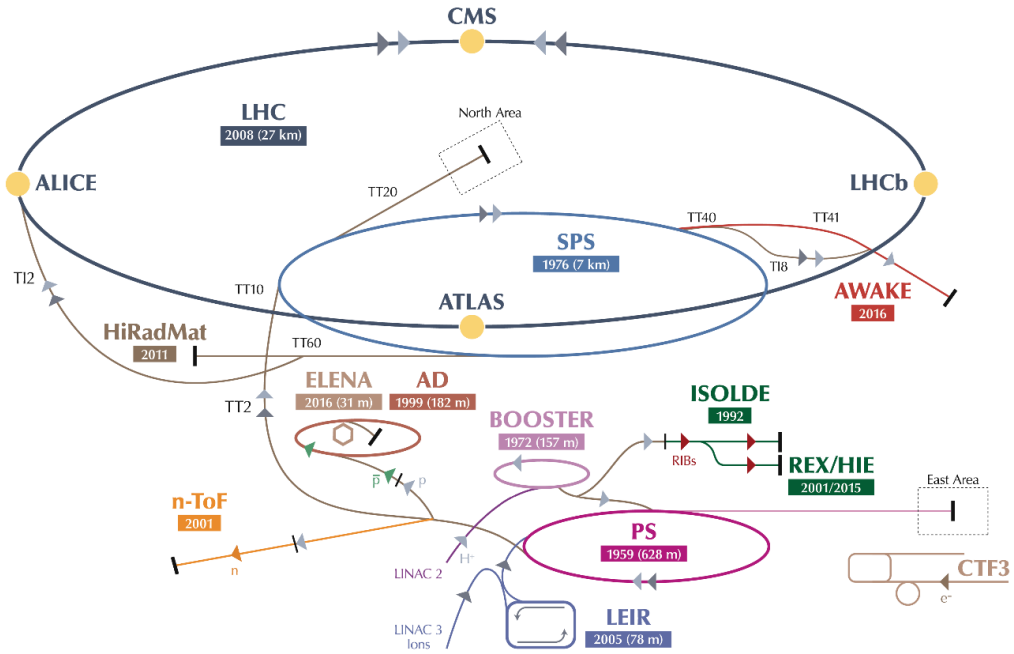
Example Actinium-225

- Extract from Thorium-229 (TerraPowerProject, ITU Karlsruhe, Obninsk, DOE)
- Irradiate Radium-226 with Cyclotron (robotic reactor technology, Prague E&Z aso)
- **Spallation** (CERN-ISOLDE, NorthStar, Troitzk etc): produce period table! 2% Ac-225

  - Problem of chemical separation: Contamination with 1% Ac-227 ($t_{1/2} \approx 20$ years)

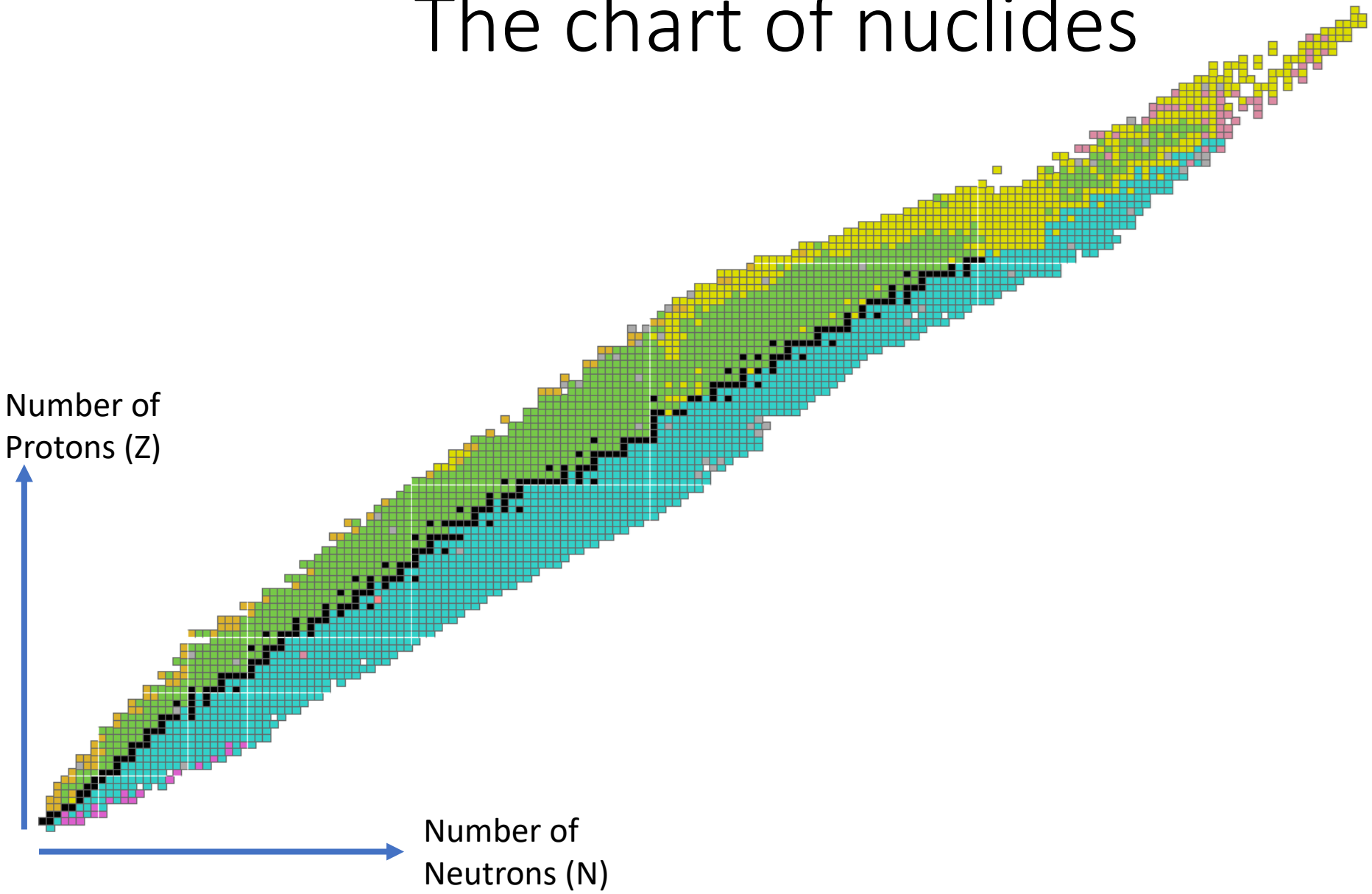
ISOLDE and MEDICIS

- **I**sotope mass **S**eparator **O**n-**L**ine **De**vice
- Located at Proton-Synchroton Booster (PSB)
- Study of fundamental atomic and nuclear physics

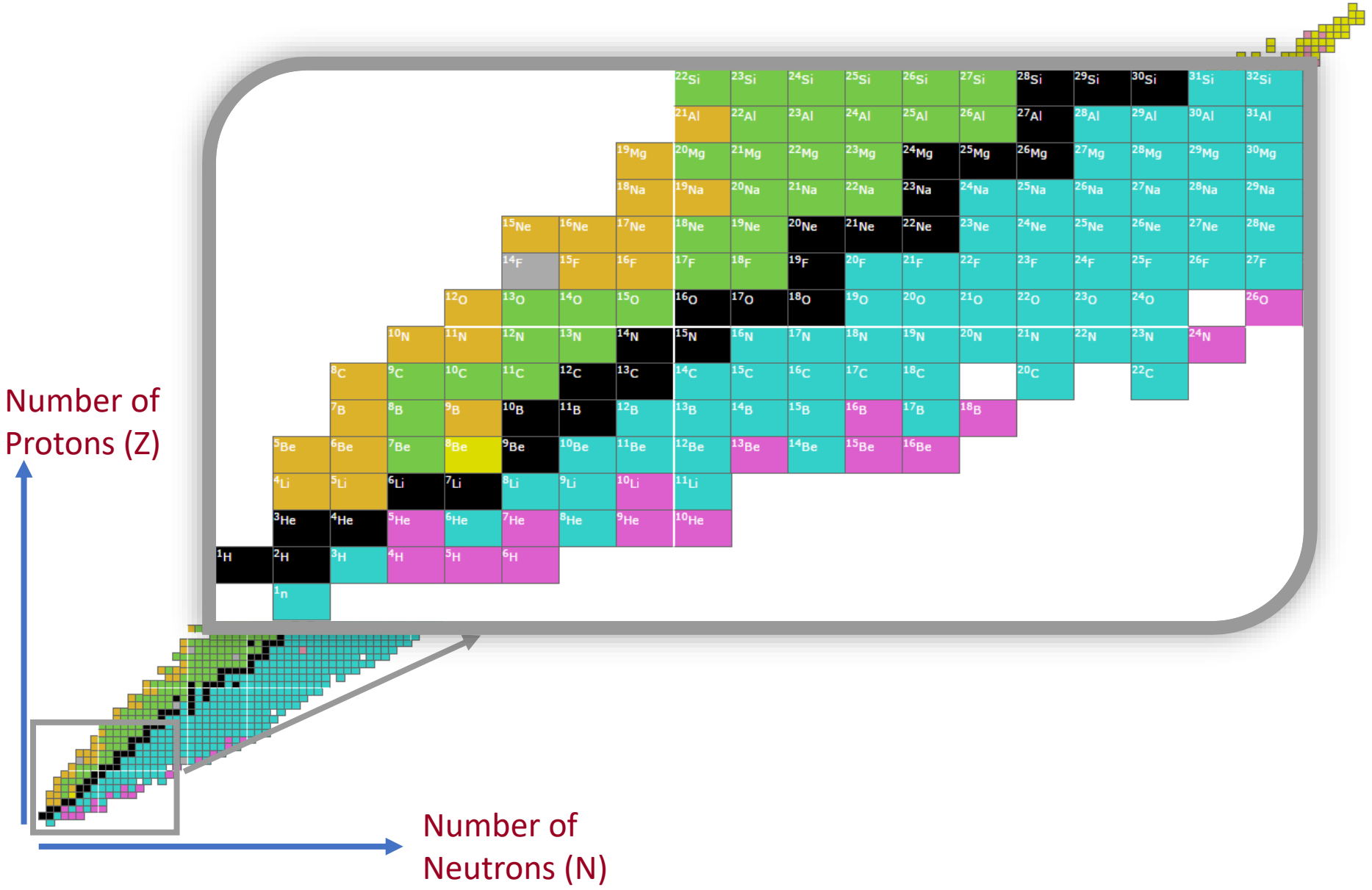


- MEDICIS (Medical Isotopes Collected from ISOLDE) → focus on medical applications exclusively!

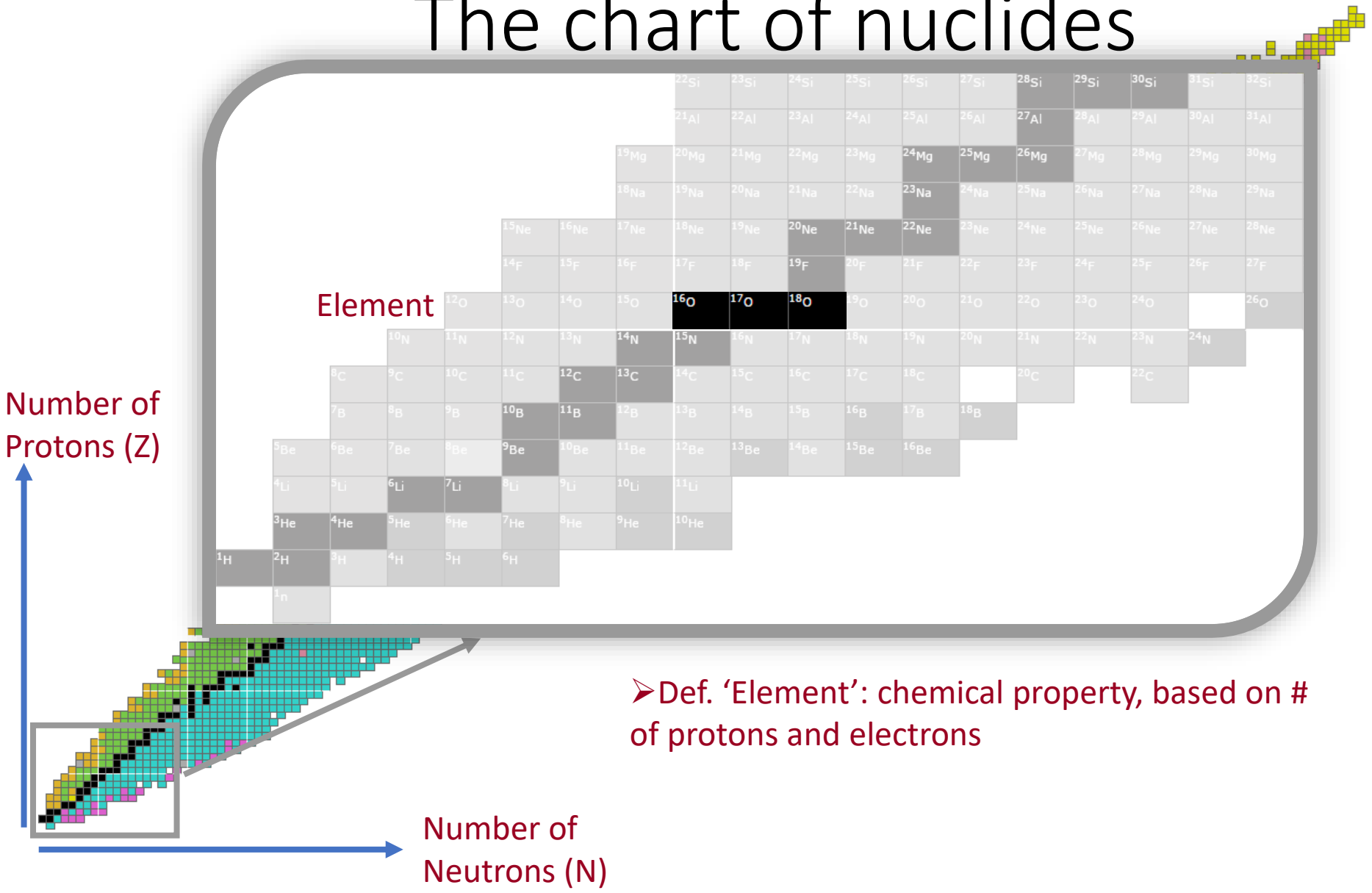
The chart of nuclides



THE CHART OF NUCLIDES

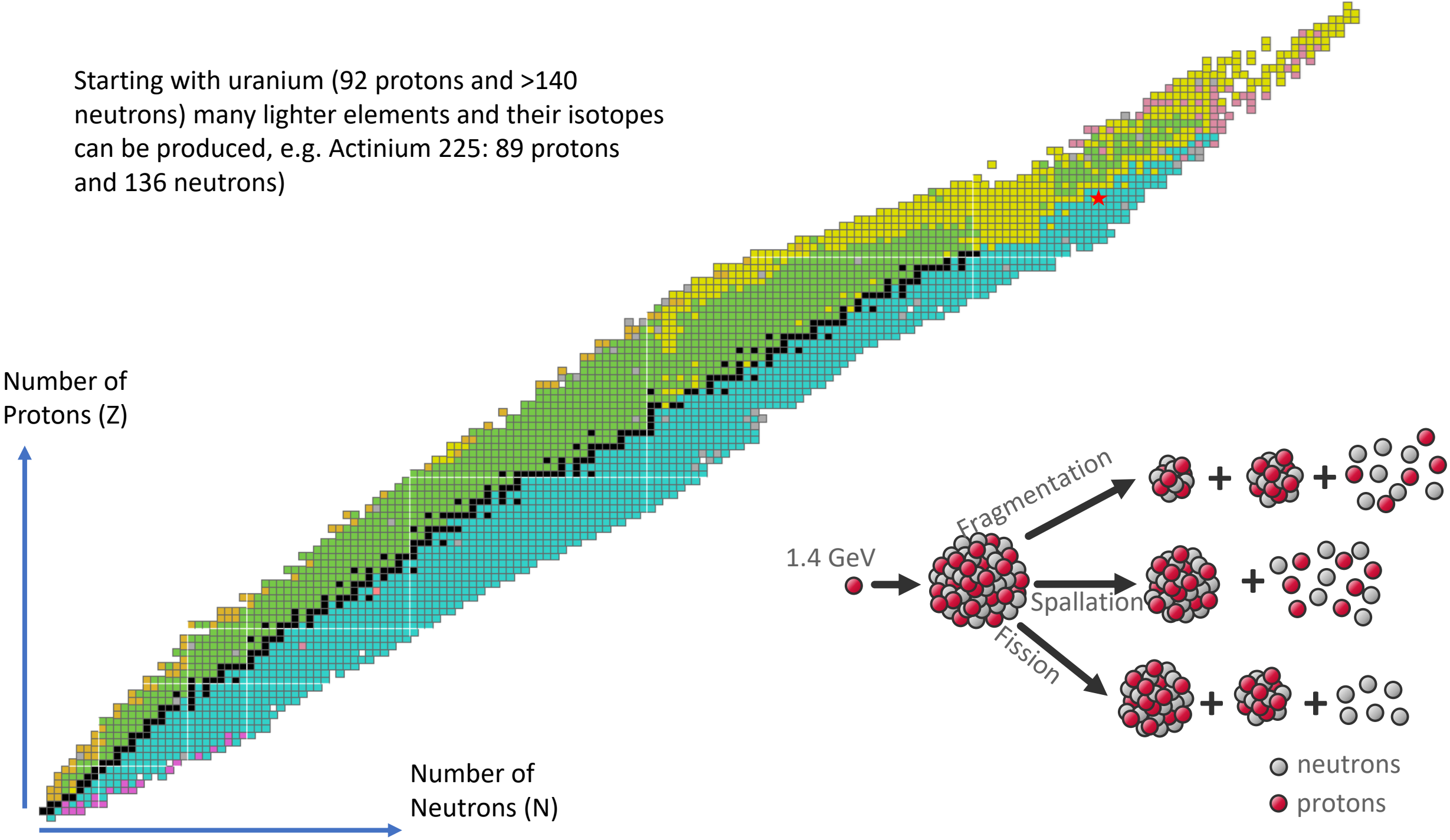


The chart of nuclides



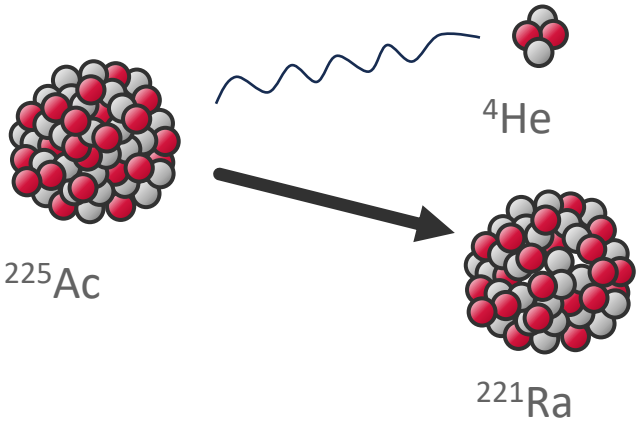
➢Def. ‘Element’: chemical property, based on # of protons and electrons

Starting with uranium (92 protons and >140 neutrons) many lighter elements and their isotopes can be produced, e.g. Actinium 225: 89 protons and 136 neutrons)

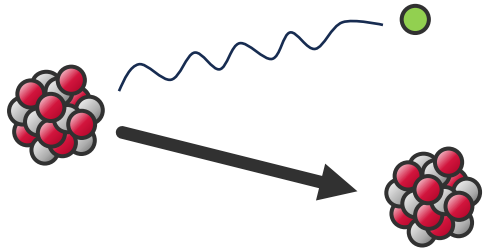


Radioactive decay

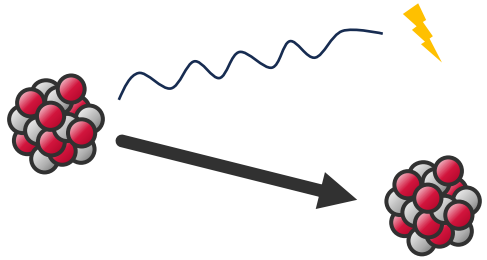
The **colorful boxes on the chart of nuclei** indicate the isotopes of an element which are not stable
What does this mean: not all configurations of protons and neutrons remain together → a **decay** follows through which the isotope loses energy and transforms into another one



Alpha decay: He atom (2p+2n) gets emitted



Beta (plus/minus) decay: electron or positron gets emitted and either proton transforms to neutron or vice versa



Gamma decay: de-excitation through emission of photon

photon
electron/positron
neutrons
protons

With a given isotopes, **the half-life** defines after which time half of the isotopes will have undergone the decay → **unit** is "Bequerel" (**Bq**) and is given in decays per second

Radioisotope production at MEDICIS - lifecycle

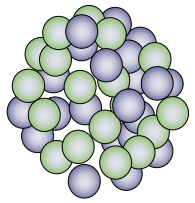
Initial sample activity 50MBq

Sample preparation

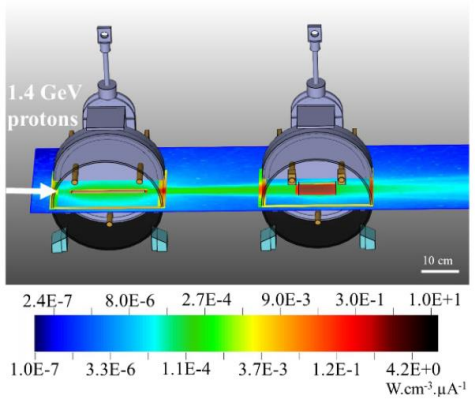
~ 1-5d

35-47 MBq remaining

^{225}Ac



$T_{1/2}$= 9.9d

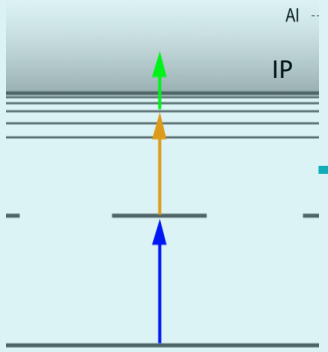


Target irradiation

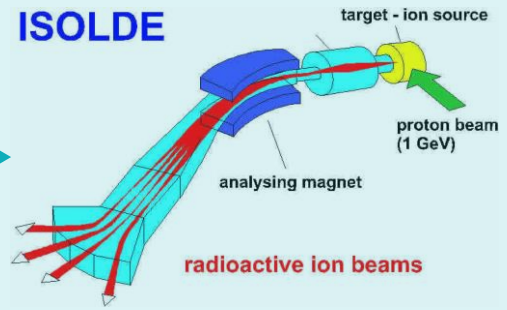
< 1d

33-44 MBq remaining

RILIS



ISOLDE



Mass separation + Ionization = ~50% efficiency

Collection at MEDICIS

~ 1-5d

23-41 MBq remaining

< 1d

Handling and transportation

Delivered sample activity 21-38MBq

How to apply targeted alpha therapy in malignant gliomas?

- **TAT for low grade gliomas: a new treatment paradigm?**
- TAT for glioblastomas, how to develop a clinical protocol?

Case presentations: low grade glioma

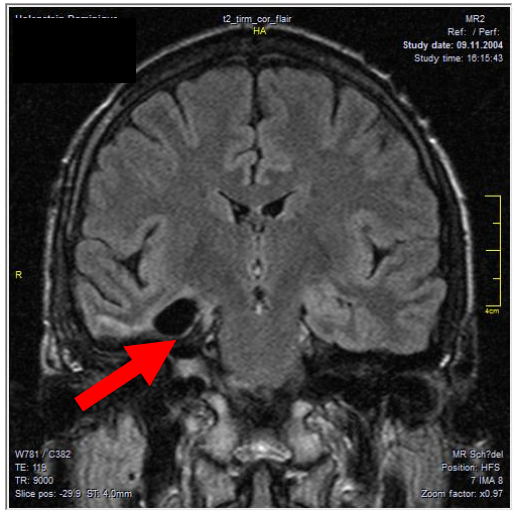
Diffuse invasive astrocytoma grade 2 (IDH-mutant)

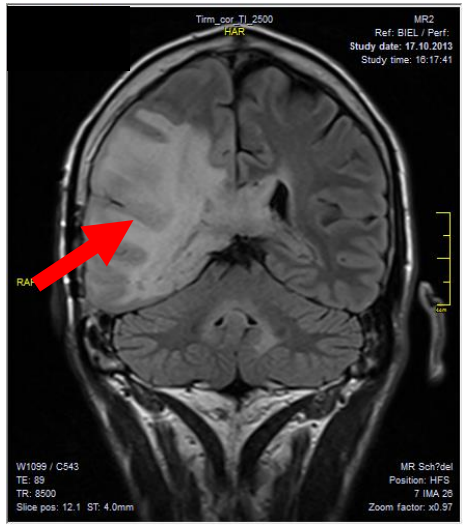
Median Survival Time: 5 years

Xuezhi *Dong et al. "Survival* trends of grade I, II, and III astrocytoma patients and associated clinical practice patterns between 1999 and 2010: A SEER-based analysis*". In: Neuro*-Oncology Practice 3 (1 Mar. 2016), pp. 29–38.

E/m (Gray) ≈ energy per tumor volume (=mass)

Small tumor volume





Large tumor volume

E_{early}/m_{early}

E_{late}/m_{late}

Early intervention: the same amount of energy is much more effective with minimal side effects

31-year old Australien computer scientist

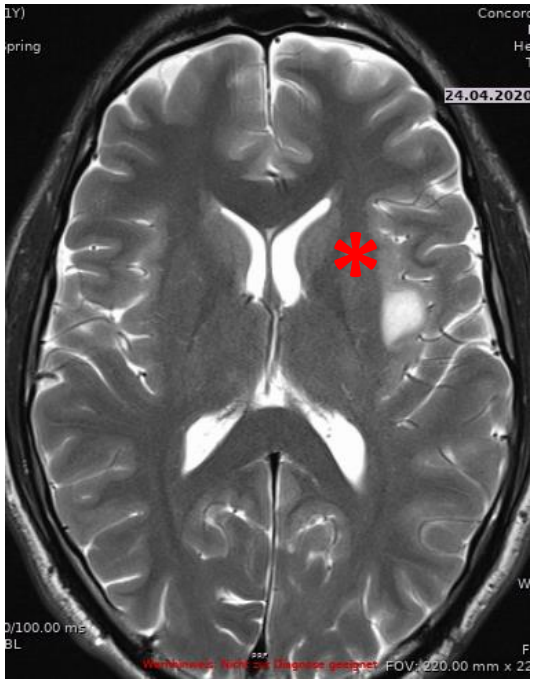
repetitive focal seizures (hand, speech)

open biopsy:

diffuse invasive astrocytoma grade 2

IDH mutant

location: Sylvian fissure

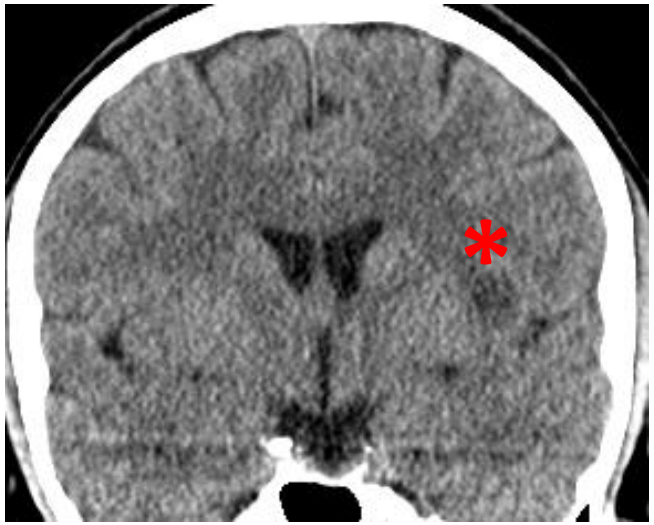


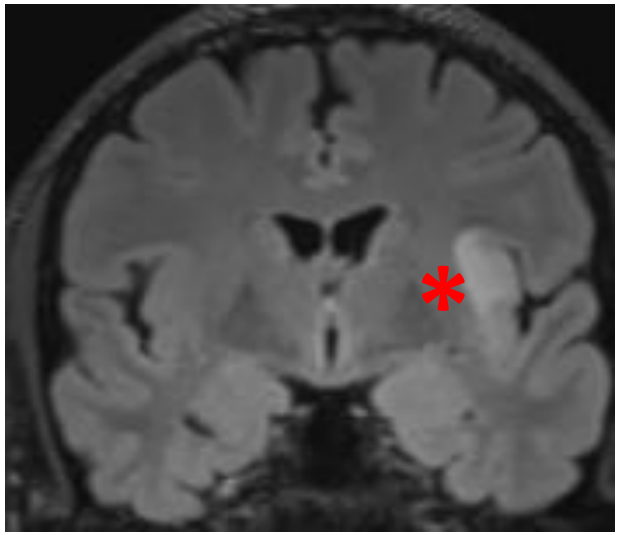
- CT/MRI 4-2020 / axial view

Surgery?

-not completely resectable

-high risk for deficits

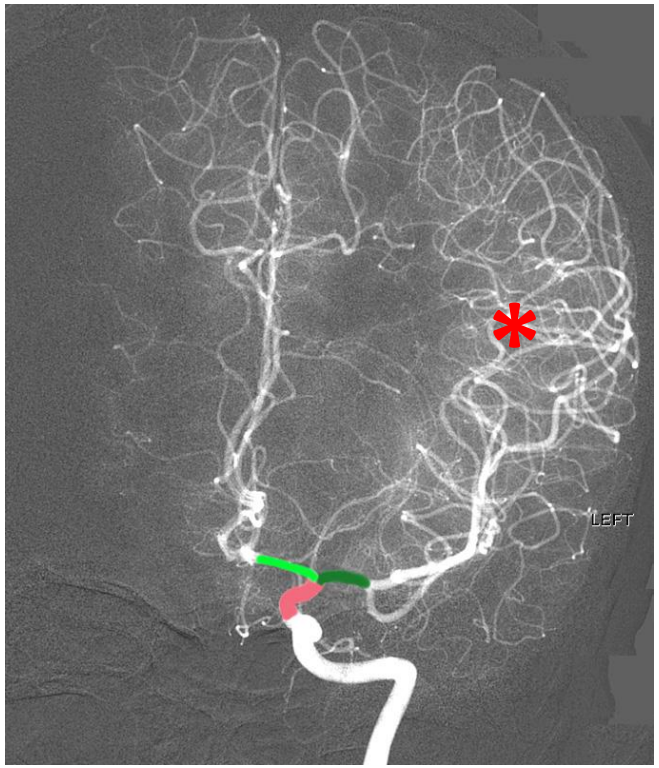


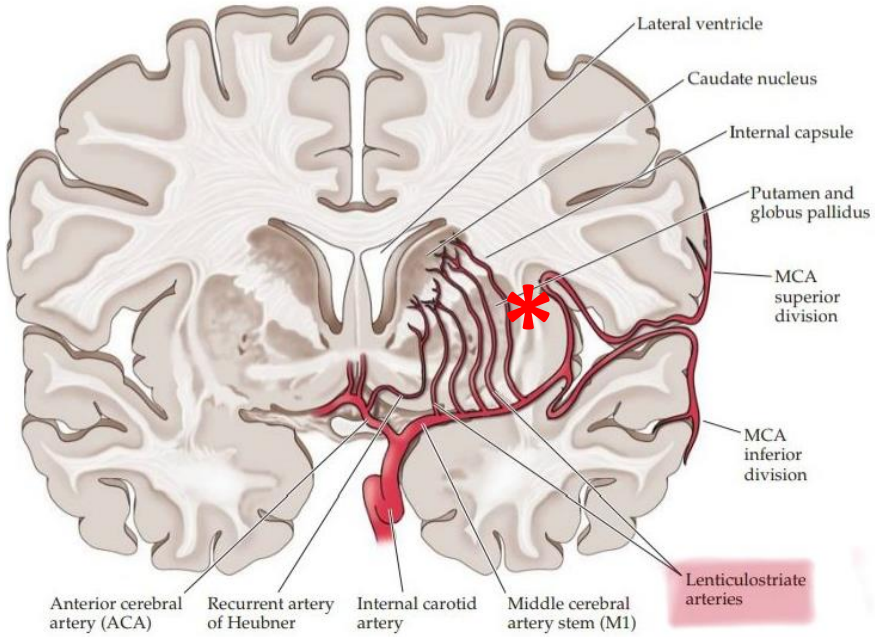


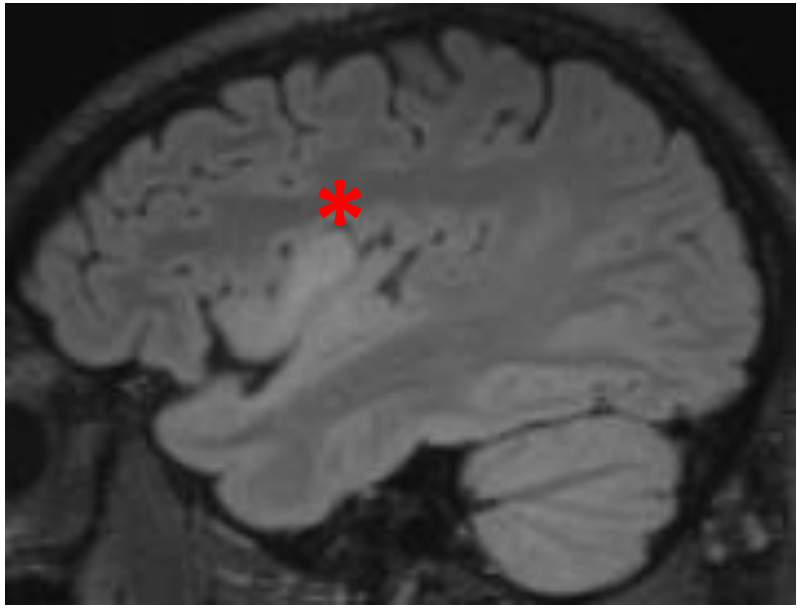
CT/MRI 4-2020 coronary view

Location

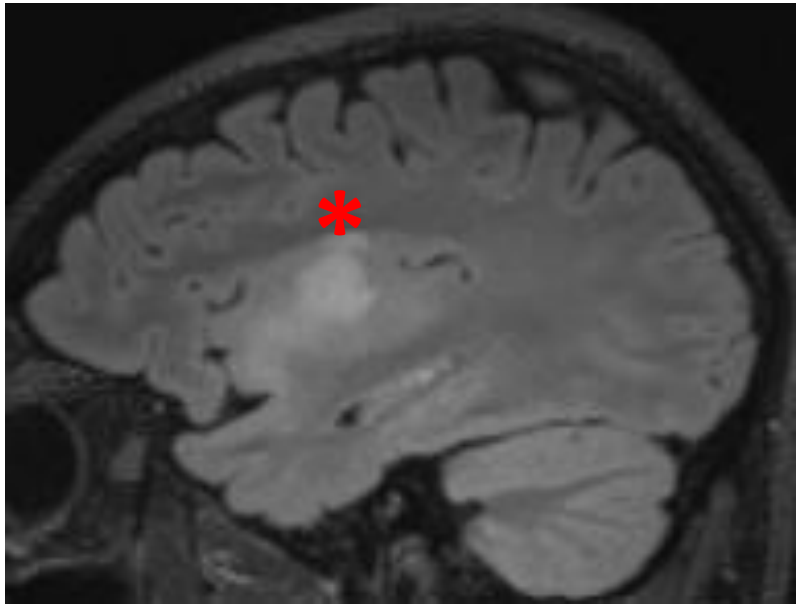
Vasculature

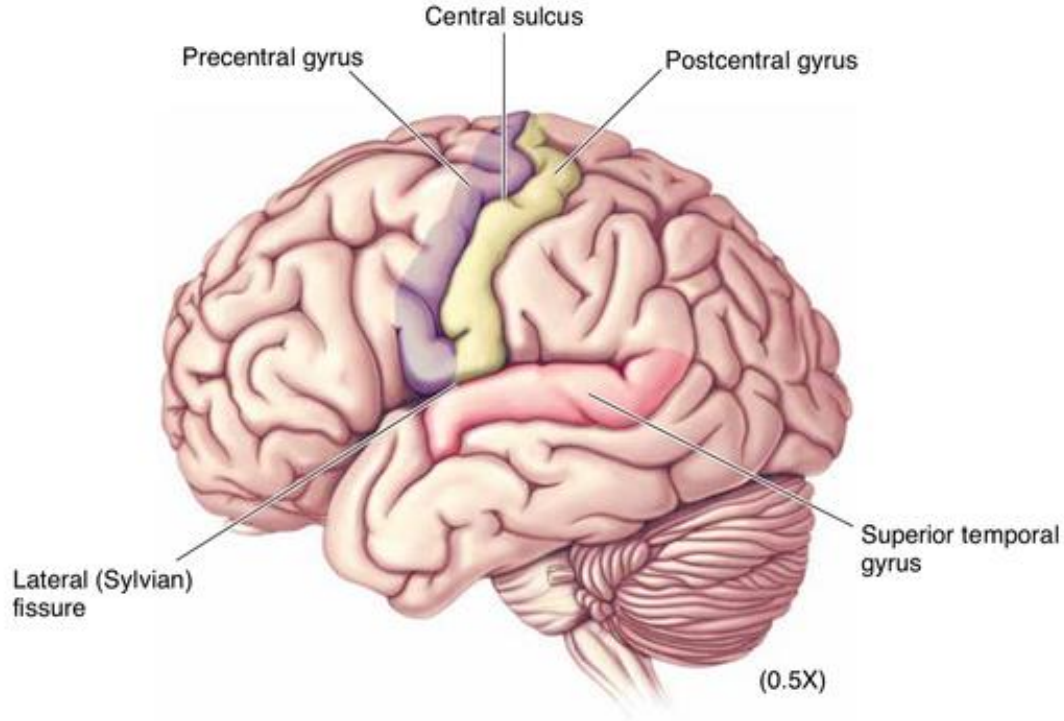


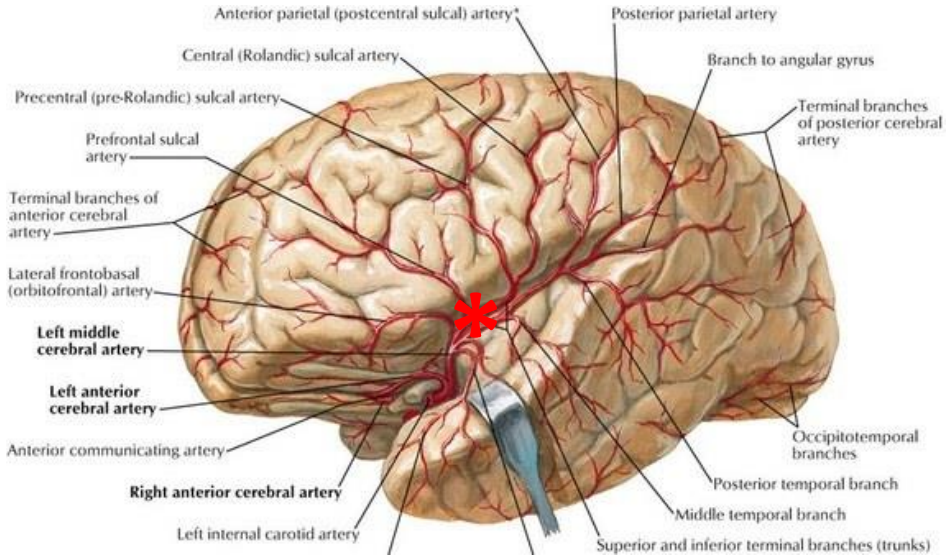


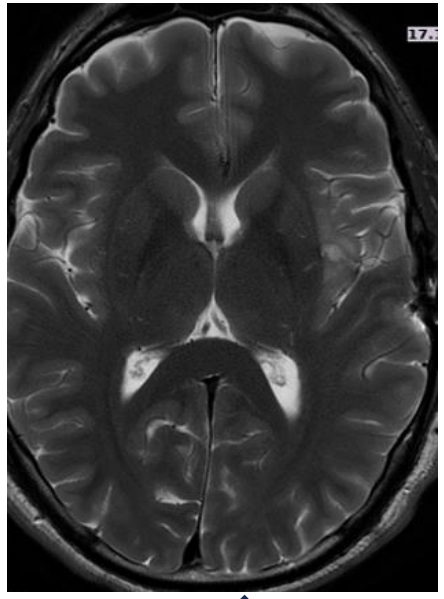


MRI 4-2020 / sagittal view

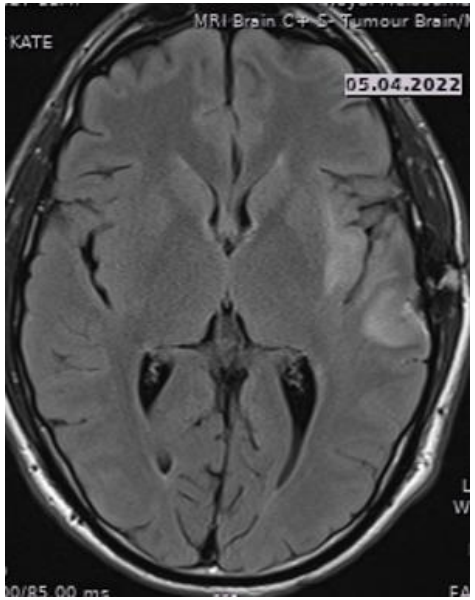




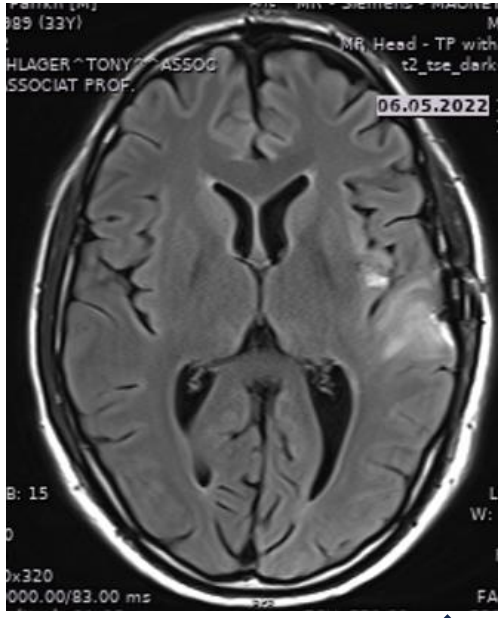




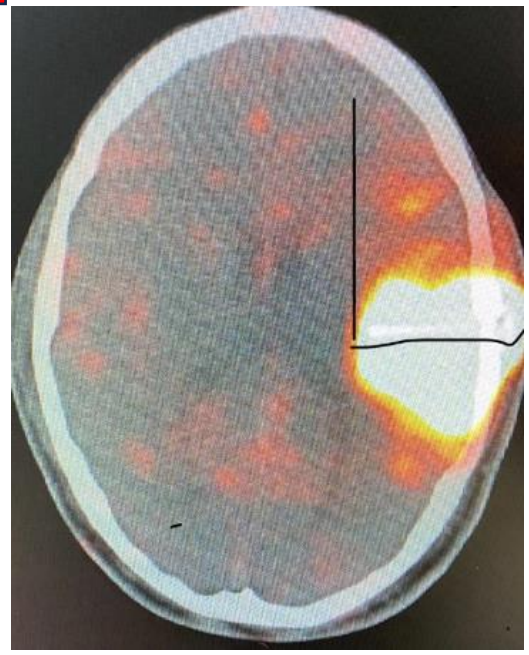
12/2021



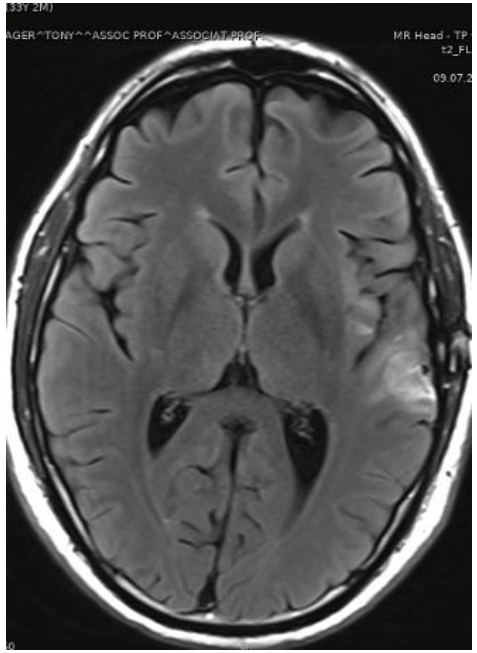
4/2022



5/2022



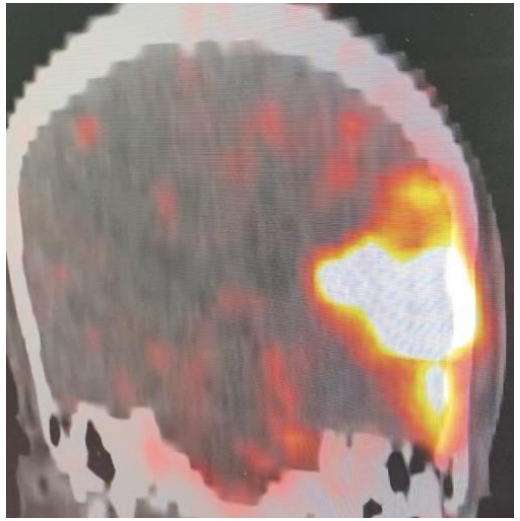
3/2022

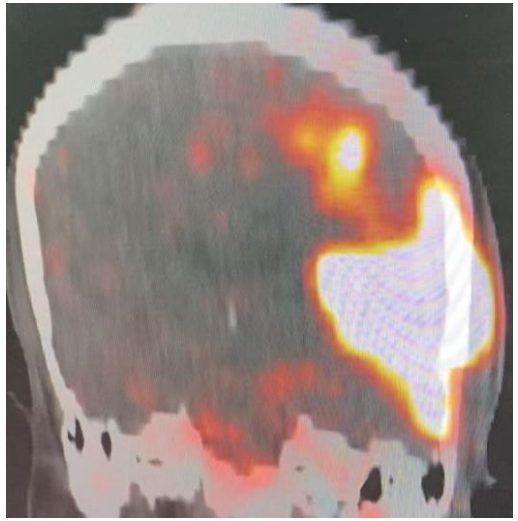


7/2022

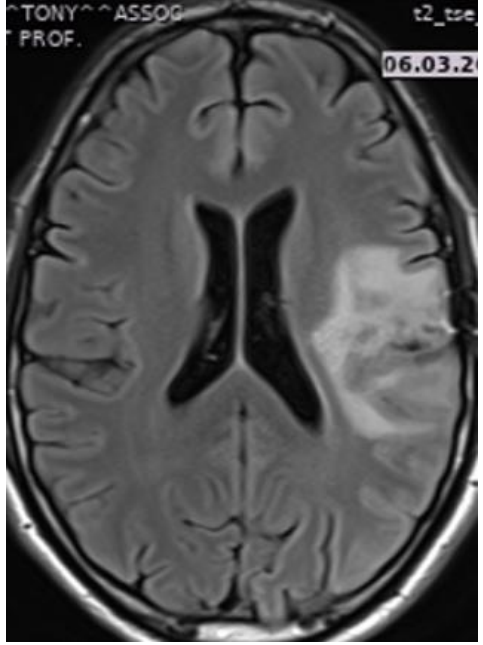
Blown up Gallium-68 DOTA-substance P signal 30 minutes after injection

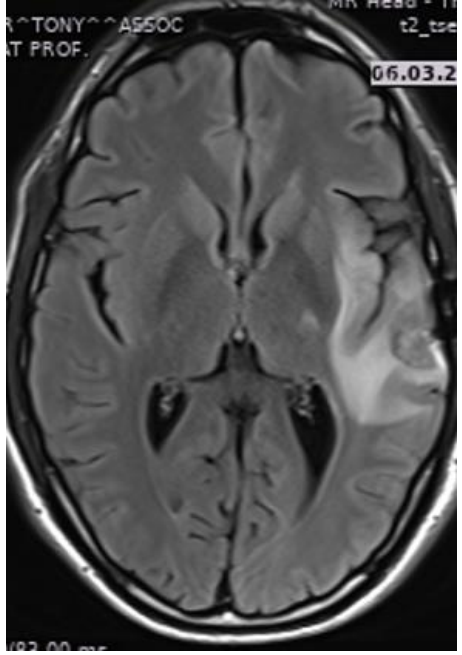
- injection volume 2 ml
- not visible in CSF
- widespread rapid diffusion (molecular weight 1800 D)





3/2023

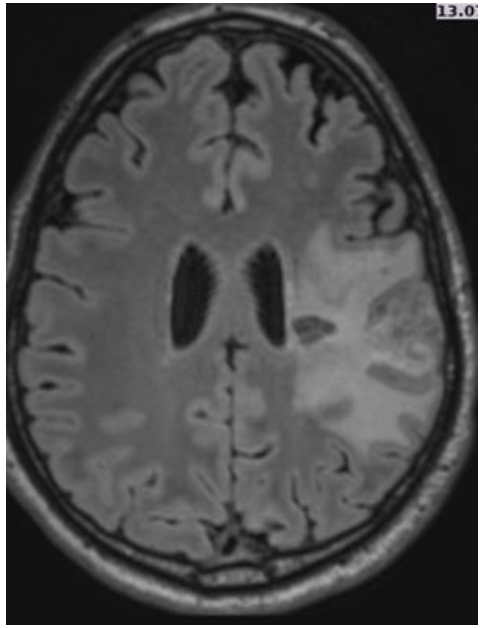


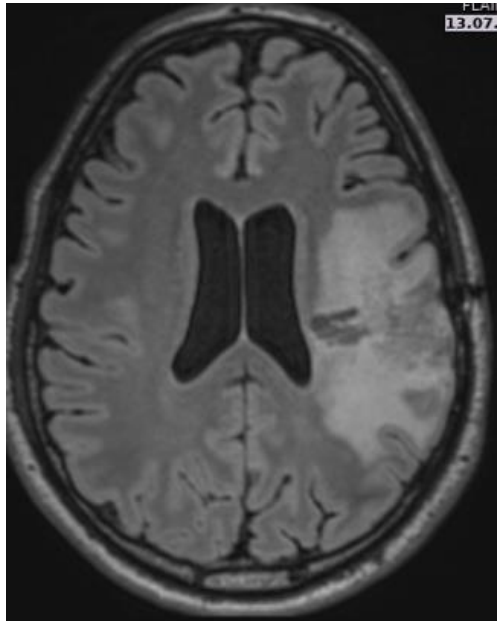


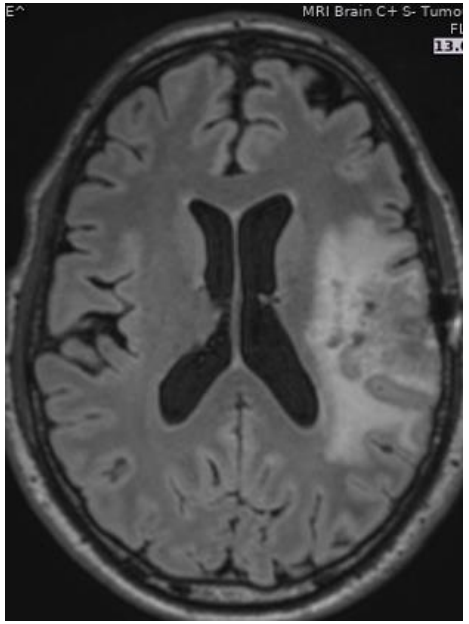
1 year after TAT, good status, mild deficit (fingers left hand,
word finding)

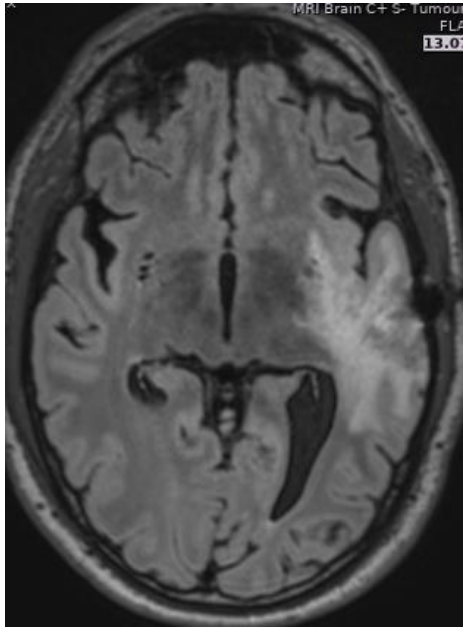
7/2024

2.3 years after TAT, good status, mild deficit (fingers left hand, word finding)





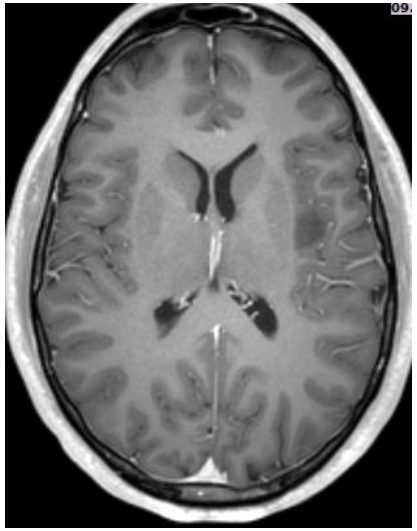




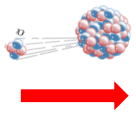
Post TAT-MRI: T1 weighted image with creates confusion

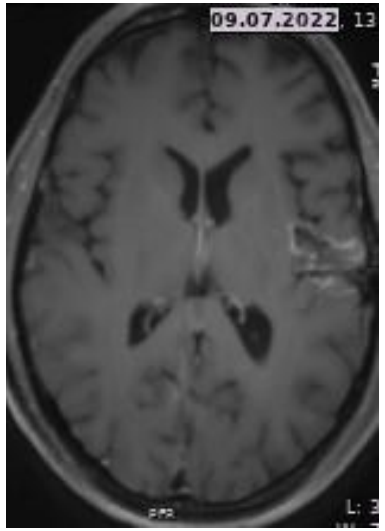
- **inflammatory** reaction towards apoptosis/necrosis

- **not** histological **upgrading** to higher malignancy!



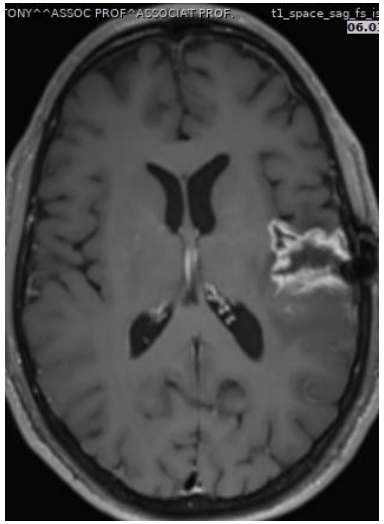
6/2020



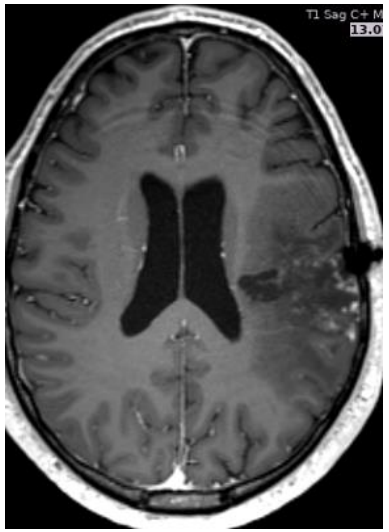


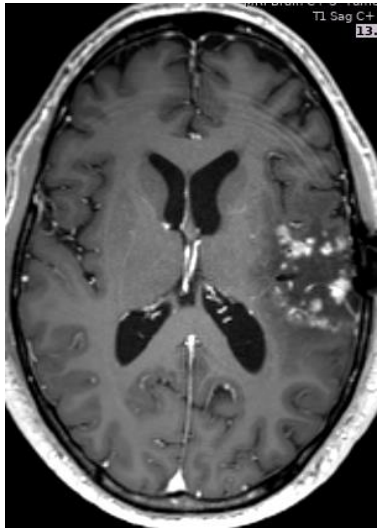
7/2022

2 years after TAT



3/2023





7/2024

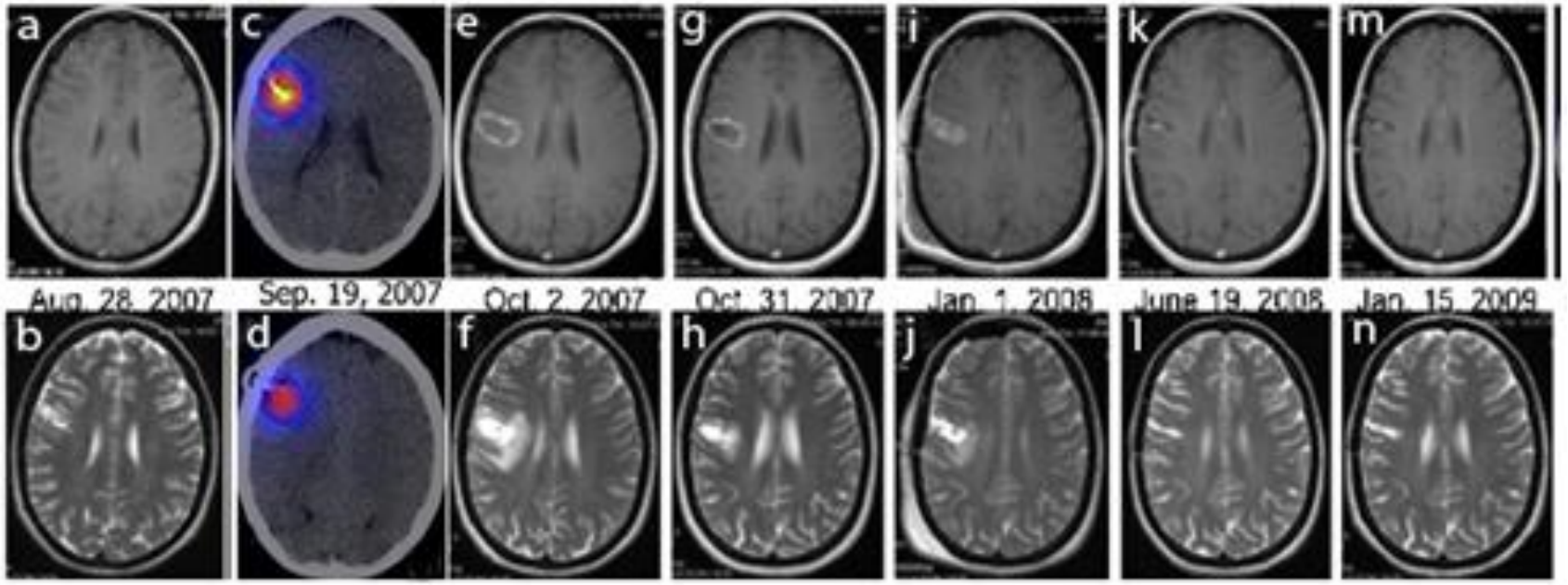
The TAT approach 2nd example

33-year old female

diffuse astrocytoma II

only using TAT and necrosectomy

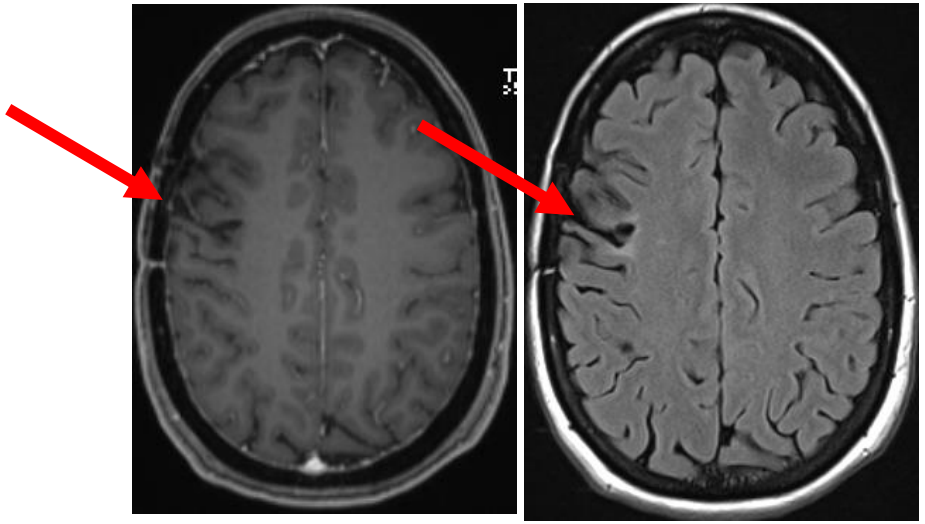
1.96GBq 213Bi-DOTAGA Substance P



2 weeks after αT

18 months after αT

t (months)



3/2014 and 2022

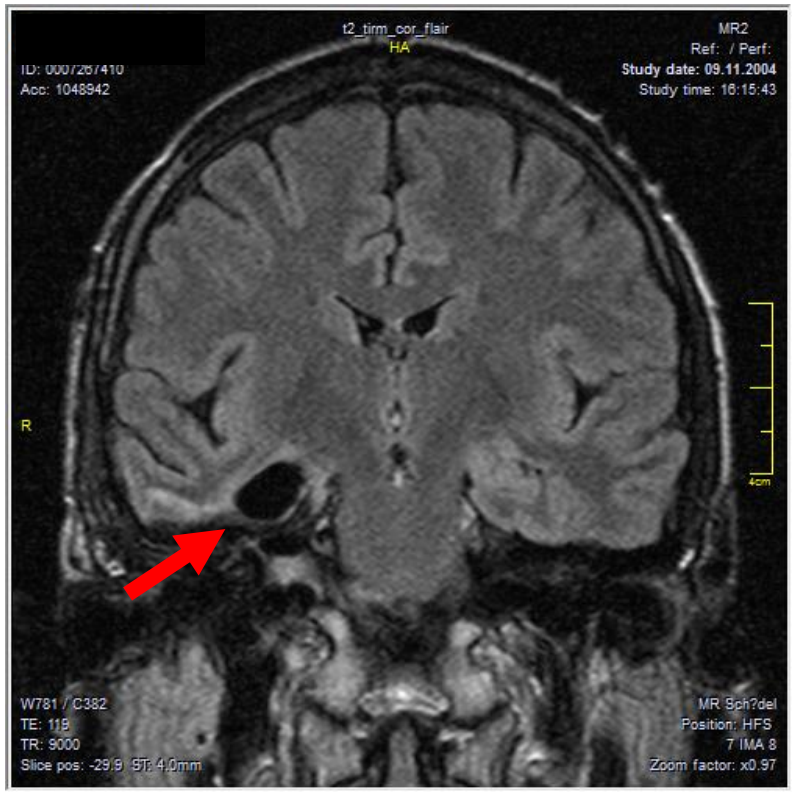
Only treatment:

neoadjuvant TAT

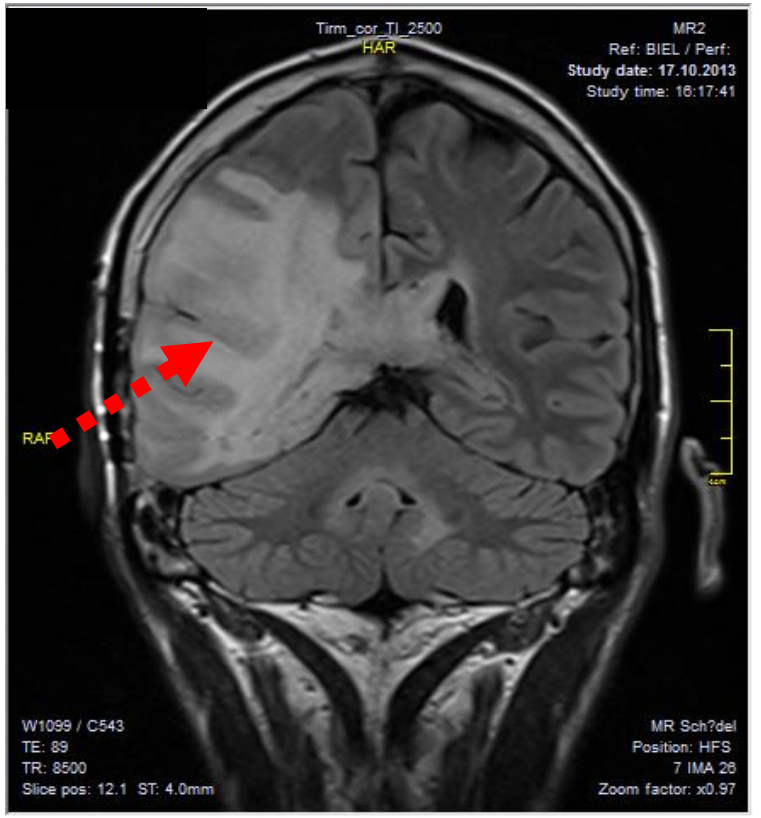
and necrosectomy

17 years recurrence-free survival in a now 50-year old woman with

diffusive infiltrative astrocytoma grade 2, no functional deficit ±“clean“ MRI



?



E_{early}/m_{early}

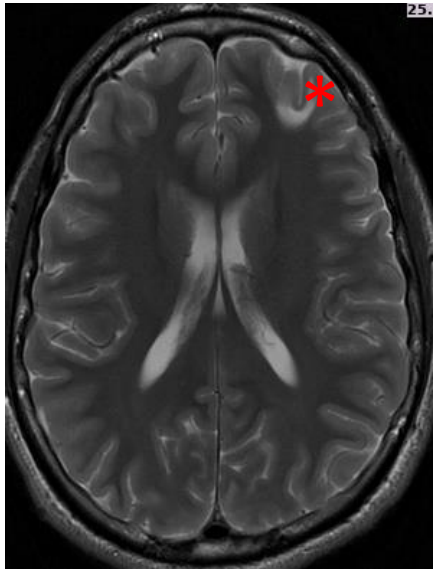
Ideal case: 2GBq Bi-213: long-term control over 17 years, no relapse, asymptomatic, no medication

Diffuse Astrocytoma II

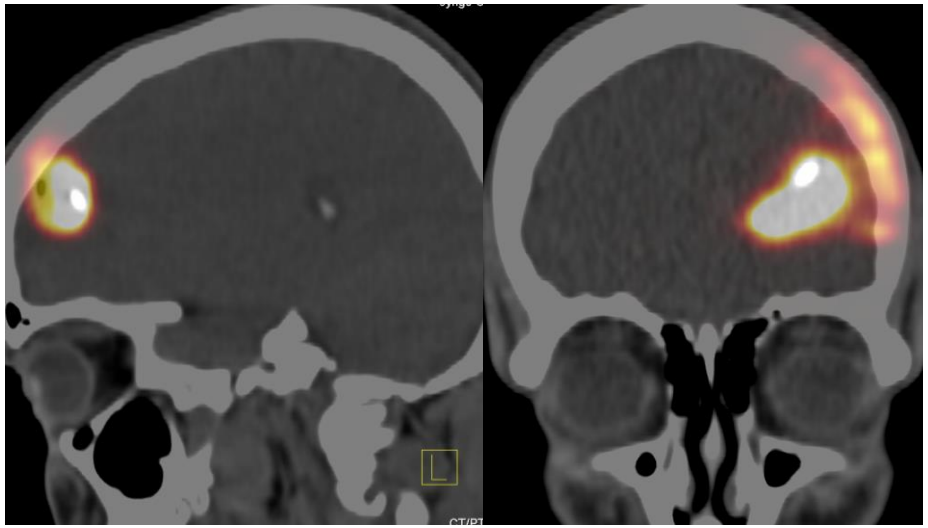
24-year old man, seizures

2 GBq Bi-213 DOTA-substance P

Sept 2015



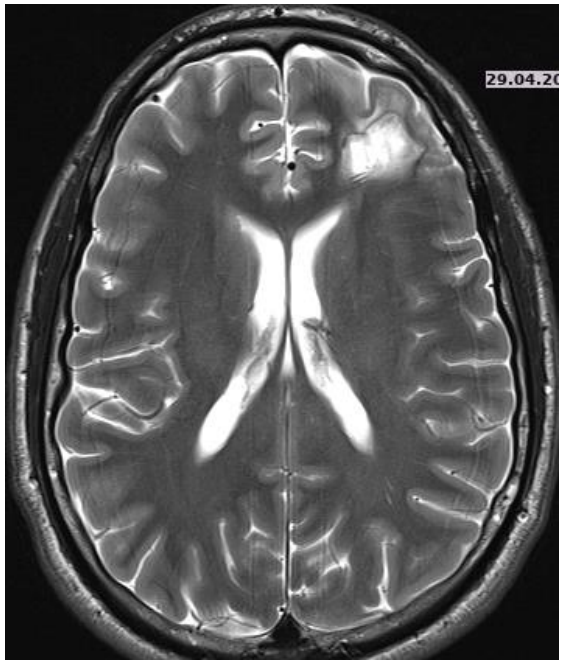
2 GBq Bi-213 DOTA-substance P



Feb 2016

4 weeks post **TAT**

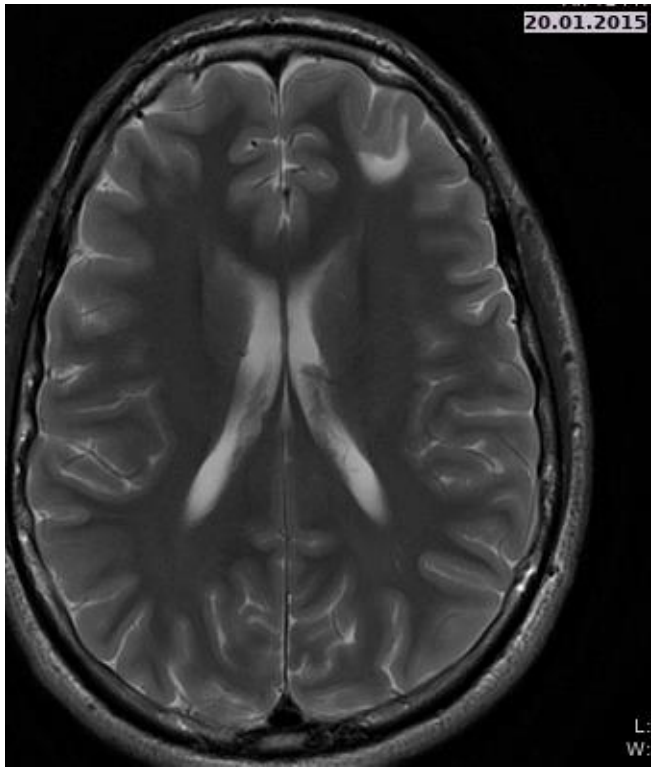
Apr 2016

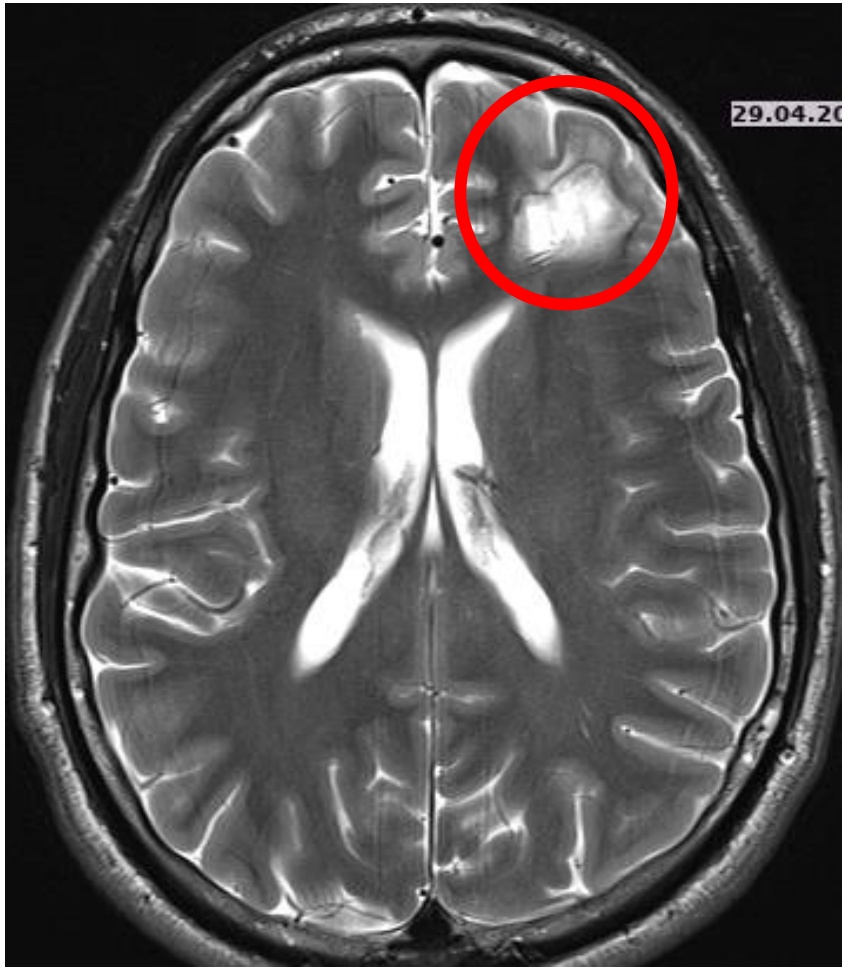


3 months post **TAT**

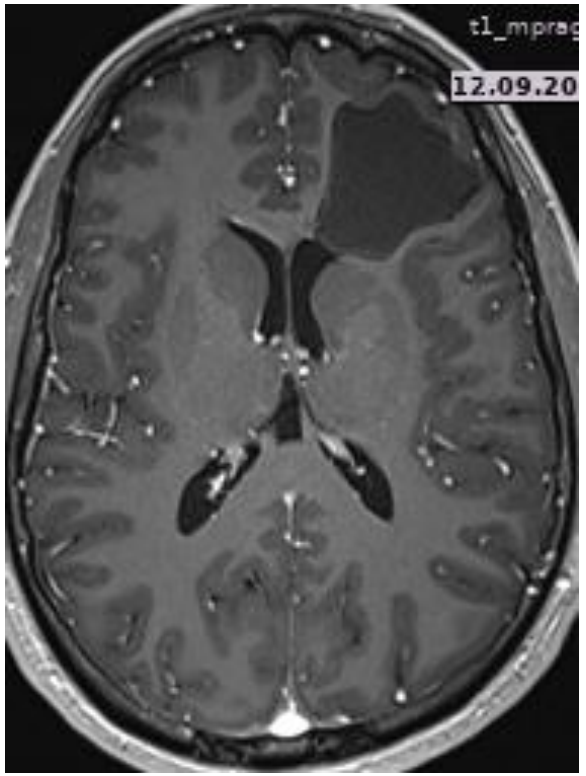
April 2016

Jan 2015





Sep 2022



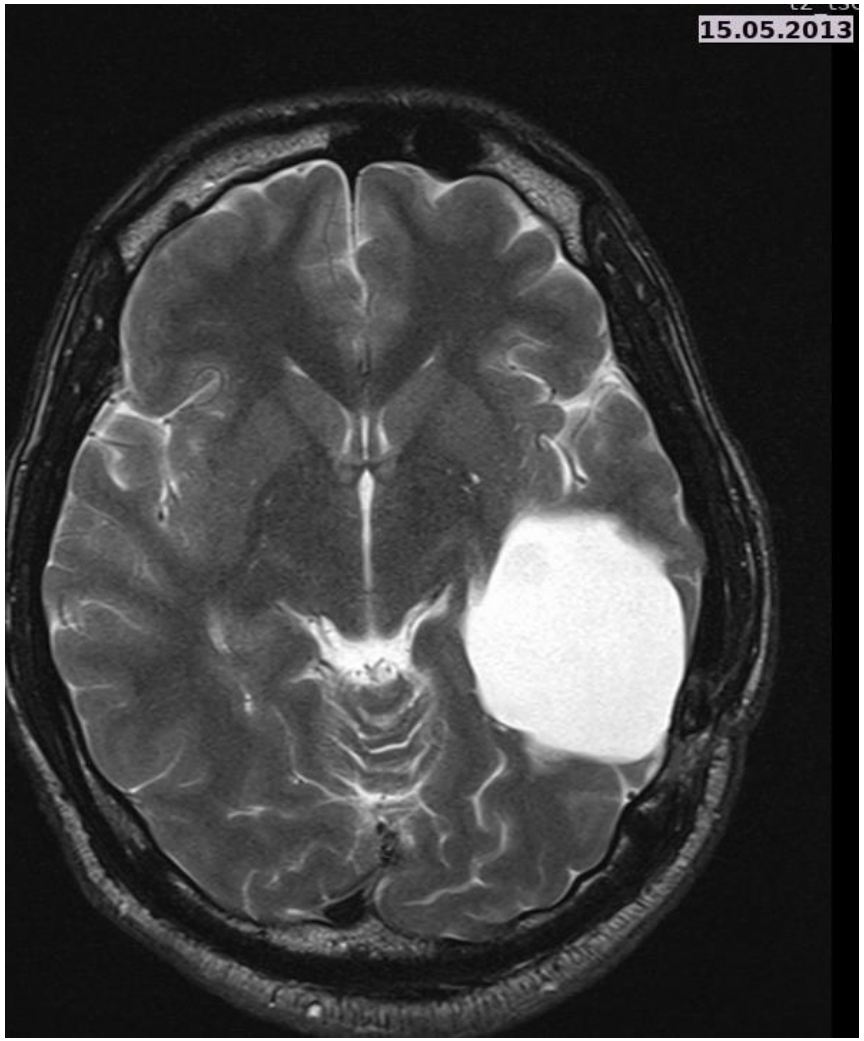
7 years recurrence-free after TαT, **Karnofsky 100**, no other therapy,

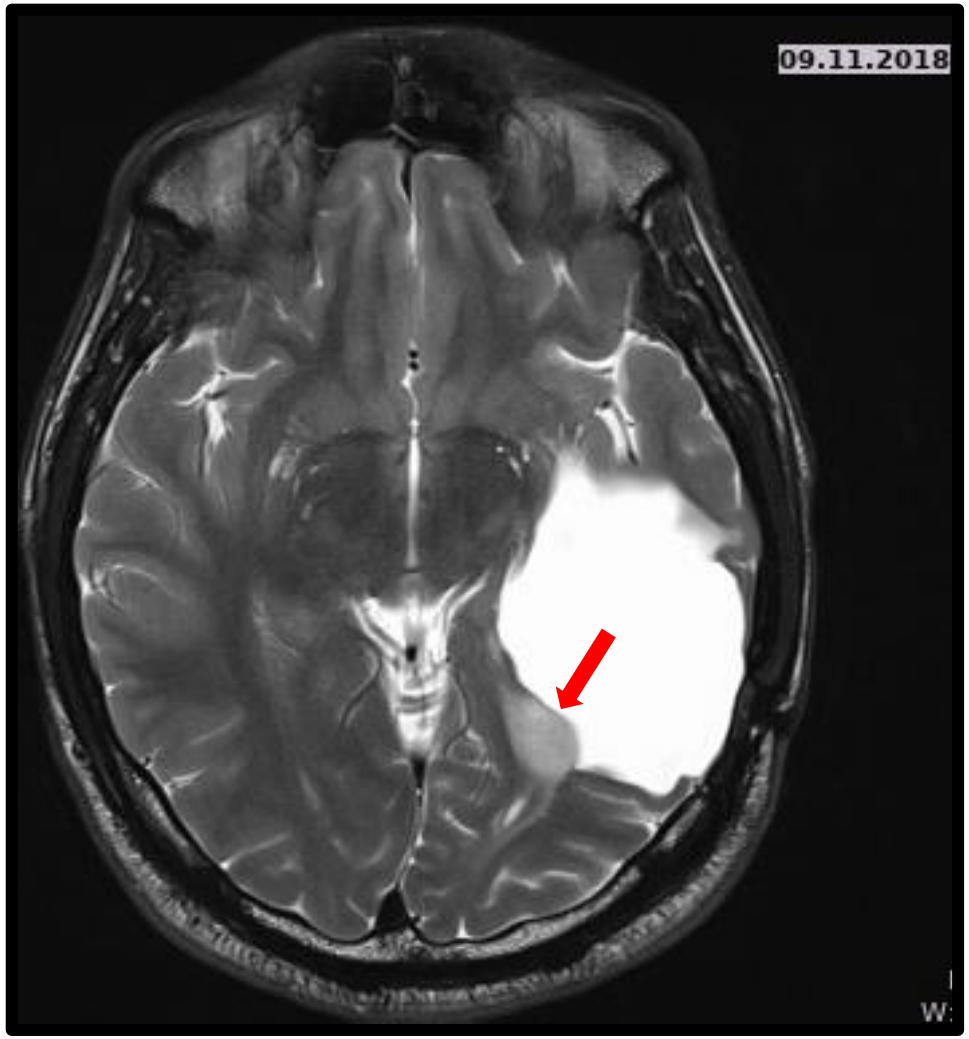
25 year-old male in 2011 diagnosis

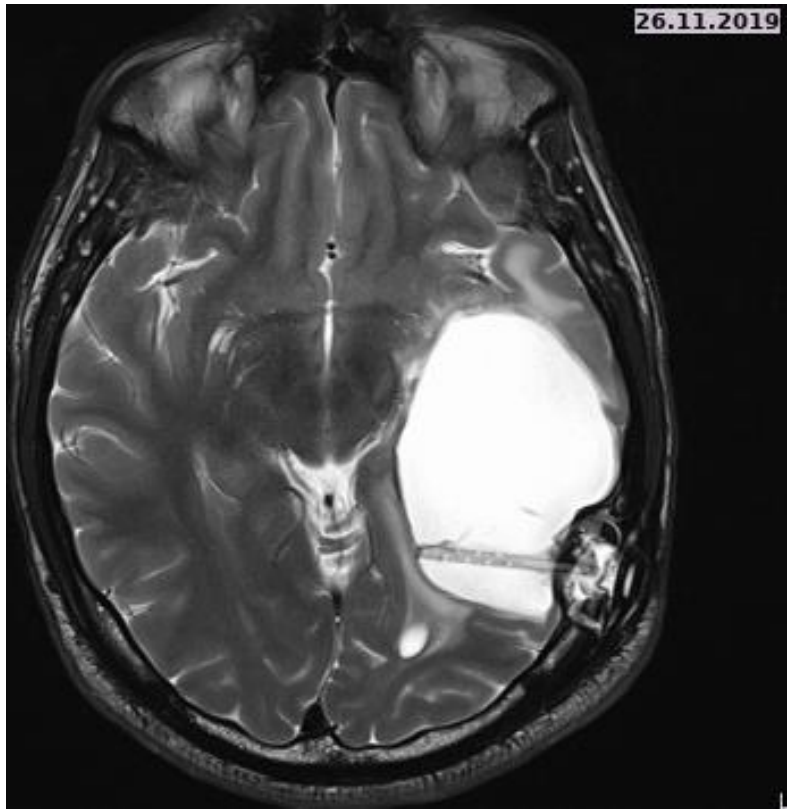
astrocytoma II left temporo-occipital

3 resections 2011, 2013, 2018

25 year-old male**, astrocytoma II** left temporo-occipital, 3 resections 2011, 2013, 2018

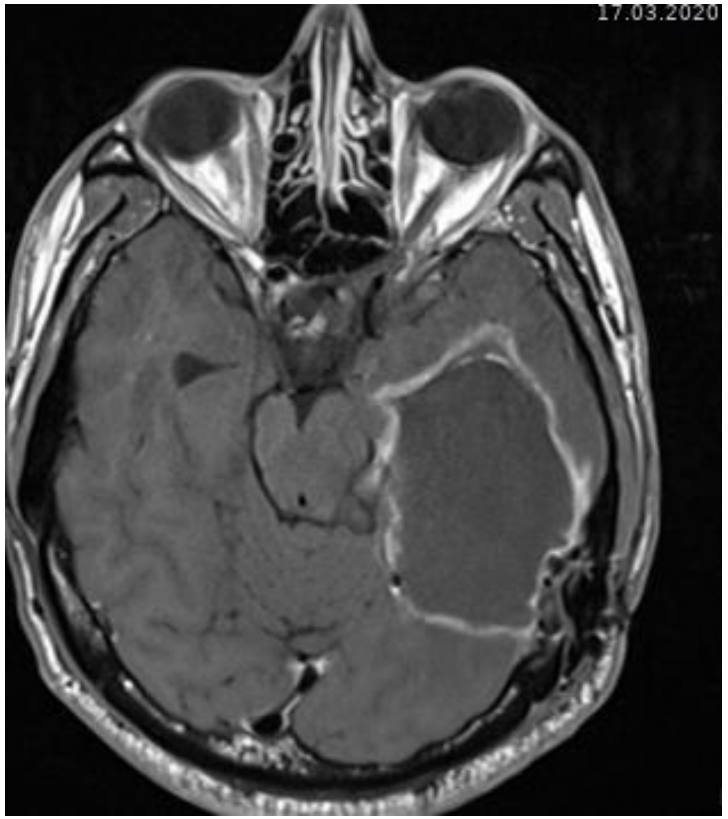






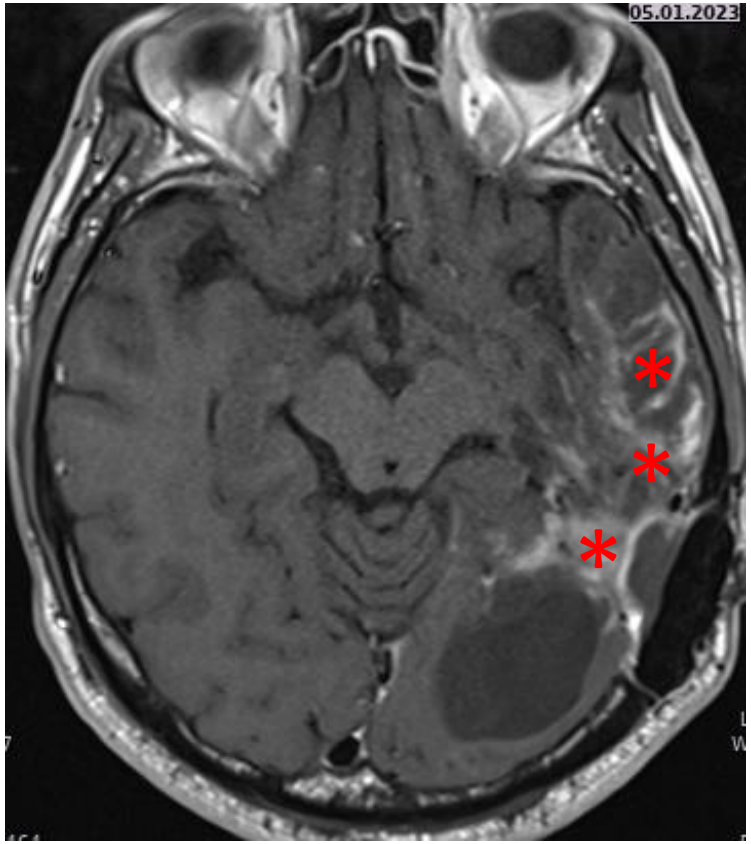
TαT in 2 fractions using Ac-225

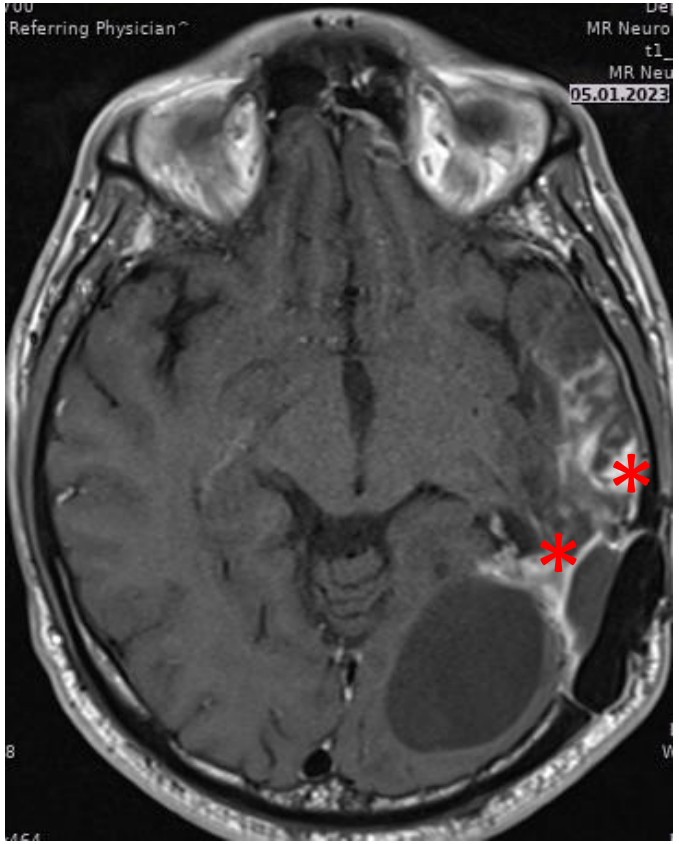
total 1.85 mCi (69.4 MBq) 2019



close interval of 8 weeks very close with large tumor burden, high dosage in advanced stage

3 years after TAT, 2 re-craniotomies (necrosis, DD progression?)
much improved, Karnofsky 80





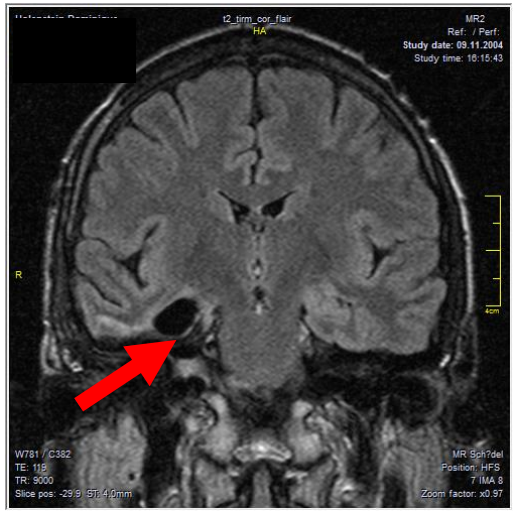
January 2023 (September 2023 similar)

Perfusion MRI:

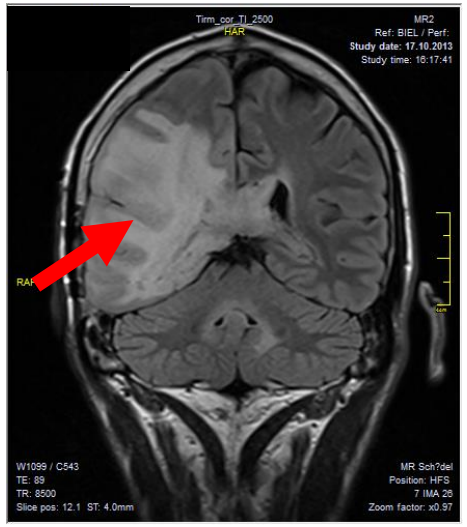
Necrosis *

not progression !

Small tumor volume



Large tumor volume



E_{early}/m_{early}

E_{late}/m_{late}

Conclusion for advanced cases

Start earlier!

Fractionation in large tumor systems?

Invasive disease +++, not visible on MRI (flair)

TAT no rescue therapy!

Age&Year Dx /Gender	*Histology/ Location*	*Genetics*	*pre-/post-a therapies*	*activity/ nuclide(cycle)*	*TAT*	*Karnofsky*	*PFS/OS (alive)*	*p*	*QALY*
43(2000)m	oligo II/pR	ND	S&Y-90SP/CT	1.9 GBq Bi-213(1)	2000	90	264+/266+	0.2	20
33(2007)f	diff astro II/fR	ND	none/S	2 GBq Bi-213(1)	2007	100	192+/194+	0.1	16
39(2008)m	diff astro II/oR	ND	none/S	2 GBq Bi-213(1)	2008	100	180+/182+	0.1	15
64(2011)m	diff astro II/centralR	IDH mut, 1p/19q wt	S/S	1.9 GBq Bi-213(1)	2011	90	*144+/150+	0.1	11
25(2011)m	diff astro II/tL	IDH-1-R132H, ATRX mut	S/S	35 MBq Ac-225(2)	9/19	80	42+/144+	0.1	10
31(2011)f	diff astro II/tL	IDH-1 mut, 1p/19qwt	S&RT/S	1.9 GBq Bi-213(1)	3/17	90	72+/146+	0.2	10
24(2015)m	diff astro II/fL	IDH2 Exon4 R172M	none/S	2 GBq Bi-213(1)	2016	100	*86+/92+	0.5	8
32(2020)m	diff astro II/fR	IDH-1 R132H, ATRX mut	S/none	20 MBq Ac-225(1)	1/22	100	12+/30+	0.8	2.5
30(2020)m	diff astro II/tL	IDH R132H, ATRX mut	S/none	17 MBq Ac-225(2)	3/22	100	14+/32+	0.8	2.7
Cross over for recurrent OGII after Y-90 SP									
SK43(2003)m	oligo II/pR	ND	S&Y-90SP	2.5 GBq Bi-213(1)	2014-18	80	48/224	0.1	6.4

Very long recurrence-free survival times in diffuse astrocytoma patients II (median survival 5 years) (Bayesian approach)

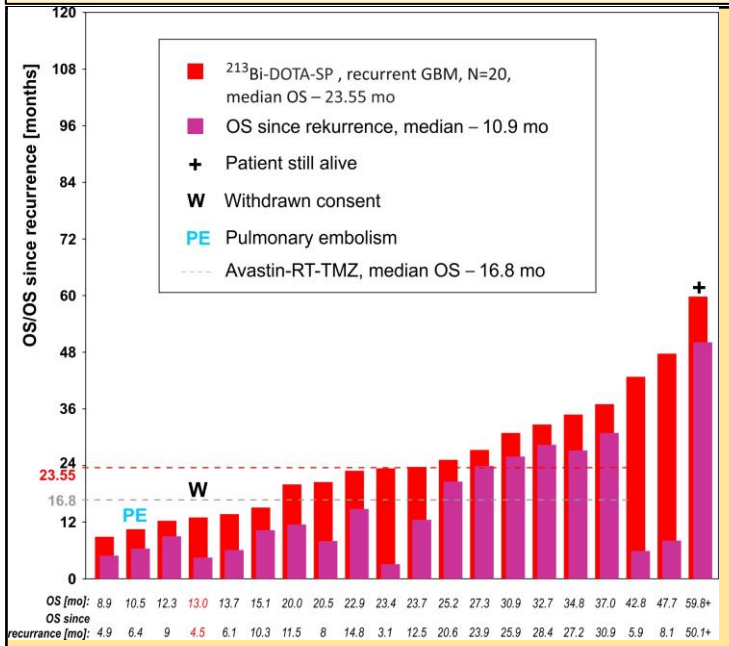
QALY: Karnofsky (0.1-1) x Survival (years), compare GBM 4 years survival gain, e.g. 0.7 x 3 = 2.8
Estimate of socio-economic impact of a given treatment

How to apply targeted alpha therapy in malignant gliomas?

- TAT for low grade gliomas: a new treatment paradigm?
- **TAT for glioblastomas, how to develop a clinical protocol?**

Results TαT on recurrent GBM

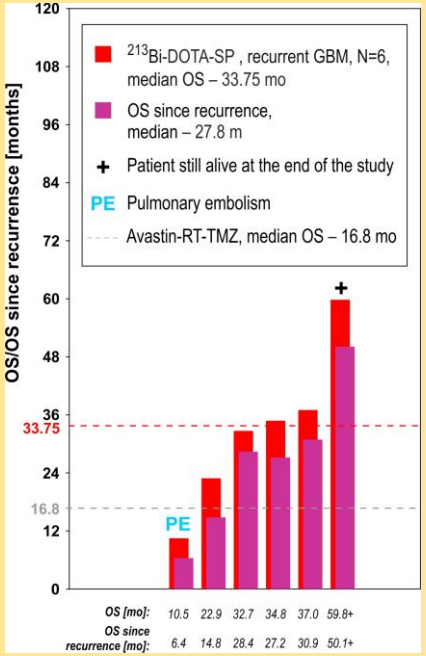
- **WUM**: > 100 GBM patients treated (**published**), phase 1 and 2 including dose finding completed, **all cases recurrent GBM**
- 3 GBM recurrent studies: a) Bi-213 labelling b) Ac-225 labelling c) secondary GBM Bi-213 labelling
- protocol for phase 3 discussed with EMA, **corrected phase 3 protocol ready to go** (early adjuvant trial)



Waterfall diagram of survival times from 20 patients treated in Warsaw with Bi-213 DOTA Substance P.

Red: total survival times since diagnosis (median 23,5 months);

Violet: survival times following start of alpha therapy (median 10,9 months)



Subgroup analysis for inclusion criteria:
define target population for phase III study

Best results obtained in patients if
tumor diameter ≤ 5 cm and
Karnofsky score **≥ 70**

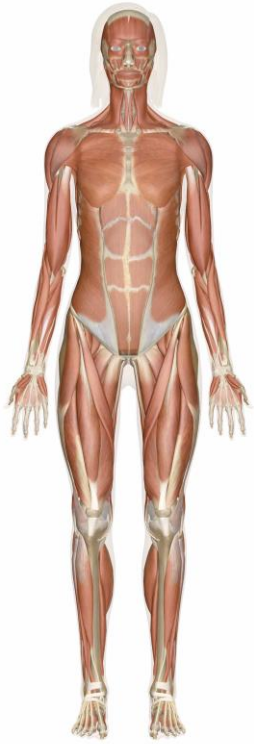
Total survival times in subgroup: 33,75 months
Survival after start of alpha therapy: 27,8 months

Standard SRCT Stupp et al: mean survival time for GBM 14.5 months

How to improve TAT response in GBM?

- Supply only every 8 weeks? Exponential growth, cell doubling < 7 days
- Do not wait until recurrence manifests in MRI!
- Acceleration of supply of Ac-225 every 2-4 weeks
- Early adjuvant TAT after end of standard RCT (supervised by EMA)

When is the best time point to apply TAT in malignant glioma?



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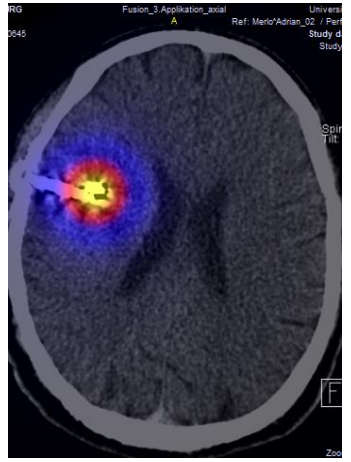
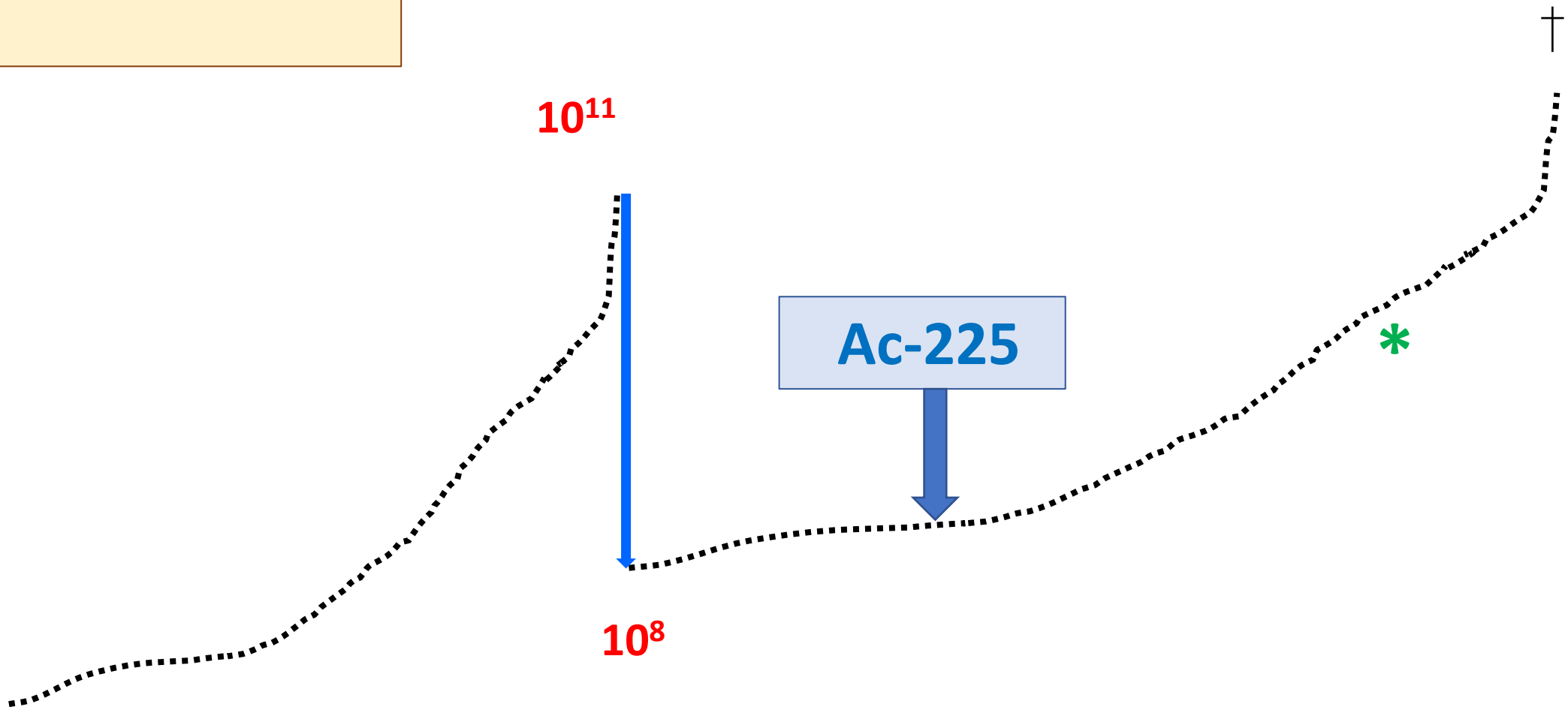


Human body (70kg) 10^{14} cells

Tumor (70g 4x4x4cm) 10^{11} cells

Surgery	99.9% removed
Residual Tumor	10^8 cells



Symptomatic

Surgery

* Recurrence ($10^{10\text{-}11}$)

How to overcome to impasse of clinical development in orphan disease

Profitability constraints and societal responsibility

Orphan disease: < 10 cases/100‘000/year

Clinical Economic Development? **„Black Hole“**

- > 200 cancer types in humans, majority are **orphans** (<10 cases/100‘000/year)
- **Ethical dilemma**: insufficient **profitability** for big pharma investors

Adam Smith
1723 – 1790

Capitalism and Free Enterprise

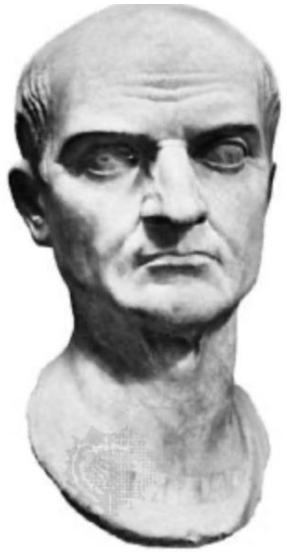
»It is not from the benevolence of the butcher, the brewer, or the baker, that we expect our dinner, but from their regard to their own interest.«

- **Disease is fate, not self-inflicted, social responsibility**

Clinical Economic Development?

- Disease: responsibility of the society as a whole (state and private benefactor)

Res publica



Caius Cilnius Maecenas 68-8 a.Chr.

Clinical Economic Development?

- **Model**: state/benefactor seed money to start ups in translational academic research units

- Incentive: **undilutible ownership in stock for the seed investor**, e.g. 30%
- **State and benefactor** assume the role of **business angel and primary investor**

- Estimate of success: 10 projects: 10 projects, 10 million seed money per project, success rate 1:10

  9 failures: loss of 90 millions
  1 success, value of 250 millions
  gain for state/benefactor: 250 Mio – 90 Mio ≈ **160 Mio**

  reinvest 100 Mio (10x10)
  60 Mio for state (health care) and University (research, infrastructure)

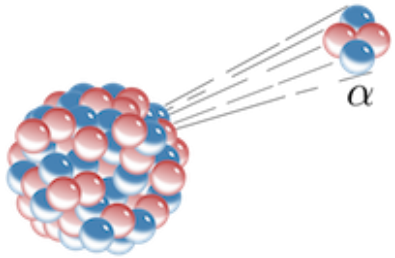
- **Invite private and public investors** for development, market expansion etc (70% share in company)

Danke!

Thank you!

Merci!

Спасибо!

Gratias!

Ευχαριστώ!