Gadolinium Specification via ICP-MS, GPC & Laserablation

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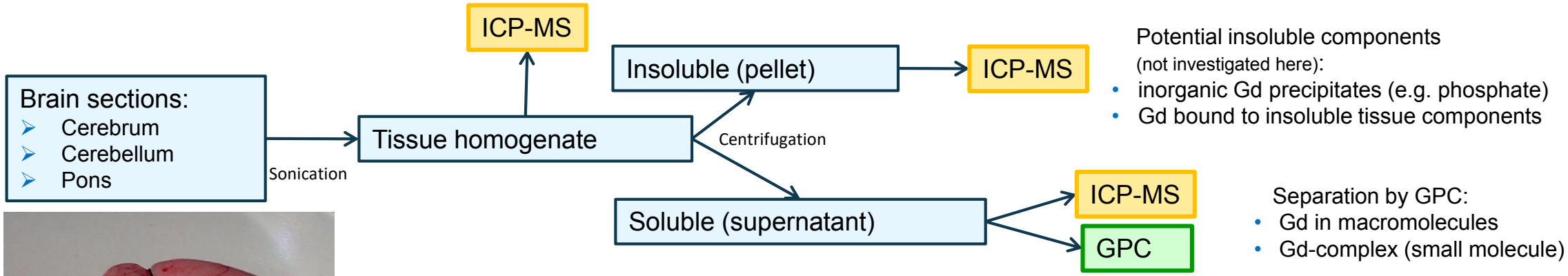
Conflicts of Interest

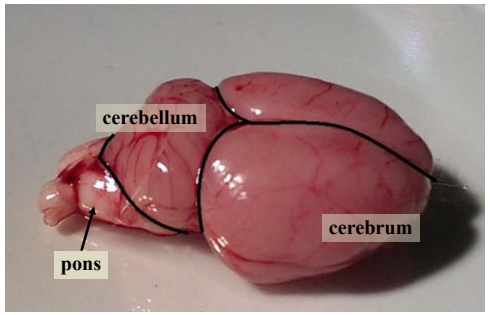
Bayer employee.

Brain Tissue Fractionation and Separation



Potential insoluble components
(not investigated here):
- inorganic Gd precipitates (e.g. phosphate)
- Gd bound to insoluble tissue components

Separation by GPC:
- Gd in macromolecules
- Gd-complex (small molecule)



ICP-MS

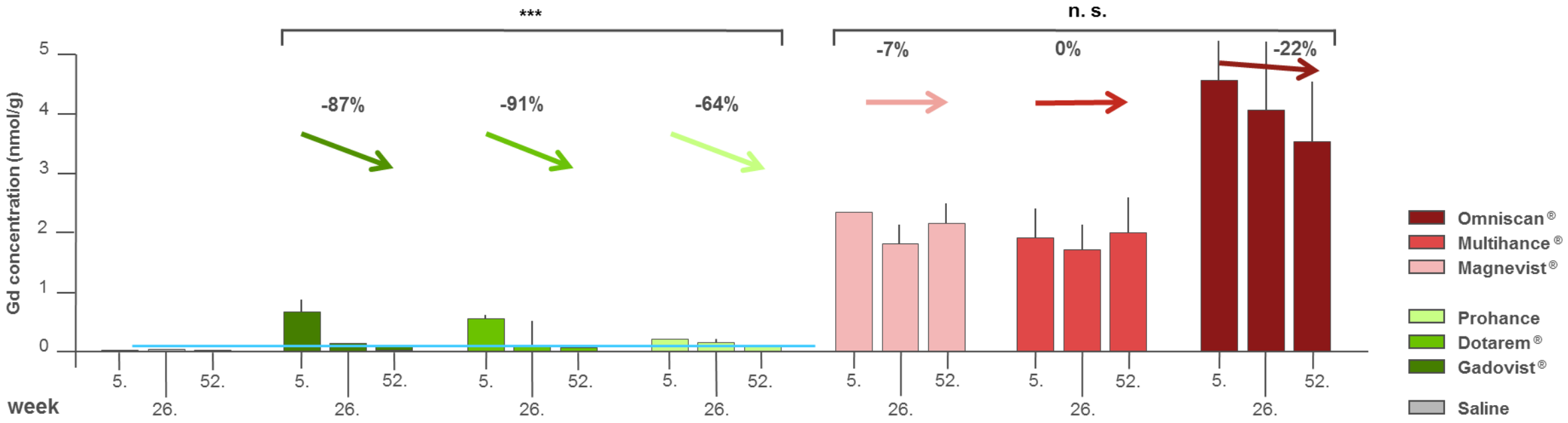
ICP-MS allows quantification of total Gd concentration, but does not discriminate between chemical species.

Gel Permeation Chromatography, GPC

GPC is a chromatographic procedure to separate high and low molecular weight components.
ICP-MS was used to identify Gd containing components (detected as chromatographically separate peaks).

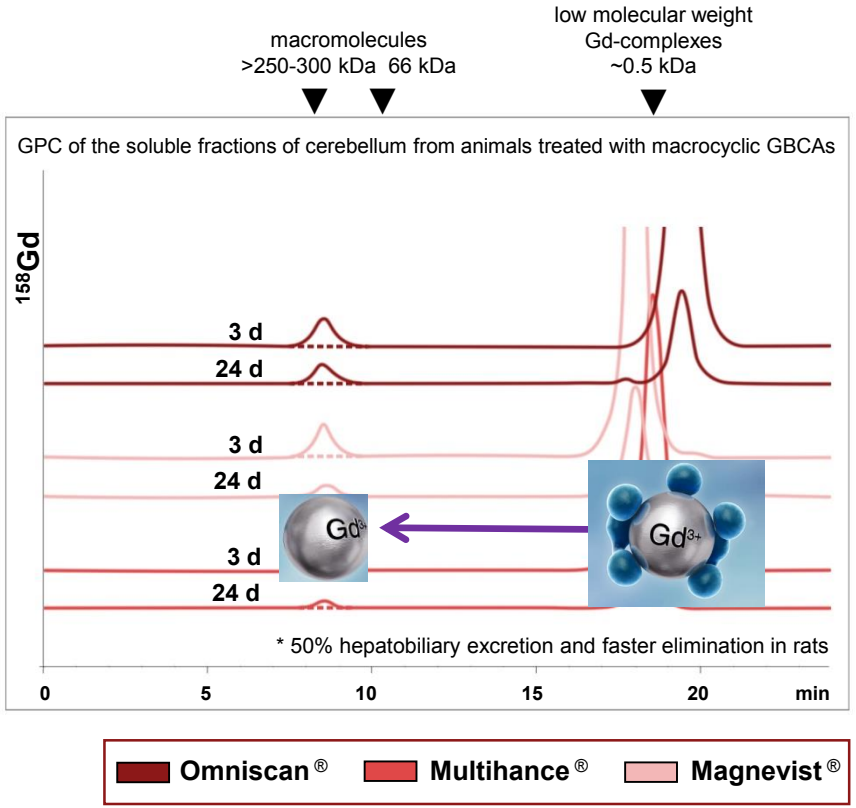
A control study was performed in a similar way with blank tissue, spiked with low amounts of all GBCAs to assess the recovery of Gd in each step.

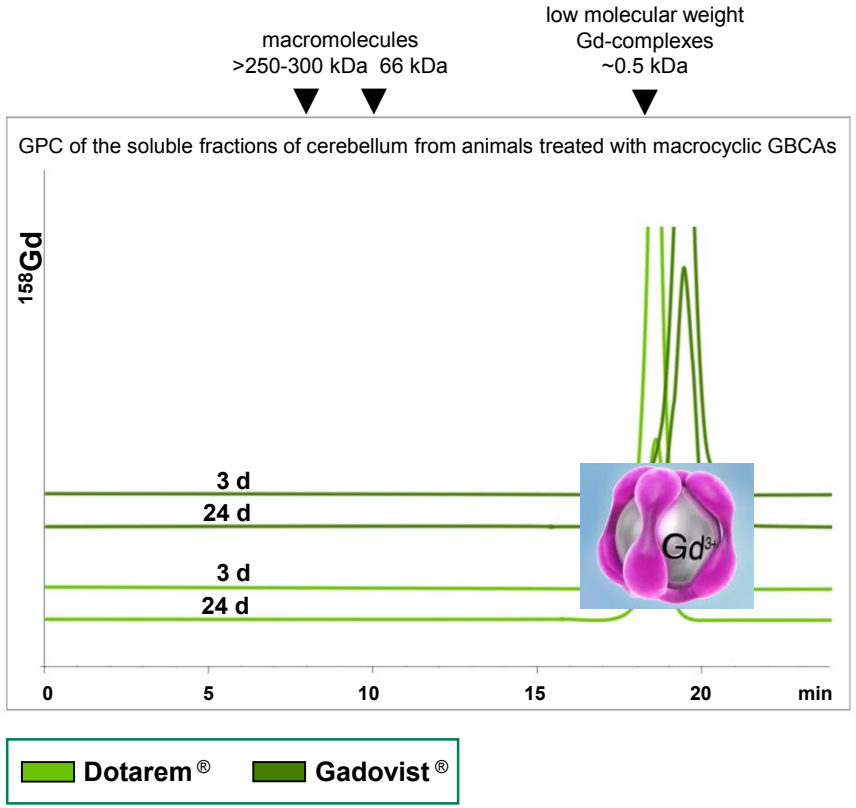
Gd Deposits in the Rat Brain: ICP-MS of the Brain 5, 26 & 52 weeks p.i.



- Significantly higher gadolinium concentrations in the cerebellum for all linear GBCAs: highest concentrations for Omniscan®, no difference between Magnevist® & Multihance® - no complete eliminiation of Gd over time
- Macrocyclic GBCA showed decreasing Gadolinium concentrations in the cerebellum between 5 weeks , 1 year and between 26 weeks and 1 year - Long-lasting, slow elimination process of GBCA from cerebellum

Differences linear vs. macrocyclic GdCAs: Chromatography of aqueous Cerebellum Homogenates divided Gd into GBCA, insoluble Gd and macromolecular Gd





Formation of Gd-macromolecules with multipurpose linear GdCAs (Magnevist®, MultiHance® & Omniscan®) results in a visual signal increase in the brain

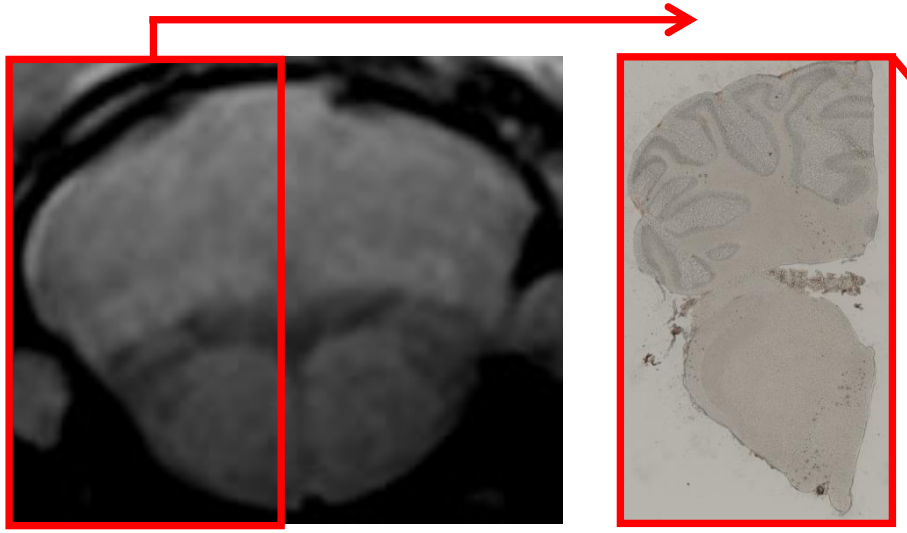
Not visible with macrocyclic agents

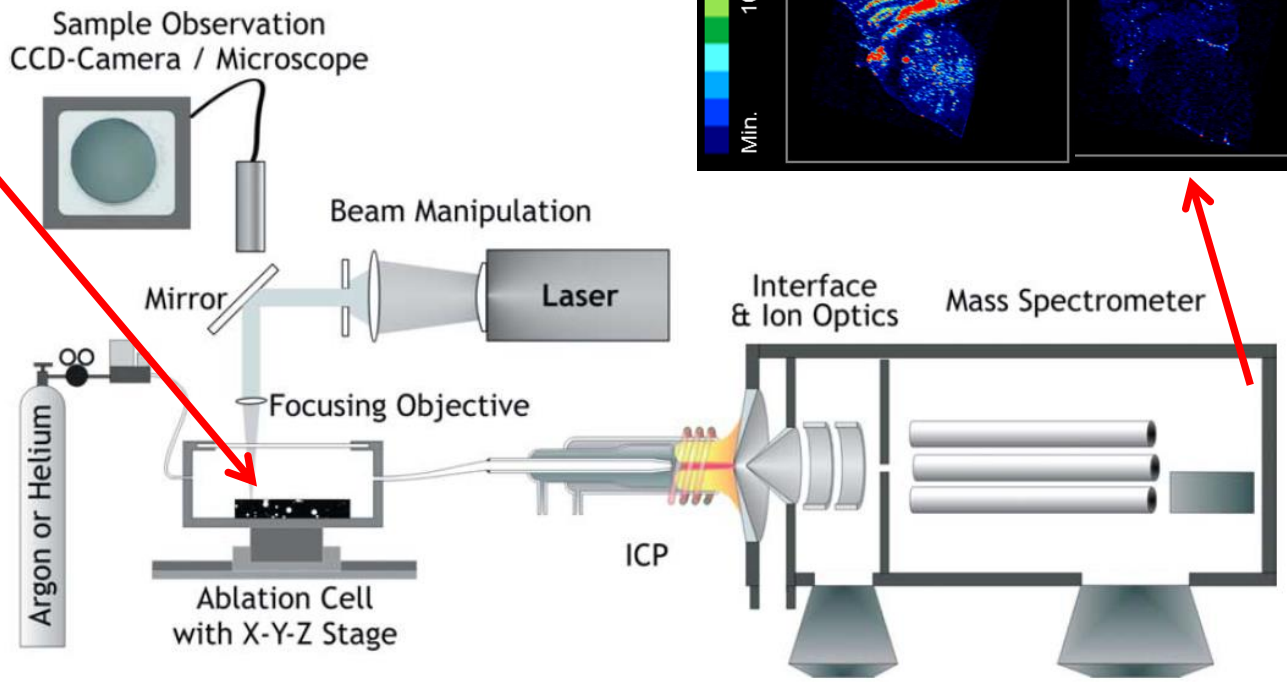
Quantification and Assessment of the Chemical Form of Residual Gadolinium in the Brain After Repeated Administration of Gadolinium-Based Contrast Agents: Comparative Study in Rats. Frenzel T, Apte C, Jost G, Schöckel L, Lohrke J, Pietsch H.

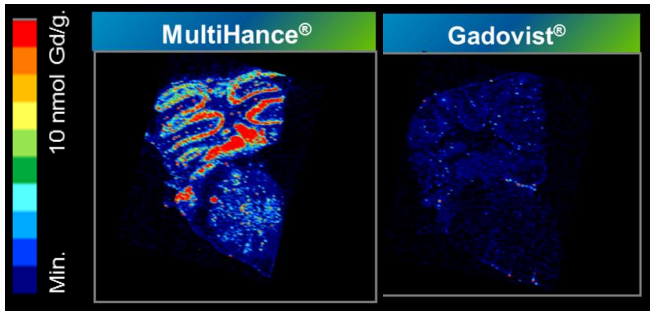
Invest Radiol. 2017 Jan 25. doi: 10.1097/RLI.0000000000000352.

Long term study of gadolinium presence Gd-distribution (Laser Ablation – ICP-MS)

Methodology: 1.) MRI 2.) Microscopy 3.) Laserablation coupled plasma mass spectrometry (ICP-MS)







Parameters:
Histological slice: 30 µm
Laser energy: 29%
Laser spot size: 60 µm Ø
Scan speed: 70 µm/s
No of lines: ca. 100, measurements per line: ca. 1000
pixel size: ca. 90 x 90 µm

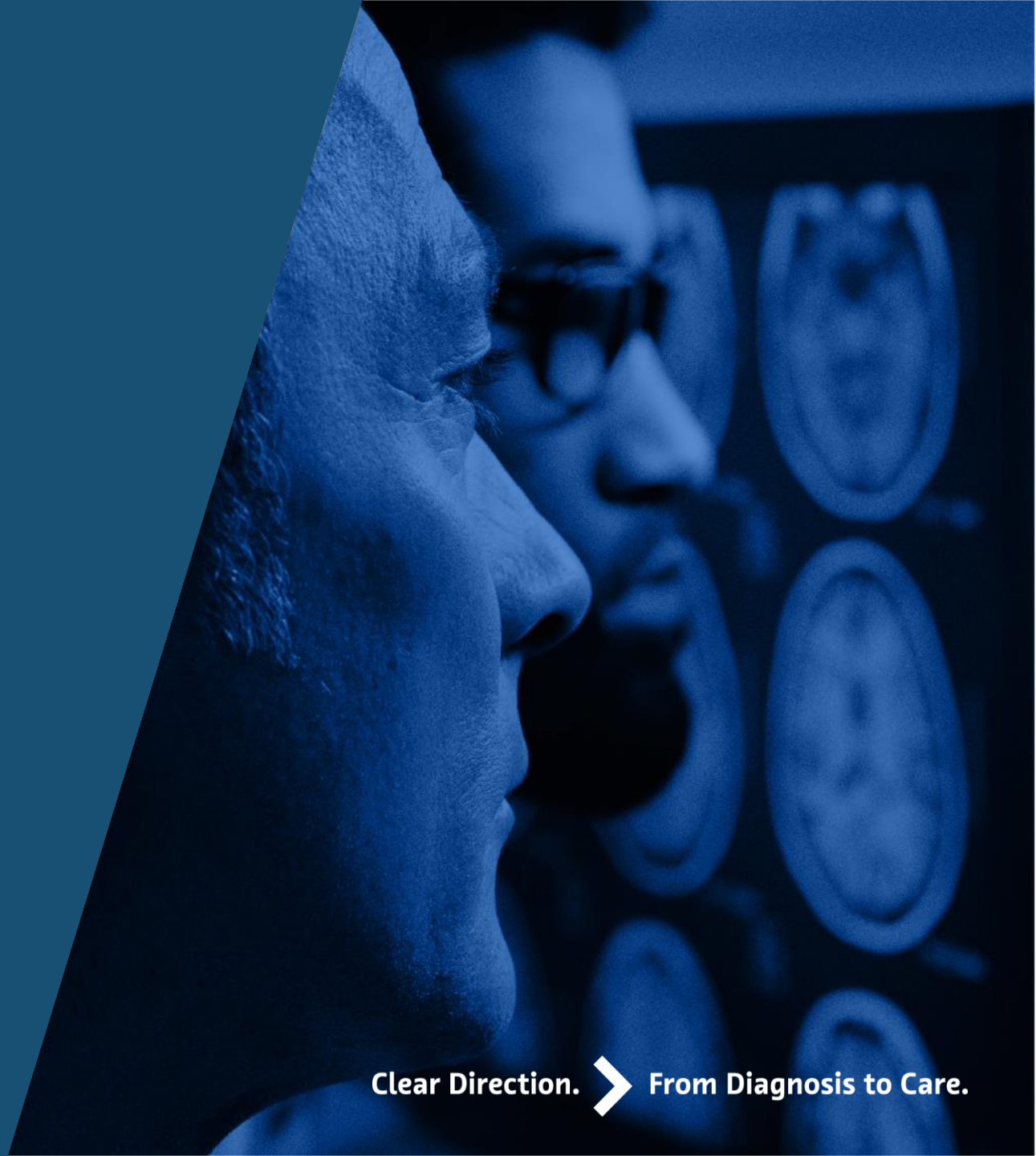
- Genuine speciation by molecular mass (mass/charge)
- Speciation maps possible
- Sample preparation involves chemical treatment, variable volatilization of species in sample & in mass spec
- Expensive equipment, experts, and is time consuming

What we could know using these methods:

- The nature of the Gd-binding macromolecules (protein, carbohydrate, pigments etc.)
- Is the Gd^{3+}ion binding to the macromolecule (most likely) or the intact GBCA (less likely, since they all do not bind to plasma proteins) – <u>isolate protein, separate Gd species, and identify.</u>
- The insoluble fraction is probably very inhomogeneous and may contain diverse species:
  - Inorganic Gd (as phosphate or hydroxide) which can be detected as dense Gd clusters in EM-EDX, co-localized with phosphorus
  - Gd^{3+} ion or GBCA entrapped in membrane vesicles or bound to insoluble tissue components, such as lipophilic macromolecules (neuromelanin or other pigments)
  - This fraction will be very difficult to analyze and characterize but contains about 50% of the Gd from linear GBCAs
- Comparison of the Gd-level found in brain with other environmental problematic elements, Cd, Al or Pb in brain.
- Speciation and trafficking of GBCA and metabolites over time / tissues

Thank you!

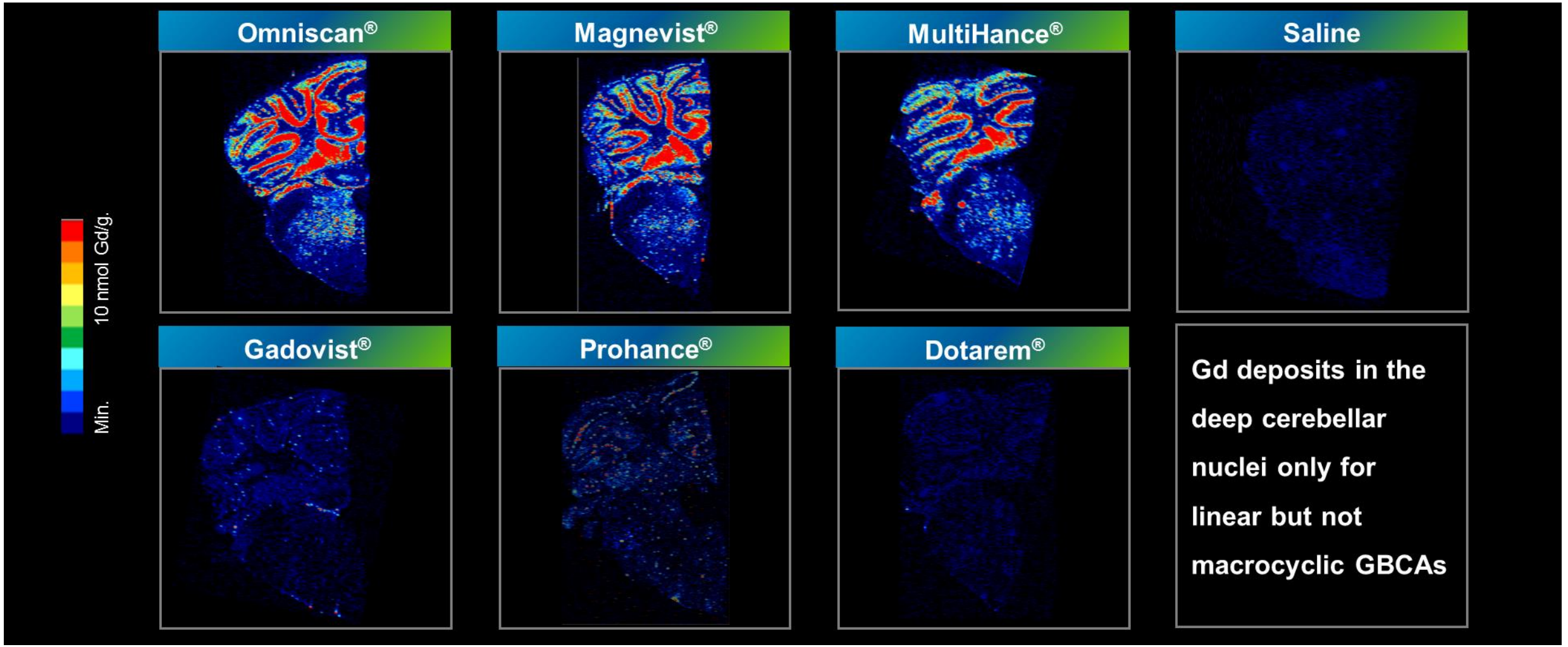
Bye-Bye



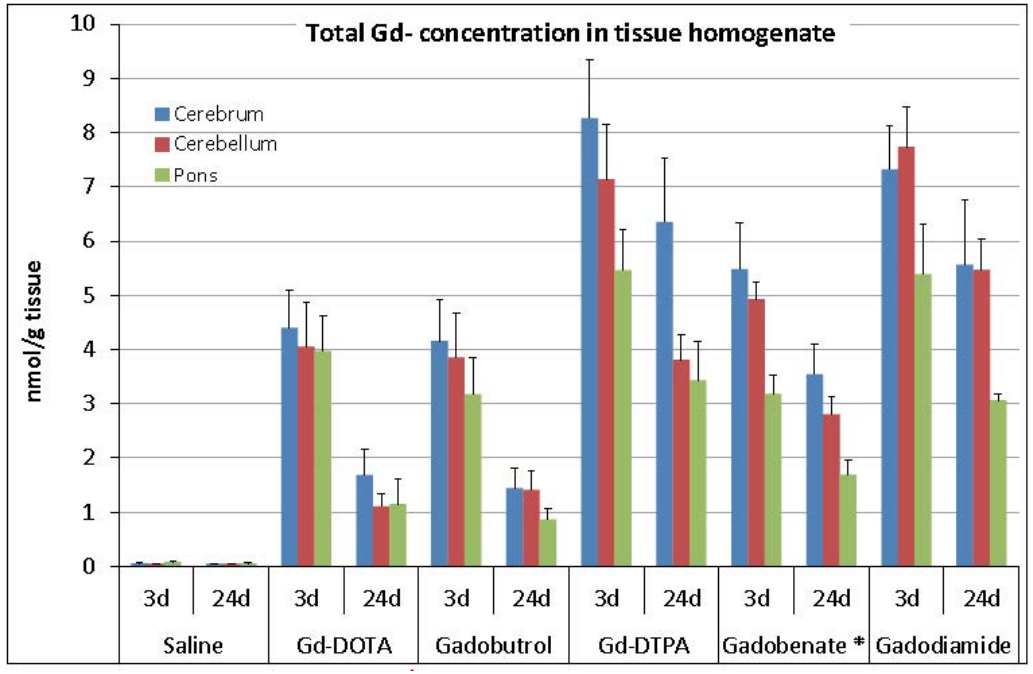
What we know about speciation:

- Gd from linear agents has been found in three different groups of chemical species
  - A soluble fraction of low molecular weight (intact GBCA) which is also excreted from brain slowly
  - A soluble fraction, bound to macromolecules, which seems to stay in the tissue for a long period of time
  - An insoluble not well characterized fraction which remains in the tissue for a long period of time
- **The total Gd tissue conc is too low to generate a visible MR signal (< 20 µmol Gd/L), if it is only the intact GBCA - > Gd species with high relaxivity must be present**
- **LA-ICP-MS demonstrated widespread presence of Gd in brain, e.g. in the granular layer and (unpublished results) other parts of the cortex of cerebellum . These areas are not visible in MRI -> These Gd species do not have an influence on T1 -> different Gd species**

Gd Deposits in the Rat Brain:
Laser Ablation coupled with ICP-MS of the Brain 52 weeks p.i.



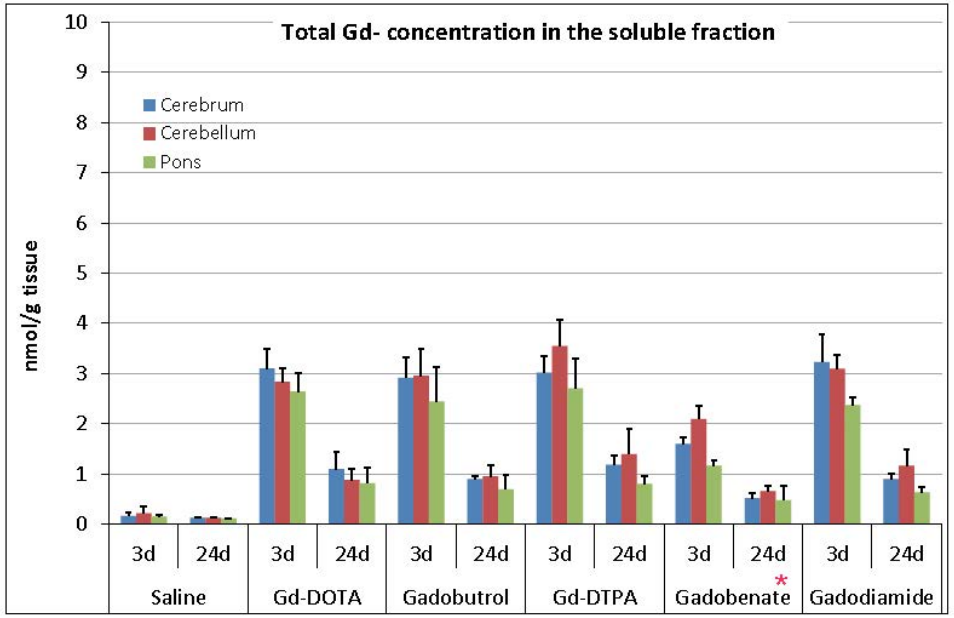
Brain Tissue Fractionation and Separation

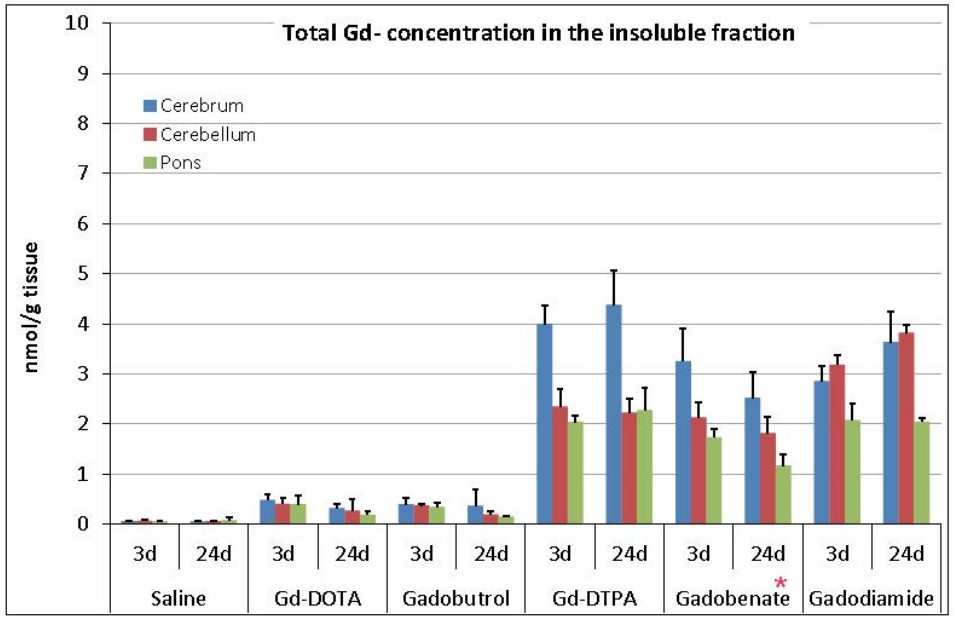


* faster elimination of gadobenate due to additional hepatobiliary excretion of ~50% in rats, which accounts for only 3% to 5% in humans.

- Total Gd concentration in brain was very low (~0.0005% id), but higher for linear than for macrocyclic GBCAs.
- No prominent difference between ionic and non-ionic linear GBCAs.
- Wash out between days 3 and 24 p.i was more pronounced for macrocyclic GBCAs (-62 to -72%) than for linear GBCAs (-23 to -47%).
- Differences between brain sections were minimal, with a trend for lower concentrations in pons

Brain Tissue Fractionation and Separation





* faster elimination of gadobenate due to additional hepatobiliary excretion of ~50% in rats, which accounts for only 3% to 5% in humans.

- About 33-60% (3 d p.i.) and 63-83% (24 d p.i.) of the Gd from linear agents was found in insoluble components
- Basically no Gd was found in the insoluble fraction after injection of macrocyclic GBCAs (comparable to control study with spiked tissue)
- The Gd conc. of soluble components on days 3 & 24 p.i. and their elimination until day 24 p.i was similar for linear & macrocyclic agents.
- High combined recovery of Gd from both fractions (87 ± 12%), similar to the control study (94-96%)