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Gadolinium Based Contrast Agents (GBCAs)

Medical Imaging Drugs Advisory Committee (MIDAC)

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LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AUC	Area Under the Curve
BBB	Blood-Brain Barrier
BW	Body Weight
CCPD	Continuous Cycling Peritoneal Dialysis
CE-MRA	Contrast-Enhanced Magnetic Resonance Angiography
CKD	Chronic Kidney Disease
CN/Pons	(Deep) Cerebellar Nuclei to Pons Ratio
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CT	Computed Tomography
CVVHD	Continuous Veno-Venous Hemodialysis
DARRTS	FDA Document Archiving, Reporting, and Regulatory Tracking System
DCN	Deep Cerebellar Nuclei
DN	Dentate Nucleus
EF	Executive Functioning
ESRD	End Stage Renal Disease
EU	European Union
FAERS	FDA's Adverse Event Reporting System
FDA	Food and Drug Administration
FLAIR	Fluid-Attenuated Inversion Recovery
FPFV	First patient first visit
Gd	Gadolinium
GBCA	Gadolinium-Based Contrast Agent
GFR	Glomerular Filtration Rate
GP	Globus Pallidus
GPC	Gel Permeation Chromatography
HD	Hemodialysis
HPLC	High-Performance Liquid Chromatography
ICP	Inductively Coupled Plasma
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICSRs	Individual Case Safety Reports
ID/G	Injected Dose per Gram of Tissue
IPMN	Intraductal papillary mucinous neoplasm
i.v.	Intravenous
KTP	Kidney Transplant
LA-ICP-MS	Laser Ablation ICP-MS
MAH	Marketing Authorization Holders
MCP	Middle Cerebellar Peduncle
MedDRA	Medical Dictionary for Regulatory Activities
mLs	Mililiters
MR	Magnetic Resonance

MRA	Magnetic Resonance Angiography
MRI	Magnetic Resonance Imaging
Multi-purpose linear	Linear GBCA that is approved for multiple imaging indications
MS	Multiple Sclerosis
NSF	Nephrogenic Systemic Fibrosis
PBRER	Periodic Benefit-Risk Evaluation Report
PD	Peritoneal Dialysis
p.i.	Post Injection
PI	Product Information / Package Insert
PK	Pharmacokinetic
PRAC	Pharmacovigilance Risk Assessment Committee
PRBC	Packed Red Blood Cells
PT	Preferred Term
PV	Pharmacovigilance
R	Relaxivity
ROI	Region of Interest
ROM	Range of Motion
SAS	Subarachnoid Spaces
SI	Signal Intensity
SII	Signal Intensity Index
MSQ	Standardized MedDRA Query
SOC	System Organ Class
T1w	T1 Weighted
TEM	Transmission Electron Microscopy
TSI	Tracked Safety Issue

NOTE: In this document certain proprietary names are used interchangeably (e.g. Gadavist® (US) or Gadovist® (outside of the US) and Eovist® (US) and Primovist® (outside of the US).

EXECUTIVE SUMMARY

Since their introduction almost 30 years ago and after more than 450 million administrations worldwide, gadolinium-based contrast agents (GBCAs) have been established as a crucial element in transforming Magnetic Resonance Imaging (MRI) into a high performance diagnostic test with beneficial and even life-saving diagnostic capabilities. Overall, GBCAs have a favorable safety profile with a very low rate of adverse events and a high therapeutic index[1].

In 2014, it was first reported that repeated administrations of GBCAs were associated with an increase in signal intensity (SI) within the brain. Since then, numerous additional studies have been conducted on this topic. Based on a compilation of the scientific data as well as original research, Bayer acknowledges that the clinical significance of these findings, if any, remains unknown. Bayer has been conducting and will continue to undertake studies needed to shed light on this question.

On May 22, 2017, the U.S. Food and Drug Administration (FDA) announced a public hearing to discuss Gadolinium (Gd) presence in the brain and body. The meeting provides an opportunity to clarify the existing knowledge base, particularly in light of some aspects being discussed controversially, identify the current gaps in understanding, and set future directions for investigations. Bayer looks forward to the Committee's suggestions, as it continues its investigation of this question.

While the clinical relevance of Gd presence in the brain and body is the ultimate question to be answered, and remains unanswered to date, Bayer has sought to thoroughly investigate these findings in animal models first in order to elucidate the underlying mechanisms that may better predict any clinical significance and further guide clinical research.

To date, animal models demonstrate:

- All GBCAs enter the brain, most likely via the choroid plexus
- Increased SI in the brain can be visualized after multiple administrations of all linear GBCAs, while no visible SI has been confirmed with any macrocyclic GBCA
- Trace concentrations of Gd have been measured in the dentate nucleus (DN) and globus pallidus (GP) of rat brains after multiple doses of multi-purpose linear GBCAs, and to a lesser extent, after macrocyclic GBCAs
    - All multi-purpose linear GBCAs show comparable Gd concentrations and distributions in the brain, including specific localization in the deep cerebellar nuclei and granular layer
    - Gd concentration in the brain is lower by a factor of about 100 compared to skin
    - Gd measurements in other organs are currently underway

[1] Therapeutic index (TI) (also referred to as therapeutic ratio) is a comparison of the amount of an agent that causes the diagnostic effect to the amount that causes toxicity.

- With all GBCAs, almost the entire injected dose is eliminated within 24 hours. However, a small amount (0.0004% linear and 0.00001% macrocyclic) may remain in the brain for at least one year.
- Macrocyclic GBCAs appear to be slowly but continuously eliminated from the brain to the limits of detection within one year. Multi-purpose linear GBCAs are not eliminated from the brain during the observation period of one year.
- All multi-purpose linear agents appear to release a small portion of Gd from the intact chelate which may then bind to soluble macromolecules and insoluble complexes. Macrocyclic GBCAs do not appear to dissociate or bind to macromolecules.
    - Control experiments indicate that intact Gd chelate does not bind to macromolecules; Gd-macromolecule formation therefore necessitates that de-chelation of linear GBCAs occurs.
- The release and binding of Gd to macromolecules correlates to the stability of the respective agent.
- No histopathological changes have been observed in the rat brain tissues after repeated administration of any of the GBCAs.

The available clinical imaging studies are consistent with these non-clinical findings.

- SI increase in the DN and GP is seen after approximately five or more administrations of multi-purpose linear GBCAs
- For the liver specific GBCA Eovist, which is injected at one quarter the dose of other GBCAs, the SI increase can only be seen after about 18-20 administrations
- No visible SI increase can be confirmed after repeated administration of macrocyclic GBCAs
- The likely reason for the SI increase is the partial de-chelation which appears to only occur with linear GBCAs
- No adverse health effects have yet to be associated with these findings

Bayer's Pharmacovigilance (PV) surveillance activities include quantitative and qualitative review and analysis concerning Bayer's GBCAs as well as other relevant patient groups represented by Bayer's large product portfolio. This includes a large number of multiple sclerosis (MS) patients receiving Betaseron.

After 30 years of clinical experience with GBCAs, and recent intensive scrutiny on the topic, to date there has been no confirmation of clinical symptoms or adverse events associated with the presence of small amounts of Gd in the brain or body other than NSF in severely renally-impaired patients. However, since such data are inherently limited, a number of open questions remain and will guide the direction of Bayer's continuing and future research.

This research will include:

- Additional non-clinical studies
- Population-based and clinical studies. For example, it may be possible to perform large screening population-based studies using large longitudinal healthcare databases, evaluating for any clinical signal detection in patients with multiple GBCA exposures, compared to unexposed controls. The retrospective population studies could also include specific a priori hypothesis testing such as evaluation for conditions such as fibrosis and arthritis in exposed patients. Bayer is currently evaluating these options.
    - In a second step any potential findings identified in those screening studies would need to be confirmed in a specifically designed study.

Bayer continues to provide appropriate communication to healthcare professionals as information evolves. As part of this ongoing process, label updates are proposed for the different classes of GBCA, i.e. multi-purpose linear, liver-specific and macrocyclic GBCAs.

Bayer believes that scientific and medical evidence to date continues to support a favorable benefit-risk profile for all of Bayer's GBCAs (and all GBCAs in general) in the vast majority of patients, in accordance with the product information. Bayer is committed to continue the appropriate and necessary non-clinical and clinical research in cooperation with health authorities to address open and relevant questions.

1. Background

To date, approximately 450 million administrations of GBCAs have been given to patients worldwide in order to support the diagnostic performance of magnetic resonance imaging (MRI). In the US, nine GBCAs have been approved (gadopentetate dimeglumine (Magnevist®), gadodiamide (Omniscan™), gadobenate dimeglumine (MultiHance®), gadoversetamide (OptiMARK®), gadofosveset trisodium (Ablavar®), gadobutrol (Gadavist®), gadoterate meglumine (Dotarem®), gadoteridol (ProHance®) and gadoxetic acid (Eovist®).

GBCAs have become an essential part of the diagnostic routine and have been recognized as having an exceptionally low frequency of adverse drug reactions. According to the American College of Radiology (ACR), the overall adverse event rate for GBCAs ranges from 0.07% to 2.4%, with most reactions mild and physiologic. Allergic like reactions are uncommon; severe life-threatening anaphylactic reactions are exceedingly rare (0.001% to 0.01%). Overall, all GBCAs are considered to have an overall favorable safety profile (ACR, 2017).

Since the introduction of GBCAs, three issues have arisen in which the stability of the different sub-classes of agents has been a factor:

- In approximately 2003, the non-ionic linear agents Omniscan and OptiMARK were found to interfere with colorimetric serum calcium measurements, resulting in a risk to patients of being inappropriately treated with intravenous calcium supplementation. It was found that the release of Gd from these two products with the lowest stability among all GBCAs resulted in competitive binding with Ca2+ for the colorimetric substrate of the test under *in vivo* conditions suggesting spurious hypocalcemia **(Prince, 2003; Prince, 2004; Lowe, 2005**). The more stable ionic linear GBCAs (Magnevist and MultiHance) showed no interference with colorimetric methods for calcium determination and neither did the macrocyclic agents. Inclusion in the label of the non-ionic linear GBCAs and educational efforts were successful in reducing this risk.

- In 2006 nephrogenic systemic fibrosis (NSF) was described for the first time in association with GBCAs in patients with severe renal impairment. Stability again appeared to be one of the factors in triggering NSF, with the non-ionic linear agents having the highest propensity to release Gd, followed by the ionic linear GBCAs and lastly the kinetically inert macrocyclic GBCAs. Identification of the population at risk, labeling changes, changes in clinical practice including the elimination of high and repeated dosing in at-risk populations, and educational efforts have been successful in all but eliminating new cases of NSF.

- In 2014, the topic of SI increase and presence of Gd in the brain again brought the role of GBCA stability into focus. The majority of imaging data suggest that SI increase in certain brain areas is primarily associated with linear GBCAs. Bayer's non-clinical research provides evidence for some release of Gd from the linear molecules and subsequent binding of that Gd to macro-molecular structures, which leads to a higher relaxivity of these new formations and is the likely reason for the visual SI on MR images. This binding of Gd to macromolecules has not been observed with the more stable macrocyclic GBCAs. Therefore, these traces of Gd in the brain are not sufficient to create a signal on MR

images for the macrocyclic GBCAs. However, and very different from NSF there are to date, no studies that confirm an association between the observed increase in SI with repeated GBCA administrations and any histopathological changes in brain tissue or with any clinical adverse events.

This document provides an overview of

- Available clinical data
- Bayer's non-clinical research activities
- Bayer's PV data
- Considerations about future clinical studies
- Suggestions for label changes and risk mitigation

2. Status of scientific clinical knowledge regarding signal intensity (SI) increase and presence of gadolinium (Gd) in the brain and in the body

2.1 Signal intensity increase in the brain

2.1.1 Overall results on published clinical imaging and post mortem studies

Signal intensity (SI) increase on T1-weighted (T1w) unenhanced MR images in the basal ganglia of the brain and the presence of Gd* in these regions after repeated administrations of GBCAs has been a topic of research and scientific discussion for more than three years. A number of publications have emerged during this time mainly describing retrospective, single-center trials reporting the effect of various GBCAs on the signal intensity on non-contrast T1w images in the brain, and Bayer is actively researching this topic. The publications related to imaging findings reporting SI increase data on the following GBCAs: Gadopentetate dimeglumine (Magnevist), gadodiamide (Omniscan), gadobenate dimeglumine (MultiHance), gadobutrol (Gadavist), gadoterate meglumine (Dotarem), gadoteridol (ProHance) and gadoxetic acid (Eovist). Only a very few articles describe actual measurements of Gd concentration in brain tissue (in patients who received Omniscan, Magnevist, ProHance, Eovist, and Gadavist) for a single patient or for very small numbers of patients (**McDonald, 2015; Murata, 2016; Kanda, 2015a; Roberts, 2017; McDonald RJ, 2017**).

The published clinical and non-clinical studies to date provide evidence of the following:

- No visual SI increase is observed after multiple administrations of macrocyclic GBCAs (Dotarem, Gadavist, ProHance) (**Schlemm, 2016; Radbruch, 2016; Radbruch, 2017a; Radbruch, 2017b; Eisele, 2016; Bae, 2017; Langner, 2017; Tibussek, 2017.**
- Pediatric studies (**Radbruch, 2017b; Flood, 2017; Hu, 2016**) with standard dose (0.1 mmol/kg/bw) administered had similar results as in adults
- When exposed to a very high number (35 or more) of administrations of multi-purpose linear GBCAs the SI increase according to one publication (**Zhang, 2017**) can also be observed in other brain areas besides the DN and GP (e.g. posterior thalamus, substantia nigra, red nucleus, cerebellar peduncle, colliculi) whereas in patients who received 20 or more macrocyclic GBCA administrations no SI increase was seen in any area of the brain (**Radbruch, 2017a**).

*Since the terms accumulation, retention and deposition imply a somewhat permanent situation (which is not scientifically confirmed), at this point in time Bayer would prefer to use "presence of gadolinium (Gd)" as a more accurate descriptor of the current state of scientific understanding.

- For the liver-specific linear agent Eovist such a SI increase has been reported in only one out of three recently published articles (**Kahn, 2017**; **Ichikawa, 2017; Conte, 2017**) and only after a significantly higher number of administrations (11-37) whereas in the other two publications no SI increase is seen after up to 15 or 18 administrations (**Ichikawa, 2017**; **Conte, 2017**).
    - The finding that an increased SI becomes visible for Eovist only after a higher number of administrations is not unexpected given that Eovist has a higher stability than all other linear GBCAs (**Frenzel, 2008**), is administered at a quarter of the dose of multi-purpose linear agents, and has a unique dual elimination pathway (50% renal, 50% hepatobiliary). All these features together lower the systemic Gd burden and thus the potential for Gd presence in the brain substantially when compared to all multi-purpose linear GBCAs.
- The SI increase or the presence of Gd in the brain is not limited to patients with impaired kidney function, which is an important differentiation from what has been observed with NSF. However, earlier appearance of a SI increase after multi-purpose linear GBCAs is seen in renally impaired patients (**Cao, 2016**).
- The influence of radiation therapy and chemotherapy on the observed SI and specifically if there is any additive affect is subject to ongoing clinical evaluations. Initial data suggest that such additive effect may exists (**Kasahara, 2011**; Kinner 2016 conference report at ISMRM).
- Some post-mortem (6 in total) studies provide explorative data on the presence of Gd in some brain areas as well as bone tissue (**McDonald, 2015; Murata, 2016; Kanda, 2015a; Roberts, 2017**; **McDonald RJ, 2017; McDonald JS, 2017**). Murata et al. reported on the presence of Gd traces from macrocyclic as well as linear GBCAs Gd in the brain areas and also bone tissue. Gd concentrations in bone tissue from all nine patients involved in this study were several times higher than brain Gd levels after the administration of linear and macrocyclic agents (**Murata, 2016**).
- Despite differences in SI, no histopathological changes to brain tissue and no adverse health effects have been confirmed to be associated with these findings.

2.2 Gadolinium presence in other tissues (e.g. skin, bone, other)

2.2.1 Introduction

Currently the understanding on Gd presence in other areas of body e.g. skin and bone is limited. There are no published data from prospective studies investigating the overall Gd presence in other tissues with the exception of studies for NSF, a specific clinical and histopathological condition, which has been associated with the stability of various GBCAs. Bayer addresses this field in its non-clinical research and is currently engaged in a collaborative (with GE HealthCare and Guerbet) study on the potential for long-term Gd presence in bone and skin required as a result of the EU Article 31 referral procedure about NSF. The following sections discuss the available literature as well as the post-marketing study.

2.2.2 Published literature on Gd presence in the body (organs, bone, skin)

Animal studies have indicated that Gd can persist for long periods of time, especially in the bone. Gd is not a naturally occurring biologic constituent and, once within the tissues of animals it might remain over time. The structure of the Gd chelate in particular determines the stability of the metal ligand and, in turn, is related to the propensity to release Gd *in vivo*. Numerous *in vitro* studies have indicated differences in Gd chelate affinities and in Gd chelate kinetic stabilities. These can be affected by a variety of factors, including the pH, the availability of other metal ions that compete with the Gd on the chelate, and the structure of the chelate.

In 2004, Gibby et al. reported on a study in human bone tissue following administration of a clinical dose of Gd chelate (0.1 mmol per kg) to patients undergoing hip joint replacement surgery to determine if measurable differences in Gd presence occur between two widely available magnetic resonance contrast agents (**Gibby, 2004**). Patient groups were compared after ProHance and after Omniscan and an age-matched control population without history of Gd administration. Bone samples were collected and analyzed by inductivity coupled plasma atomic emission spectroscopy (ICP-AES). Tissue retention was 1.18 ± .787 µg Gd/g bone (n = 10) for Omniscan and 0.466 ± .387 µg Gd/g bone (n = 8) for ProHance measured by ICP-AES. For Omniscan 2.5 times more Gd in bone was measured than for ProHance. The tissue samples in this study were all collected 3-8 days after the administration of either Omniscan or ProHance. With such a short interval between exposure and tissue collection one cannot reach any conclusions on any permanent retention. Moreover, differences between the products or classes of products cannot be concluded solely based on these data as it is to date still unknown if the agents are continuously (though slowly) eliminated and if the elimination rate and pace is the same for all the GBCAs or not. Furthermore, the study does not provide data on the molecular form of the Gd found. As far as prior exposure to Gd is concerned the authors describe that at least for the control group no such prior exposure was known, however, for the treatment groups they could not exclude the possibility of prior exposure for all patients.

Darrah et al. (2009) investigated the Gd concentrations in trabecular and cortical bone tissues in 13 patients who underwent total hip replacement surgery and had received GBCAs. 18 patients who had not received GBCAs served as controls to establish the "natural background level" of Gd and other rare earth elements in human bone (**Darrah, 2009**). Gd and other rare earth elements were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Of the 13 patients in the Gd-exposed group, 12 had anomalously high bone concentrations of Gd with respect to the other rare earth elements. Gd concentrations in the exposed group ranged up to 31 nmol/g in cortical bone and up to 39.51 nmol/g in trabecular tissues and were significantly higher (up to 4,800 times greater) than in the controls, indicating to the authors that a small percentage of the administered dose (they estimate < 0.3%) is not eliminated and may be incorporated into human bone. Concentrations and anomalies had no correlation to the time elapsed between Gd exposure and surgery, which ranged up to 8 years. No difference was observed in bone Gd concentrations and anomalies between patients dosed with Omniscan (n = 6) and ProHance (n = 5). Osteoporotic fracture patients exposed to Gd had significantly lower Gd concentrations than osteoarthritis patients. Authors concluded that this suggests different mechanisms of metal incorporation and/or retention in osteoporotic bone tissues, and may signal an increased risk of endogenous Gd release for patients with increased rates of bone resorption

(e.g. osteoporosis patients and menopausal, pregnant, and lactating women) who are exposed to GBCAs.

In summary, it appears that traces of Gd can be found in other areas of the body such as bone and skin. Any relative comparison between the various GBCAs, in particular any quantitative conclusion, is not reliable based on the existing data. There is, however, evidence that even years after GBCA exposure, traces of Gd can be measured in skin and bone, which is suggestive of a very long storage in certain tissue compartments.

In 2016, one postmortem study by **Murata et al. (2016**) provided explorative data on the presence of Gd in some brain areas as well as bone tissue. Tissue samples of nine patients who received one or more injections of a single type (linear or macrocyclic) of GBCA (five patients received macrocyclic ProHance, two received macrocyclic Gadavist, and one each received the linear GBCAs MultiHance and Primovist). Decedents with only non-contrast MRI or no MRI served as controls. Multiple brain areas, including GP and DN, as well as bone (from 8 patients) and skin (from 3 patients), were sampled and analyzed for Gd using ICP-MS (**Murata, 2016**).

Gd concentrations measured in bone were approximately 23 times higher (median) than those measured in the brain ($P = 0.008$ for bone vs GP) and showed a significant correlation ($r = 0.81$, $P = 0.022$). In controls, Gd levels in the brain were at or below limits of measurement and were significantly lower compared with study cases ($P = 0.005$ for GP). The authors concluded that Gd levels in normal brain and bone tissue occur with macrocyclic and linear agents in patients with normal renal function. Presence of Gd in cortical bone occurs at much higher levels compared with brain tissue and shows a notable correlation between the two.

- Limitations as acknowledged by the authors are:
    - The small sample size.
    - The varying time intervals between GBCA administration and tissue sampling, ranging from 5 days to more than two years in this study, clearly limit any quantitative comparison. A time interval of at least 6-8 weeks between last GBCA administration and tissue sampling is recommended (**Pietsch, 2009a; Pietsch, 2009b**).
    - The lack of sufficient control for confounding factors especially prior exposure to other GBCAs.
    - One patient had a higher Gd amount in the bone but also had a greater impairment of renal function than all other patients.

2.2.3 Ongoing Study of Gadolinium Retention in Bone and Skin ("Bone Study")

As a result of the Article 31 procedure concluded on July 01, 2010, Bayer and other marketing authorization holders (MAHs) of GBCAs were asked to conduct a study of the potential for long-term retention of Gd in human bone and skin. This study (Study No. ALS-Gd64/001/ EudraCT No: 2012-001439-30) is ongoing in collaboration with G.E. HealthCare and Guerbet. The products involved are Magnevist, Gadovist, Primovist, Omniscan, OptiMARK and Dotarem.

The primary objective of the study:

- Prospectively explore the potential for long-term retention of Gd in the bones of patients with moderate or severe renal impairment or stable renal function (eGFR >60 ml/min/1.73 m^2) at the time of GBCA injection who have received a single dose of a GBCA or multiple doses of the same GBCA.

Secondary objectives of the study:

- To evaluate skin samples for the concentration of Gd
- To evaluate bone and skin samples for the concentrations of calcium, phosphorus, sodium, iron, zinc, and potassium
- To evaluate skin samples for any dermatopathological changes that may be associated with NSF
- To describe potential co-factors for NSF, susceptibility factors, and drug treatments with potential impact on bone metabolism

The first site initiation took place on April 2013; first patient first visit (FPFV) occurred in May 2013. As of April 2017, 29 study sites have been initiated; 16 of these sites are open for recruitment. A total of 94 subjects have been recruited so far, of which 75 have been stratified. To date safety assessments in this study did not give rise to any concerns. No reports of NSF have been received from this ongoing study. According to the revised estimated study timelines the submission of the final study report is expected by the 2nd quarter of 2018.

Bayer's comment: *The study has experienced slow recruitment for a number of reasons. First, the combination of contrast enhanced MR in a population undergoing hip surgery is not that frequent. Secondly, the study aims to quantify concentrations of gadolinium in products which the Health Authority has contraindicated in renally impaired patients until an amendment was put in effect which removed that requirement recently.*

Additionally, the study has a possibility of generating unreliable, random results, since patient history (including prior GBCA exposure) may be unreliable.

The time interval between administration and tissue sampling has been reduced from 6 to 4 weeks to facilitate recruitment. Besides that, it did not have an effect on recruitment yet, the non-standardized time interval is also a factor that is likely to make reliable quantitative comparisons on tissue concentration of Gd impossible.

2.2.4 NSF

With NSF, a specific clinical and histopathological condition has been associated with the stability of the various GBCAs. It is important to note that the exact mechanism and in particular other co-factors that are likely needed to trigger NSF are still unknown.

Worldwide regulatory authorities issued warnings and /or defined risk categories for GBCA related to NSF resulting in contraindications for use in patients with severe renal impairment for the two non-ionic linear GBCAs Omniscan and OptiMark as well as for the ionic linear agent Magnevist, primarily based on the absolute number or reports (not ratios relative to utilization) and in particular the number of "unconfounded" or "single agent" reports. For all other agents, a box warning in the US is in place. While the regulatory/labeling and further educational efforts

apparently were effective in containing and almost eliminating the clinical occurrence of NSF the risk classifications and selection of GBCAs being contraindicated for certain patient groups however could be challenged because using "numbers" of reports or number of "single agent reports" as the primary criterion for risk may result in an inaccurate risk assessment for many reasons further discussed in **Section 4.3** Nephrogenic Systemic Fibrosis

2.2.5 Study limitations / confounders / technical parameters

The more than 35 clinical imaging studies addressing SI increase in the brain as well as the five post-mortem studies measuring Gd in brain tissue provide interesting aspects and insights but all also have certain limitations. Those limitations or confounding factors need to be recognized when interpreting the data as some of them are more relevant than others.

All studies have in common that they are retrospective. This per se is not necessarily a major concern, but the retrospective nature of the trials is associated with some more or less inevitable other potential problems that may have an effect on the robustness of the data.

- Among the most relevant problems are the often-existing lack of control and/or knowledge of administration of GBCAs prior to the published study period. This may include an unknown prior exposure of the same or a different GBCA or different class of GBCA. This is particularly problematic for post-mortem studies. As long as we do not have a full understanding if the Gd persists in brain tissue for some or all GBCAs and as long as there is not a way to identify from which GBCA the Gd originated every prior administration may have an effect on the result and potentially lead to erroneous conclusions. Especially any quantitative comparative conclusions have to be made with extreme caution.

  Although the same problem exists also for the imaging studies it is less of a concern because in order to reach the threshold to cause a visible signal increase several administrations are needed.

- Another frequent and relevant problem is the lack of standardization in those retrospective studies. Especially the use of different scanners, including different field strengths between scans (baseline and final) as well as varying MR sequence parameters needs to be considered.

  - Significant variation of MR sequences between baseline and final scan limit the comparability between different time points. For example, the difference in sequence parameters potentially obscures an already existing SI at baseline while it becomes visible later simply because of that variation in sequences and/or sequence parameters and is then interpreted as a drug-related effect

- A related but slightly different problem is the lack of standardization as far as the Region of Interest (ROI) setting and positioning is concerned. Especially in studies claiming a SI that can be measured but is not visible on the MR scan this might be sufficient to explain the findings.

- Specifically, in post-mortem studies measuring Gd in tissue, a sufficient time-interval between GBCA administration and the analysis of Gd in tissue (at least 6 weeks) (**Pietsch, 2009a**; **Pietsch, 2009b**) is needed. Since the initial research for NSF it is known that during those at least 6 weeks equilibrium has not been reached and Gd can be

measured for all GBCAs in tissue. In order to do any quantitative comparisons this interval should also be comparable.

  - In the publication by Murata for example the time interval between administration and tissue sampling ranges from 5 days to more than 2 years (**Murata, 2016**).
  - **Gibby (2004)** reports a time interval of about 1 week only between GBCA administration and tissue sampling.

- Lack of (an appropriate and matched) control group is a frequent limitation in the published retrospective data

The statistical approaches are not always appropriate to demonstrate or exclude an effect. For example, if in a population more than 80% of the patients receive 2 or 3 administrations of a linear GBCA and only a few patients receive more than 5 or 6 injections (which appears to be the necessary threshold to lead to a visible effect) the presentation of results as statistical mean across all patients is likely to obscure an effect, even if it exists (**Ramalho, 2015; Ramalho, 2016).** There is an ongoing debate regarding the question of SI also after repeated administration of macrocyclic GBCAs. The data are limited (two publications and unpublished data presented at scientific conferences) but can serve as examples to illustrate the important role that confounding plays in contributing to misleading results

- The study by **Stojanov** (**2016a**) has been disputed and publicly discussed (**Agris, 2016**). **Stojanov** (**2016a**) and the new study by **Rossi-Espagnet** (**2017**) report a measured SI increase after macrocyclic GBCA exposure, but do not show any visual effect of SI increase. First of all, the lack of standardized ROI placement needs to be considered and raises concern about the robustness of the measurements as a true effect. As with many other studies, it cannot be ruled out that some patients had previously received administrations of linear GBCAs, which is has been acknowledged by **Stojanov** (**2016b**). With the exception of those two studies all other studies showed no increased T1w SI after repeated administration of macrocyclic GBCAs (**Kanda, 2015b; Radbruch, 2015; Cao, 2016; Radbruch, 2016; Eisele, 2016; Schlemm, 2016; Radbruch, 2017a; Radbruch, 2017b; Bae, 2017; Langner, 2017; Tibussek, 2017; Müller, 2017; Yoo, 2017**).
- Another recent report presented during the ECR 2017 (J Moreno) illustrates the importance of patient history and standardization. The initial presentation of their data during a smaller meeting (EGREC) claimed a SI increase after studying 129 patients with exposure to a macrocyclic GBCA. The data were questioned during the meeting and after a more careful exclusion of all patients with potential prior GBCA administration and the acknowledgment of non-comparability of sequence parameters between baseline and follow-up, eventually at ECR the authors included only 26 patients and found no significant SI increase.

Authors now are more frequently recognizing the importance of a consistent approach of imaging acquisition. The problems related to those clinical imaging studies is one of the reasons that Bayer's current focus is on non-clinical research. Non-clinical studies can provide non-confounded results to generate a better understanding of the underlying patho-mechanisms. The clinical focus is less on imaging studies. Those are considered to be more like a surrogate for changes occurring on a molecular level in the brain and are not expected to answer the question of

clinical relevance. Bayer's focus on clinical data is therefore currently on post-marketing and PV evidence, including some selected signal detection approaches.

Until today, the available data do not indicate an association of Gd presence in the brain or most other organs with clinical adverse effects. It is important to note that based on the available published literature, no specific clinical symptoms or adverse health effects has been confirmed to be associated with the reported SI increase and / or Gd presence in the brain.

3. Non-clinical research strategy – Completed, ongoing and planned studies

3.1 Introduction

In order to answer the open questions with regard to Gd presence in the brain Bayer has initiated a number of non-clinical studies, several of which are already published (**Jost, 2016a; Jost, 2016b; Lohrke, 2017; Frenzel, 2017)**. In a very first step it had to be shown that an animal model is suited to reproduce and study the imaging findings that have been observed in humans. Bayer's research approach was to use the same models that were established to study the effects and mechanisms involved in triggering NSF. Overall, results confirm most of the clinical observations and the suitability of the chosen animal models.

The focus of Bayer's non-clinical studies in rats was to explore the extent, the location and potential resulting histopathological changes as well as the time course and the molecular form of Gd present in the brain after repeated high dosages of linear and macrocyclic GBCAs.

Another study focused on the mechanisms involved for GBCAs to enter the brain. The potential influence of impaired renal function and an incompletely developed BBB are also part of the program.

In order to increase and advance the knowledge on SI increase and Gd presence in the brain, Bayer has carried out a number of additional non-clinical studies (**Jost, 2016a; Jost, 2016b; Lohrke, 2017; Frenzel, 2017**). A rat animal model has been established to investigate the presence of Gd in the brain more systematically, especially under controlled and reproducible conditions.

Bayer´s non-clinical studies have already revealed a number of important findings:

- SI increase in the cerebellar nuclei of rats up to 24 days post-injection (p.i.) was noted after repeated, high-dose administration of multi-purpose linear agents, but not with macrocyclic GBCAs.
- Traces of Gd were detected in brain tissue following repeated exposure to both multi-purpose linear and, to a lesser extent, macrocyclic GBCAs (~ 0.00004% of the injected amount of Gd in the whole brain for multi-purpose linear).
- All GBCAs enter the brain via the blood-CSF barrier to a similar and very low extent. Therefore, CSF is a potential pathway of GBCA entry into the brain.
- Administration of multi-purpose linear GBCAs leads to the formation of Gd containing macromolecular structures in the brain which explains the over proportionally high SI increase on the MR images. This is not observed for macrocyclic agents, which appear to remain as an intact GBCA molecule (i.e. Gd is completely chelated).
- Different from NSF skin lesions, no histological changes were detected in the brain after repeated, high doses of either linear or macrocyclic GBCAs.

3.2 Detailed overview of Bayer's non-clinical findings

The clinical studies on Gd presence are to some extent limited by their retrospective study design, confounding factors and the lack of access to human brain tissue. Since 2016, several non-clinical studies have been published as they allow for the control of confounding factors and may allow for better evaluation and understanding of the patho-physiological mechanisms and to potentially see symptomatology (**Jost, 2016a; Jost, 2016b; Robert, 2015; Robert, 2016; Smith, 2017; Kartamihardja, 2016; Rasschaert, 2016**).

A tabular overview of Bayer's non-clinical study program is provided in **Table 1** followed by additional details on the results of each study.

Table 1: Ongoing and published studies in rats to explore the topic of Gadolinium presence

Study No.	Study description	Investigated GBCA	Administered dose [mmol Gd/kg bw]	Repeated dose	Recovery time before analysis	Analytical methods
1.	Repeated high dose injection into healthy rats (**Jost, 2016a**) T1w brain MRI	Omniscan Magnevist MultiHance Gadovist Dotarem Saline (control) (n=10 per GBCA)	2.5 (Brain MRI)	10x, daily	2 groups: A) 3 days (n=10) and B) 24 days (n=5) after last application	- T1w whole brain MRI at baseline and 3 and 24 days after last administration at clinical 1.5T scanner - Fluid attenuated brain MRI baseline and 1 min after injection
		(n=3 per GBCA for CSF analysis)	1 (CSF analysis)	1x (CSF analysis)		

Study No.	Study description	Investigated GBCA	Administered dose [mmol Gd/kg bw]	Repeated dose	Recovery time before analysis	Analytical methods
2.	Single high dose in healthy rats (**Jost, 2016b**) • MR cisternography • Gd-concentration in CSF	Omniscan Magnevist MultiHance Gadovist Dotarem ProHance Saline (control) Gadomer (experimental CA) (n=12 per GBCA, 2 groups)	1.8	1x	2 groups: A) MRI (0-240 min) CSF Gd-conc. (260 min) B) CSF Gd-conc. (24h)	- Fluid attenuated brain MRI (baseline, 9, 25 and 240 min p.i.) - analysis of the Gd concentration (260 min and 24 h after injection) in the CSF using ICP-MS
3.	Repeated high dose in healthy rats (**Lohrke, 2017**) • Tissue Gd concentration and distribution	Omniscan Magnevist ProHance Gadovist Saline (control) Untreated (n=10 per group)	2.5	20x, daily	8 weeks after last application	- Histopathology - Gd tissue concentration of brain sections by ICP-MS - tissue distribution of Gd using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) of brain cryo-sections

Study No.	Study description	Investigated GBCA	Administered dose [mmol Gd/kg bw]	Repeated dose	Recovery time before analysis	Analytical methods
4.	Repeated high dose in neonatal/ juvenile rats • Tissue Gd concentration and distribution	Omniscan Magnevist ProHance Gadovist Saline (control) (n=18 per group)	2.5	5x, daily	8 weeks after last application	- Histopathology - Gd tissue concentration of brain sections by ICP-MS - tissue distribution of Gd using LA-ICP-MS of brain cryo-sections
5.	Repeated high dose in healthy rats (**Frenzel, 2017**) • Fractionation and speciation of deposited gadolinium • (Add-on to Study No.1)	Omniscan Magnevist MultiHance Gadovist Dotarem Saline (control) (n=10 per GBCA, 2 groups)	2.5	10x, daily	2 groups: A) 3 d (n=5) and B) 24 d (n=5) after last application	- Separation of brain in cerebellum, pons and cerebrum - Separation of each tissue in insoluble, soluble and protein free fraction - Gd concentration in each fraction by ICP-MS - GPC and HPLC of soluble fractions
6.	Repeated high dose comparing healthy and renally impaired rats (5/6 nephrectomized) • Brain MRI • Tissue Gd concentration in skin and brain (cerebellum, pons, cerebrum)	Omniscan MultiHance Gadovist Dotarem Saline (control) (n=6 per GBCA, 2 groups)	1.8	8 x (4 days per week)	2 groups: A) healthy B) 5/6 nephrectomized rats	- T1 weighted whole brain MRI (clinical 1.5 T MRI) before dissection - Separation of brain in cerebellum, pons and cerebrum - Gd tissue concentration by ICP-MS

Study No.	Study description	Investigated GBCA	Administered dose [mmol Gd/kg bw]	Repeated dose	Recovery time before analysis	Analytical methods
7.	Repeated high dose in healthy rats: long term study • Brain MRI • Tissue Gd concentration	Omniscan Magnevist MultiHance Primovist Dotarem ProHance Gadovist Saline (control) (n= 6 per GBCA, 3 groups)	1.8 additional doses: MultiHance: 0.60 Primovist: 0.15	8x (4 days per week)	Group 1: 5 weeks Group 2: 26 weeks Group 3: 52 weeks	- T1 weighted whole brain MRI (clinical 1.5 T MRI) before dissection - Separation of brain tissue in cerebellum, pons and cerebrum - Gd tissue concentration by ICP-MS
8a.	Repeated dose in healthy rats: intracisternal administration • Brain MRI • Tissue Gd concentration	Omniscan Artificial CSF (n=8 per group)	0.00125 mmol per animal	2x (1 per week)	5 weeks after last application	- T1 weighted brain MRI (clinical 1.5 T MRI) at baseline and - Dissection and separation of brain in cerebellum, pons and cerebrum - Gd tissue concentration by ICP-MS
8b.	Single dose in healthy rats: intracisternal administration • Brain MRI Tissue Gd concentration	Omniscan MultiHance Gadovist Artificial CSF (n=8 per group)	0.00125 mmol per animal	1x	5 weeks after last application	- T1 weighted brain MRI (clinical 1.5 T MRI) at baseline and once per week - Dissection and separation of brain in cerebellum, pons and cerebrum - Gd tissue concentration by ICP-MS

Study No.	Study description	Investigated GBCA	Administered dose [mmol Gd/kg bw]	Repeated dose	Recovery time before analysis	Analytical methods
9.	Repeated high dose in healthy rats: Transmission electron microscopy (TEM) study (**Lohrke, 2017**)	Gadovist Magnevist Omniscan Saline (control) (n=3 per group)	2.5	20x (4 daily injection per week)	8 weeks after last application	- TEM: evaluation of the brain with focus on the globus pallidus and lateral cerebellar nucleus (which is equivalent to the dentate nucleus in humans)
10.	Repeated high dose/dose escalation in healthy rats: behavioral studies	Gadovist Omniscan	0.6 1.2 1.8	8x (4 days per week)	- 4-7 weeks - 9 weeks - 14 weeks - 30 weeks - after last application	- Behavioural tests for alterations in motor function: - SHIRPA - Open Field Test - PhenoMaster - CatWalk - Rota Rod - Behavioural tests for alterations in cognitive function: - Acoustic startle response (ASR/PPI) - Operant wall - Stair case paw reaching - Stereologic analysis of brain tissue after last behavioural examination
11a.	Repeated high dose in healthy rats: Biomarker study (long term)	Gadovist Omniscan Saline	1.8	8x (4 days per week)	- Group 1: - 4 days - Group 2: - 35 days	- Luminex Multiplex analysis: Evaluation of 32 analytes that are associated with an immune response (cytokines, chemokines) in full brain homogenates

Study No.	Study description	Investigated GBCA	Administered dose [mmol Gd/kg bw]	Repeated dose	Recovery time before analysis	Analytical methods
11b.	Single high dose in healthy rats: Biomarker study (acute)	Gadovist Omniscan Saline Naïve	1.8	Single dose	- Group 1: 6 hours - Group 2: 24 hours	- Luminex Multiplex analysis: Evaluation of 32 analytes that are associated with an immune response (cytokines, chemokines) in full brain homogenates
12.	Retrospective study of healthy mini-pigs that have been involved in several preclinical GBCA studies: Gd accumulation study in a clinical like setting	Gadovist Magnevist	0.01 – 0.32	Total accumulated dose: 7-129 mmol/animal	- 8 – 38 months	- Dissection and separation of brain in cerebellar cortex, cerebellar nucleus, cerebral cortex, globus pallidus and pons - Gd tissue concentration by ICP-MS

3.3 Body and Brain

Study 1: Repeated high GBCA dose in healthy rats (in total 60 rats)

Bayer's first brain MR imaging study by **Jost et al. (2016a**) in rats investigated the differences between the contrast agent classes (linear versus macrocyclic, ionic versus non-ionic GBCAs). In this study two different time points (3 days and 24 days) after the last GBCA administration were investigated. In a second part of the study, cerebrospinal fluid (CSF) spaces were evaluated for contrast enhancement by fluid-attenuated (FLAIR) magnetic resonance imaging (MRI).

As a result, increased SI in the deep cerebellar nuclei (which are equivalent to the DN in humans) was found up to 24 days after multiple, extended doses of linear GBCAs, confirming clinical results (**Figure 1**). The elevated SI changes remained persistent over the entire observation period (**Figure 2**). In contrast, no SI changes in either the cerebellar nuclei or the GP were observed for macrocyclic GBCAs. An additional finding was the enhancement of the CSF with all GBCAs investigated, which triggered the next study in MR Cisternography.

Figure 1: T1w MRI of the rat brain (cerebellum) before (baseline) and after (day 3 / day 24 p.i.) multiple injections of high GBCA doses.

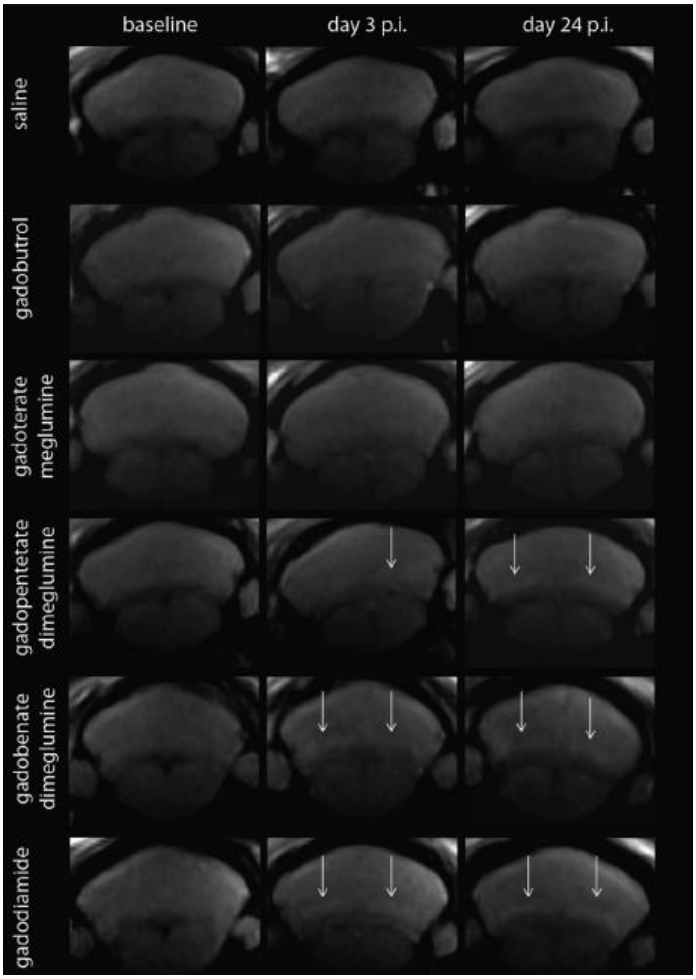
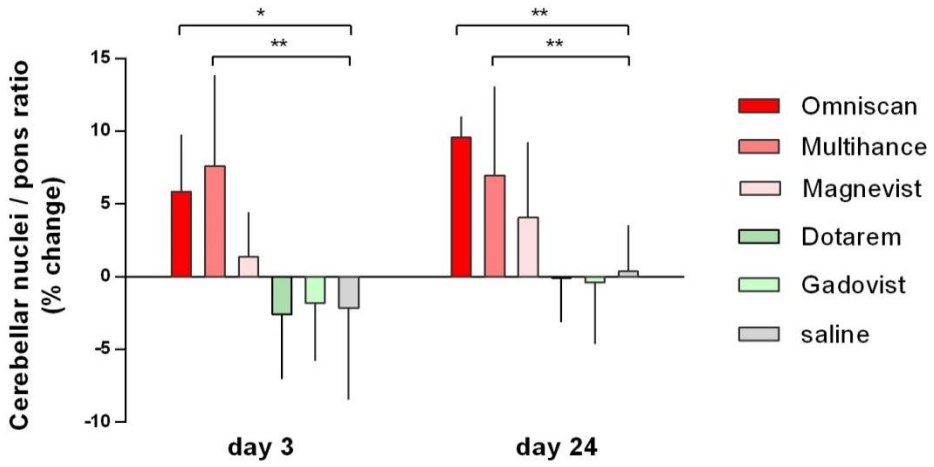


Figure 2: Percent change in SI ratio (deep cerebellar nuclei / pons) for day 3 and day 24 p.i. compared to baseline. No increased cerebellar nuclei / pons ratios compared with baseline were observed in the MRI scans of rats that received Gadovist, Dotarem or saline.
"*" ($p<0.05$) and "" ($p<0.01$) indicate statistical significance of GBCA group compared to saline**

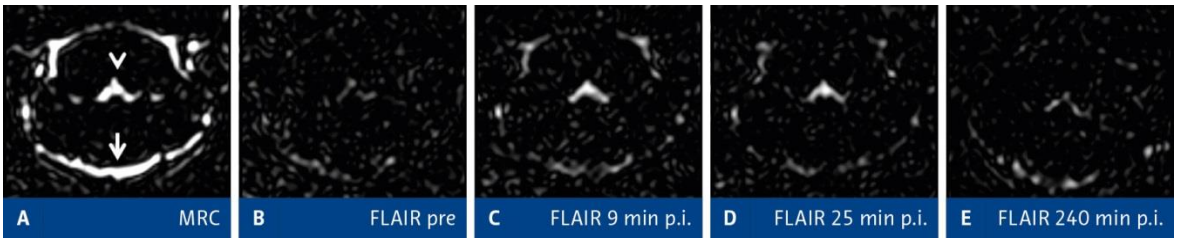


Study 2: Single high GBCA dose in healthy rats (in total 96 rats)

The aim of this MR Cisternography study by **Jost et al (2016b**) was to systematically evaluate one of the potential initial pathways of GBCA entry into the brain – the penetration of GBCAs from the blood into CSF and the distribution kinetics within different CSF cavities – by investigating the CSF enhancement after administration of linear and macrocyclic GBCAs.

The contrast enhancement in the CSF is being determined by FLAIR MRI. As a result, a SI increase of the CSF spaces was observed at comparable levels in FLAIR images after all GBCAs (**Figure 3**). Bayer observed that the kinetics of signal enhancement differ between the inner CSF cavities (3rd and 4th ventricle, aqueduct) and subarachnoidal space. The faster SI increases in the inner cavities demonstrates that the primary location of GBCA infiltration is most likely the choroid plexus located in the ventricles. The choroid plexus continuously secretes CSF, and the choroid plexus epithelium forms the blood-CSF barrier. The fenestrated capillaries of the choroid plexus are relatively permeable to smaller substances, such as GBCAs, which can pass into the choroid plexus interstitium. With this regard, it is important to note that, the blood-CSF barrier is known to be physiologically more permeable than the BBB (**Sage, 1994; Pardridge, 2011**). After penetrating the blood-CSF barrier, further GBCA distribution within the CSF is driven by diffusion, convection and CSF flow that are directed through the ventricles to the subarachnoidal space of the cortex and spinal cord.

Figure 3: Representative images (A – E).
The CSF spaces were visualized by MR Cisternography (MRC), for example the 4th ventricle (arrowhead) and the arachnoidal space (arrow) (A). In the fluid attenuated (FLAIR) images the respective CSF signal is almost completely attenuated before GBCA injection
(B). After GBCA administration a clear signal enhancement of the CSF spaces was found in the FLAIR images up to 240 min p.i. (C - E).



Analytical determinations of the Gd concentrations in the CSF and blood were conducted 4.5 hours and 24 hours post GBCA injection by ICP-MS. At 4.5 hours, the CSF Gd concentrations for all GBCAs were found at very low (range 18.8-27.4 nmol Gd/ml) but comparable levels confirming the findings of the MR Cisternography. After 24 hours GBCAs are almost completely cleared from the CSF.

In summary, this study shows that GBCAs can penetrate from blood into the CSF independent of their chemical structure or physicochemical properties. However, the mechanism of further GBCA distribution from the CSF into the brain tissue and specifically to the DN and the GP could not be evaluated in this experiment and needs further investigations.

Study 3: Repeated high GBCA dose in healthy rats (in total 60 rats)

The aim of our third mechanistic study published by **Lohrke et al**. (**2017**) was to systematically examine potential histopathological changes and Gd presence in the skin and brain of rats after repeated high dose administrations of linear GBCAs and macrocyclic GBCAs. Linear and macrocyclic GBCAs were compared to each other to assess potential differences in Gd concentration, distribution, and their effects on tissue morphology.

Laser ablation coupled with ICP-MS was used to visualize the tissue distribution pattern of Gd. The detected Gd concentration in the brain for linear GBCAs was about 15-times higher than for the macrocyclic agents (**Figure 4**). Eight weeks after the last injection of repeated high doses, very small amounts (<0.0002 %ID/g) were detected in the brain. Similar Gd concentrations were detected also in other organs / tissues for example the heart muscle. In none of the groups morphological changes in the brain were detected by routine haematoxylin and eosin microscopic examination, immunohistochemistry or special staining methods (**Figure 5**). In agreement with previously published non-clinical NSF studies, some of the animals injected with Omniscan (gadodiamide) but none of the other groups showed NSF-like skin lesions macroscopically as well as histologically. These findings underline that the rat model used is able to reliably mimic effects related to GBCA stability, at least in the skin.

Figure 4: ICP-MS results; Gd-concentration (µmol Gd/kg tissue) in skin, brain and skeletal muscle is given for different GBCA.
(A) Eight weeks after the last injection, high Gd concentrations were measured for the linear agents gadodiamide and gadopentetate dimeglumine in the skin and bone.
(B) The average concentration (mean ± SD) of residual Gd in the brain was approximately 100-fold lower compare d with the skin in gadodiamide-injected rats and approximately 15-fold higher for linear than for macrocyclic GBCAs. Low Gd concentrations were measurable when both classes of GBCAs were used.

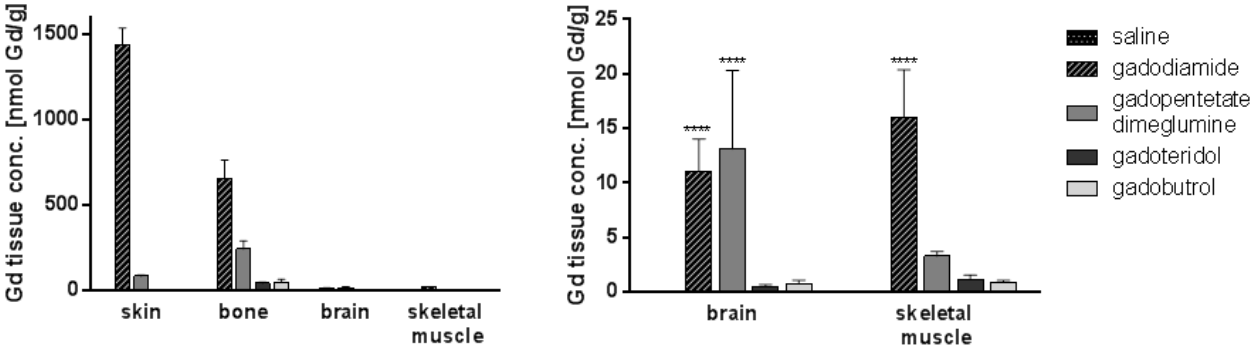
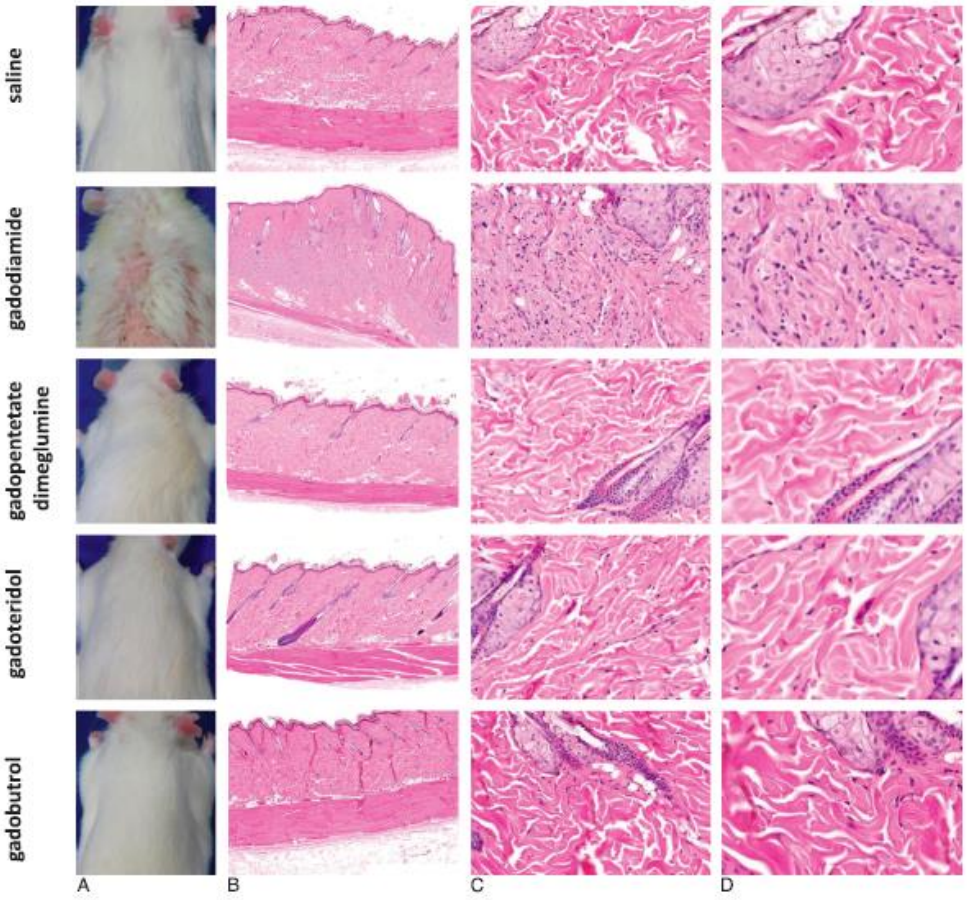


Figure 5: (A) Macroscopic skin appearance and
(B) an overview of the skin tissue and
(C and D) enlarged H&E skin sections of animals administered saline, gadodiamide, gadopentetate dimeglumine, gadoteridol, and gadobutrol. All animals in the gadodiamide group showed fibrosis and mononuclear cell infiltration and an increase in dermal cellularity compared with the saline group and all other investigated GBCA groups.



Study 4: Repeated high GBCA dose in neonatal/ juvenile rats (in total 90 rats)

The study design is comparable to the described Study 3, but in this study neonatal/ juvenile rats will be investigated to evaluate the influence of an incompletely developed BBB. Please note that in those rats, the BBB fully matures relatively late (postnatal weeks 3-4), which is in contrast to the human situation, where the in-permeability of the BBB matures during gestation and is completely finished by the end of the third term of gestation. The evaluation/investigation of the data is still ongoing.

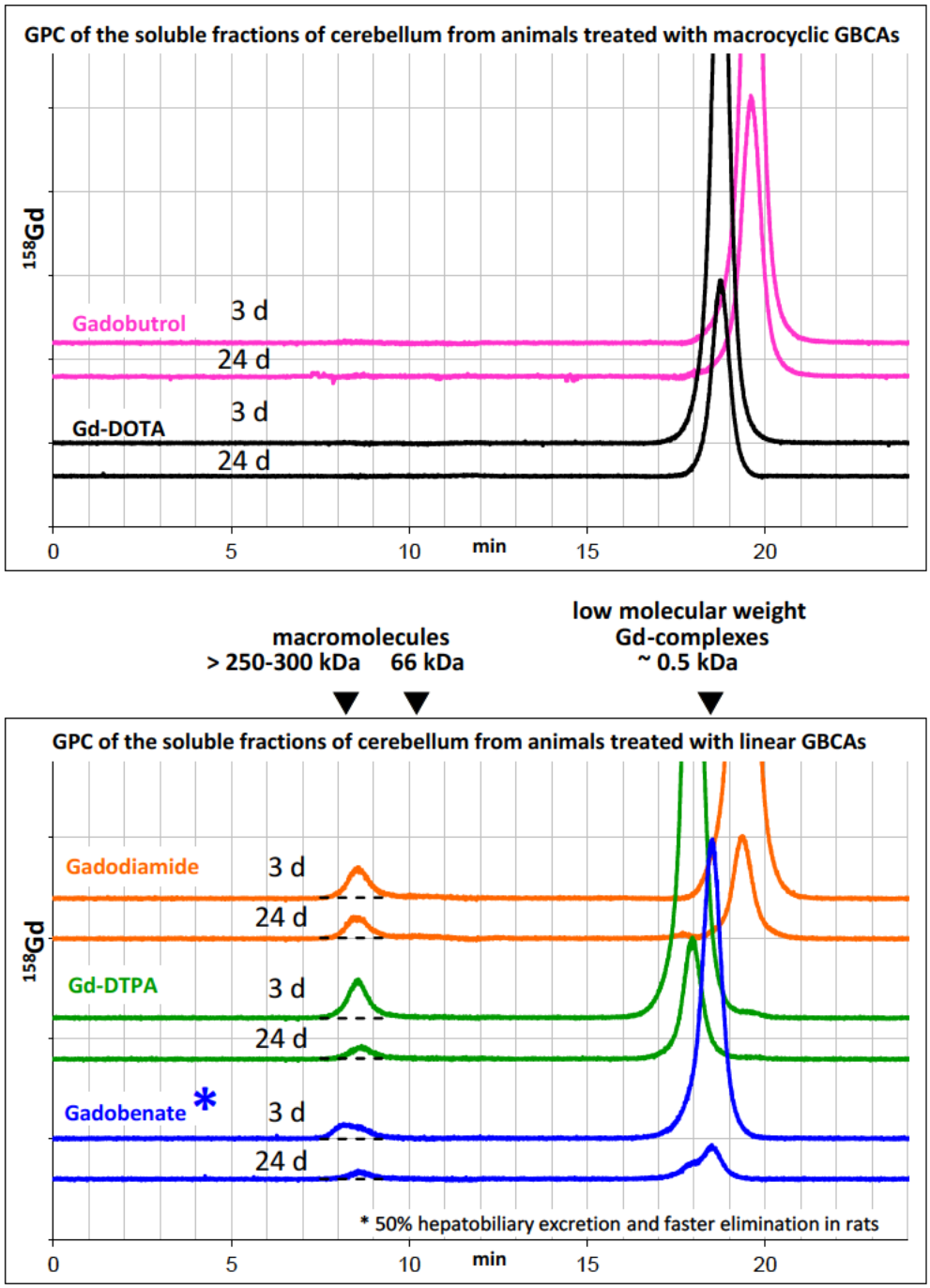
Study 5: Repeated high GBCA dose in healthy rats (in total 60 rats)

The purpose of this already published study by **Frenzel et al. (2017)** was to investigate the molecular form of Gd present in the brain tissue after repeated high dose administrations of linear GBCAs and macrocyclic GBCAs by using tissue fractionation followed by chromatography. Study 5 was a continuation of Study 1.

At three and 24 days after the last injection, the brain tissue was fractionized and the soluble fraction of the tissue extraction was analysed by gel permeation chromatography (GPC). The separation of the components by GPC is based on differences in molecular weight.

Figure 6 shows representative chromatograms of the soluble fraction of brain tissue homogenates after linear and macrocyclic GBCAs. A substantial difference in the detection of early and late peaks was seen when comparing the chromatograms of brain tissue sections from animals administered with linear versus macrocyclic agents. According to GPC, a smaller portion of the Gd in the soluble fraction of the linear GBCAs groups was bound to macromolecules larger than 250 to 300 kDa while this was not the case for the macrocyclic GBCA group.

Figure 6: Examples of Gd-specific GPC chromatograms of cerebellum homogenates from animals 3 and 24 days after injection with (A) linear GBCAs OmniscanTM (gadodiamide), Magnevist (Gd-DTPA) and MultiHance (gadobenate) and (B) macrocyclic GBCAs Dotarem (Gd-DOTA) and Gadovist (gadobutrol). The chromatograms show the intensity of Gd in arbitrary units.



* Smaller peak area likely due to faster elimination of MultiHance because of relevant hepatobiliary excretion which is about 50% in rats but only 3-5% in humans (**Kirchin, 1998; de Haen, 1996**).

It should be noted that the total peak area in the MultiHance group was slightly smaller than in the Magnevist and Omniscan group which is due to the very efficient additional hepatic excretion pathway of MultiHance in rats (about 50%) and which is only 3 – 5% in humans (**Frenzel, 2017; Robert, 2015**). The relative portion of Gd bound to macromolecular structures increased from day 3 to day 24 for all linear agents.

Explanation of the control experiment (Supplement from Frenzel, 2017)

The control experiment consisted of the extraction and analysis of blank brain tissue spiked with the same low amount of GBCAs that was found in the treated animals (10 nmol Gd/g).

The control study was performed for several reasons:

1. To demonstrate nearly complete recovery of all Gd present in the tissue. This was not done in many other studies published before **(Frenzel, 2017**)
2. To demonstrate that intact GBCAs are not part of the observed soluble Gd containing macromolecules (e.g. by suspected protein binding of the entire GBCA).
3. To demonstrate that the very low amount of Gd which was found for the macrocyclic GBCAs in the insoluble fraction was due to incomplete washout, incomplete separation between soluble and insoluble fraction or vesicle reformation of membrane fragments, which may encapsulate some parts of the soluble fraction.

The results of the control experiment verified all of our assumptions with one exception: Omniscan did lead to Gd containing macromolecules during the analytical process. This was surprising and is mentioned in the paper as a potential source of bias. This finding indicated that Omniscan is highly instable in a biological environment like the tissue homogenate (Note: the homogenate contains some intracellular components which usually do not get into contact with the GBCA). The conditions of the test were very mild: pH 7.4 and 4°C for about 5 hours and intracellular components are not regarded as having a much more aggressive transmetallating capacity than multi-purpose components.

Nevertheless, the observed instability could have led to much higher amounts of degradation products, such as the Gd containing macromolecules in the samples from animal treated with Omniscan. However, the amount of Gd-containing macromolecules was not much higher after the treatment with Omniscan than after the treatment with Magnevist, which was stable during the control study. In addition, we found almost identical amounts of Gd in the soluble fraction of samples form animals treated with Gadovist, Dotarem, Magnevist and Omniscan. Therefore, the observed instability had very likely only limited effect, if at all, on the results of the treated animals.

In summary, a significant formation of Gd-containing macromolecular structures in the brain was only observed for linear GBCAs which are likely the cause for the increase of the SI in the deep cerebellar nuclei after administration of the linear agents. Although final proof is still missing, the explanation for this finding may be the partial dechelation of Gd from linear GBCAs and immediate binding of that small Gd portion to not yet characterized macromolecules. It might be also possible that Gd in the insoluble fraction could contribute the SI increase. In the contrary: this phenomena was not observed for macrocyclic agents: no release of Gd – no binding to macromolecules and no increase in the SI in the animal experiments.

Study 6: Repeated high GBCA dose comparing healthy and renally impaired rats (5/6 nephrectomized) (in total 60 rats)

In this study, the influence of renal impairment was investigated by comparing 5/6 nephrectomized rats to healthy rats after the administration of linear and macrocyclic GBCAs (**Pietsch, 2017**). Eight weeks after the last GBCA administration, MRI examinations were performed and the Gd concentration in the brain was measured by ICP-MS.

As a result, rats administered with linear GBCAs exhibited significantly higher cerebellar nuclei to pons ratio (CN/pons) SI ratios compared to the saline group (**Figure 7**). No distinct difference was observed between healthy control and renally impaired animals. Rats treated with macrocyclic GBCAs showed no difference in CN/pons ratios compared to saline independent of renal status. ICP-MS revealed higher Gd concentrations in the cerebellum for all exclusive renally excreted GBCAs in the animals with renal impairment compared to the animals with normal renal function (**Figure 8**). Substantially higher Gd concentrations were found after administration of linear compared to macrocyclic GBCAs. For healthy animals, the highest Gd content in the cerebellum was found after administration of gadodiamide. Compared to the other linear GBCAs a twofold higher concentration was detected (**Figure 8**). However, in animals with renal insufficiency the Gd concentration for gadodiamide and gadopentetate dimeglumine treated animals were almost comparable. For gadobenate dimeglumine the Gd concentration in the cerebellum remained almost unchanged between healthy and renally impaired rats. However, this result is strongly biased by the different excretion profile between humans and rats. While in humans 3-5% of this agent is taken up by hepatocytes this rate is 50% in rats (**Kirchin, 1998; de Haen, 1996**). Thus, in contrast to the other GBCAs investigated an effective hepatobiliary excretion pathway exists for gadobenate dimeglumine in rats.

Figure 7: Cerebellar nuclei to pons (CN/pons) signal intensity ratio for healthy (left) and renally impaired (right) animals 8 weeks after repeated high-dose application of different GBCAs. gadoterate = gadoterate meglumine; gadopentetate = gadopentetate dimeglumine; gadobenate = gadobenate dimeglumine; error bars represent standard deviation

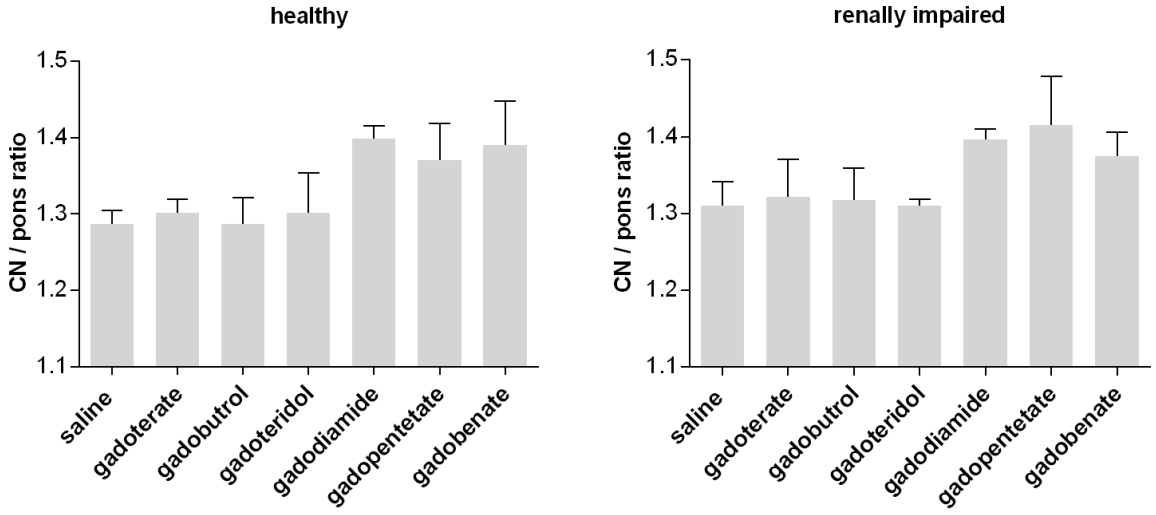
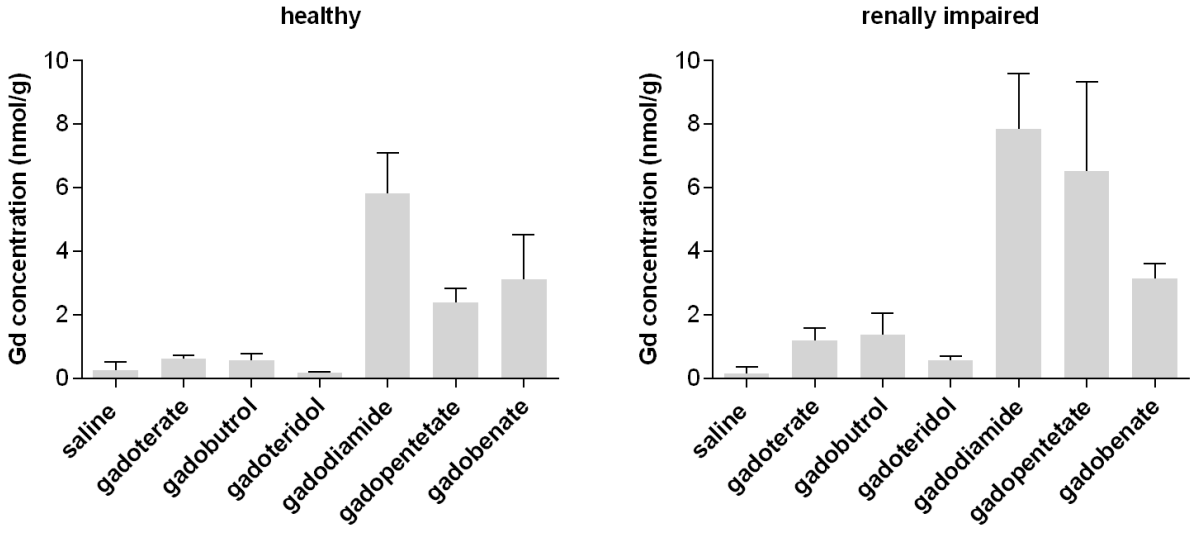


Figure 8: Gadolinium (Gd) concentration in the cerebellum of healthy (left) and renally impaired (right) animals 8 weeks after repeated high-dose application of different GBCAs.
gadoterate = gadoterate meglumine;
gadopentetate = gadopentetate dimeglumine;
gadobenate = gadobenate dimeglumine;
error bars represent standard deviation



In conclusion, renal impairment does not clearly affect the T1w SI increase 8 weeks after repeated high-dose GBCA administration in rats. However, the Gd concentration in cerebellum was moderately increased.

Study 7: Repeated high GBCA dose in healthy rats: long term study (in total 162 rats)

To date, only limited knowledge exists regarding the long-term presence of Gd in the brain or the potential for eventual elimination. Therefore, the aim of this non-clinical study in healthy rats was to investigate the long-term presence of MRI SI and the presence of Gd in the brain 5, 26 and 52 weeks after the last administration of linear and macrocyclic GBCAs.

As a result, rats that were administered multi-purpose ionic linear GBCAs exhibited higher CN/pons SI ratios at all investigated time points compared to the respective saline control. As expected, the CN/pons SI ratio for macrocyclic GBCAs did not differ from the saline control group at any time-point (**Figure 9**). ICP-MS revealed substantially higher Gd concentrations in the cerebellum for multi-purpose linear GBCAs compared to macrocyclic GBCAs (**Figure 10**). For all linear GBCAs, no systematic change in the Gd concentration was found over the time period of one year. In contrast, macrocyclic GBCAs showed distinctly lower Gd concentrations in the cerebellum at week 26 (-81%) and 52 (-87%) compared to week 5.

Figure 9: Cerebellar nuclei to pons (CN/pons) signal intensity ratio after repeated high-dose application of linear (red) and macrocyclic (green) GBCAs relative to saline control. Error bars represent standard deviation.

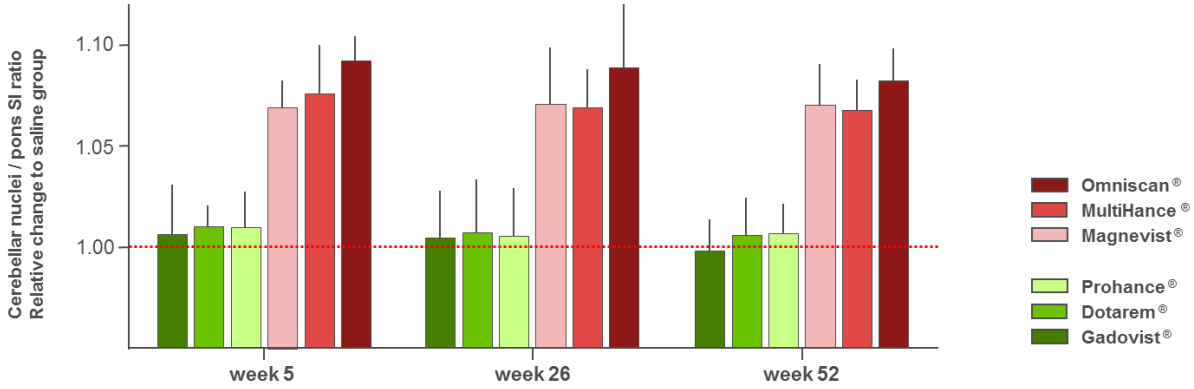
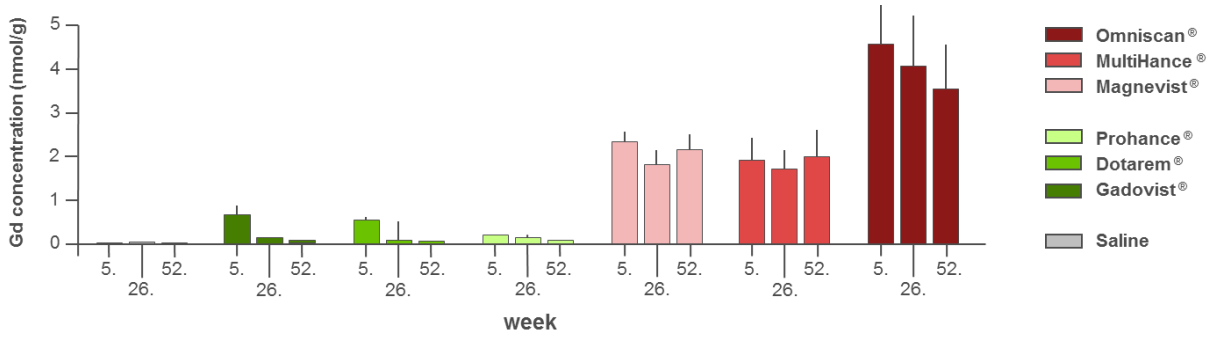


Figure 10: Gadolinium concentration in the cerebellum after repeated high-dose application of linear (red) and macrocyclic (green) GBCAs. Error bars represent standard deviation



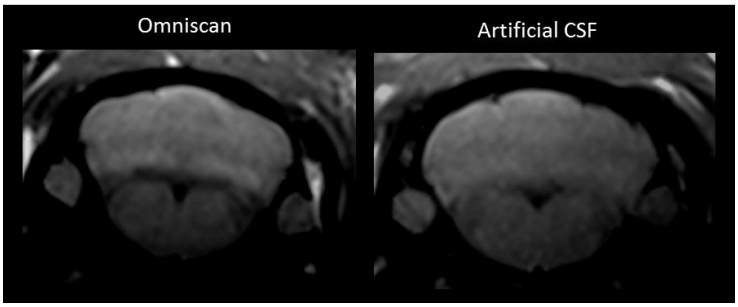
Overall, the Gd concentration in the brain for linear agents did not change significantly within one year. For macrocyclic agents, the Gd concentration was much smaller and there was a continuous decrease over time that further underlines the continuous elimination over one year.

Study 8a: Repeated GBCA dose in healthy rats: intracisternal administration (in total 16 rats)

It has been shown that the penetration of GBCAs from the blood into the CSF represents a potential pathway of GBCA entry into the brain (Study 2). The scope of the study is to investigate the distribution of Gd within the brain after repeated intracisternal injection of a linear GBCA (Omniscan). A signal enhancement in the brain deep cerebellar nuclei (DCN) was observed 5 weeks after the last administration of Omniscan but not after injection of artificial CSF (**Figure 11**). The respective Gd concentration in the cerebellum determined by ICP-MS was in the same range as the concentrations found after repeated intravenous administration of Omniscan in other studies (Study 6 & 7). A similar pattern of Gd distribution in the brain exists after repeated intravenous and intracisternal administration. This demonstrated that the route of

the GBCA via the CSF into the brain is at least one pathway of GBCA entry into the cerebellum (deep cerebellar nuclei). The results triggered a second study to evaluate the Gd distribution in the brain after intracisternal administration in more detail.

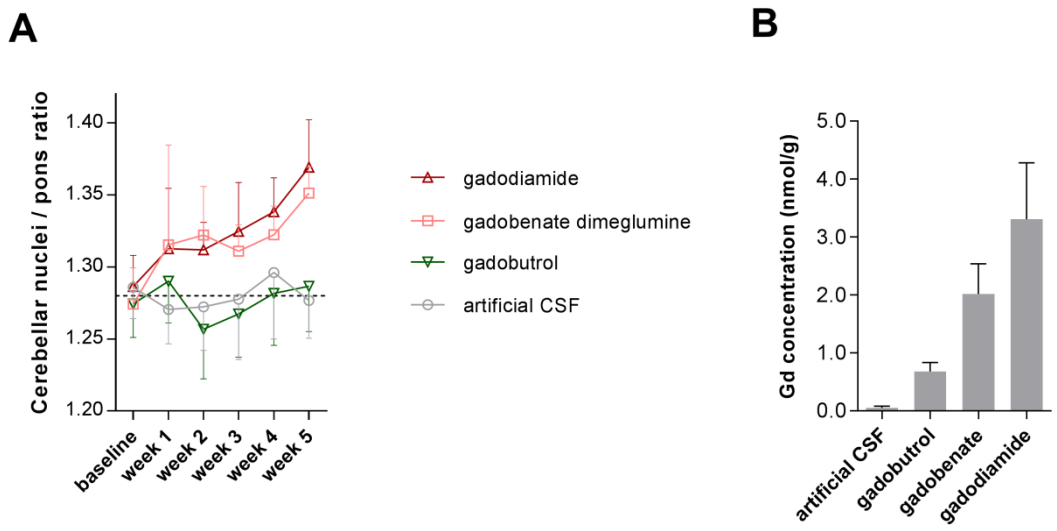
Figure 11: T1w MRI of the rat brain (cerebellum) 5 weeks after repeated intracisternal injections of Omniscan or equivalent volume of artificial CSF



Study 8b: Single GBCA dose in healthy rats: intracisternal administration (in total 32 rats)

The purpose of this study was the comparison of linear GBCAs (Omniscan, MultiHance) and a macrocyclic GBCA (Gadovist) after intracisternal administration. A single administration of Omniscan and MultiHance results in an increasing MRI signal enhancement of the deep cerebellar nuclei in rats over the observation period of 5 weeks (**Figure 12A**). Omniscan and MultiHance showed also the highest Gd concentrations determined by ICP-MS in the cerebellum 5 weeks after intracisternal GBCA administration. No enhancement effects and much lower cerebellar Gd concentrations were observed after administration of macrocyclic Gadovist.

Figure 12: A) Time course of MRI SI ratio between cerebellar nuclei and pons after one single intracisternal application of Omniscan (gadodiamide), MultiHance (gadobenate dimeglumine), Gadovist (gadobutrol) and artificial CSF. B) Gd concentration determined by ICP-MS in the cerebellum 5 weeks after GBCA administration.

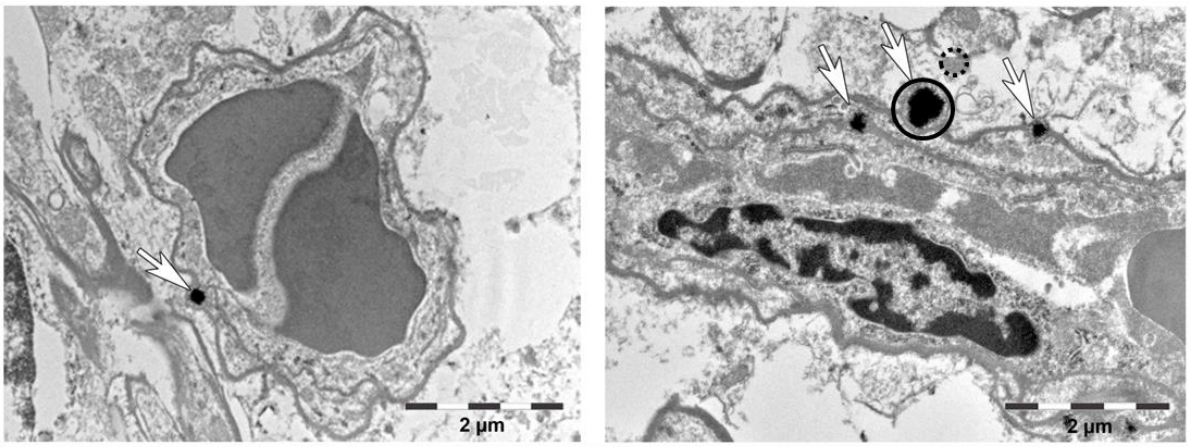


Study 9: Repeated high dose in healthy rats: Transmission electron microscopy (TEM) study (in total 12 rats)

No histologically evident alterations were found in brain of rats after repeated high dose administrations of linear and macrocyclic GBCAs (see results of Study No. 3). Published by **Lohrke et al (2017)** brain tissue slides were additionally analysed by transmission electron microscopy (TEM). The focus of the TEM analysis was on regions which are clinically described with increased SI: the pallidus and the lateral cerebellar nuclei (which are equivalent to the dentate nuclei in humans).

As a result, TEM revealed several Gd-containing spots in the region of the dentate nuclei in the brain of one animal injected with gadodiamide which is consistent with the results of **McDonald et al. (2015)** in humans (**Figure 13**).

Figure 13: Transmission electron microscopy (TEM) tissue localization of Gd-containing spots in the region of the lateral (dentate) cerebellar nuclei in the brain after the repeated high-dose application of gadodiamide. TEM evaluation showed several positive signals (white arrow). The location indicates intracellular Gd presence within endothelial cell of blood vessels



The Gd-positive high electron-dense structures in TEM could only be detected in the endothelial wall of several micro-vessels in the brain of one gadodiamide administered rat and not in neurons, neutrophil, or other glial cells, raising the possibility that Gd may not have passed the BBB. This finding has to be further examined on more samples and animals, as well as after the administration of other GBCAs than gadodiamide.

Study 11a (long term) and 11b (acute): Repeated high dose in healthy rats: Biomarker study

The scope of the study is to investigate a potential immunological response within the brain after exposure to linear GBCA (gadodiamide) and macrocyclic GBCA (gadobutrol). In this study, the protein expression of 32 immune mediators is measured in brain homogenates using a multiplex luminex system (RodentMAP, Ampersand Biosciences, USA). The 32 analytes include well-known chemokines and cytokines, which are released molecules that play an important role in orchestrating the immune environment. The RodentMAP panel comprises pro-inflammatory, anti-inflammatory, angiogenic and hematopoietic cytokines and chemokines mediating chemotaxis of macrophages, neutrophils, eosinophils and T-cells. The study evaluates the chemokines/cytokines present in the brain and whether the chemokine/cytokine profile changes after GBCA administration.

The first part of the study (Study 11a) investigates long-term immune alterations associated with Gd presence in the brain. Brain tissue is analyzed after repeated high dose GBCA exposure 4 and 35 days after the last application. The second part (Study 11b) investigates acute immune reactions in the brain after a single GBCA injection (6 and 24h p.i.).

The study is still ongoing.

Expression levels of following cytokines will be measured by using an established technology (RodentMAP): CRP, Eotaxin/CCL11, GM-CSF, IFNβ, IFN, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-17A, IL-18, IL-28, Insulin, IP10/CXCL10, KC/CXCL1, MCP-1/CCL2, M-CSF-1, MDC/CCL22, MIP-1β, MMP-9, PAI-1, SAP, SCF, Thrombopoietin, TNFα, TSLP, VEGF-A

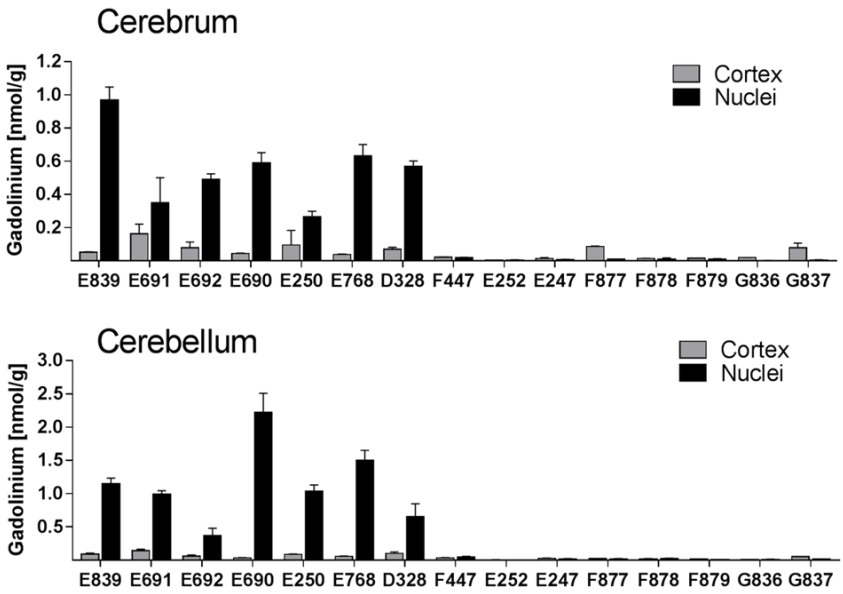
Study 12: Retrospective study in mini-pigs (in total n=15)

The aim of this retrospective study was to determine the Gd concentration in different brain areas in a mini-pig cohort that received repeated i.v. administrations of several GBCA at standard doses over several years comparable to a clinical setting. The mini-pig cohort included a group of animals which received multiple doses of both linear gadopentetate dimeglumine and macrocyclic gadobutrol and a group which received gadobutrol only. The animals received at least 4 and maximal 48 injections of GBCA resulting in an accumulated dose ranging from 7 to 129 mmol per animal, time of examination was between 8 months and 3 years after the last application.

Multiple exposures to macrocyclic gadobutrol are not associated with Gd presence in brain tissue of healthy pigs. The Gd presence in the areas of dentate nucleus and globus pallidus in gadobutrol-only animals was ≤ 0.02 nmol/g tissue and comparable to control animals with no record of GBCA history (**Figure 14**). In animals that have received both gadopentetate dimeglumine and gadobutrol, the median Gd concentration was 50 times higher than gadobutrol-only animals. The amount of accumulated Gd correlated with the administered dose of gadopentetate dimeglumine but not with the total Gd dose, including gadobutrol (**Figure 15**).

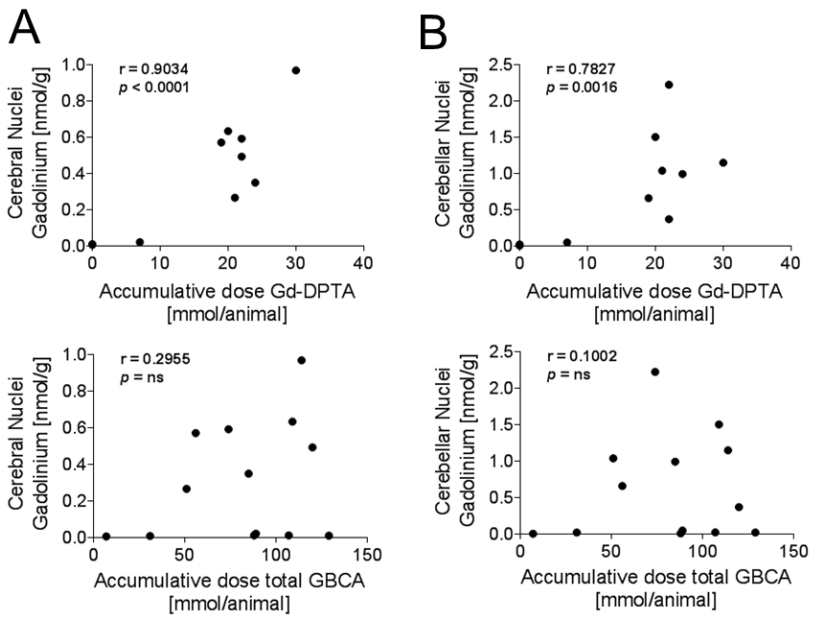
A single additional administration of linear gadopentetate dimeglumine in the GBCA record is sufficient for Gd accumulation in the dentate nucleus and globus pallidus. The median Gd concentration in cortical tissue and in the pons were very low (≤ 0.07 nmol/g tissue) in all animals analyzed.

Figure 14: Gadolinium concentration (in nmol Gd/g tissue) in homogenates obtained from different neuroanatomical regions. Bars represent individual animals with n = 3 technical replicates, the GBCA accumulative dose (in mmol Gd/animal) for each animal are displayed below the bars.



Magnevist® [mmol/ animal]	30	24	22	22	21	20	19	7	-	-	-	-	-	-	-
Gadovist® [mmol/animal]	84	61	98	52	30	89	37	82	7	31	129	107	88	-	-

Figure 15: The association between Gd concentration and the parameters accumulative dose of linear GBCA and accumulative dose of total GBCA is shown with Pearson correlation coefficient (r) and the associated P value. A) Cerebral nuclei (Globus Pallidus). B) Cerebellar nuclei (Dentate Nucleus).



3.4 Neurocognition

Study 10: Experimental studies to investigate potential neurological effects associated with Gd-presence in the brain

After multiple injections of multi-purpose linear GBCAs, Gd can be found in deep cerebellar nuclei and basal ganglia in rats. To investigate whether this Gd presence has any functional effect on neurons, rats are being tested for potential behavioral alterations after multiple dosing with the linear gadodiamide in comparison to the macrocyclic gadobutrol. The cerebellar nuclei and GP contain neurons involved in motor and cognitive function. Respective behavioral paradigms are being tested. Testing for behavioral paradigms is challenging, therefore Bayer outsourced this study to an external expert laboratory, which has experience in performing behavioral studies in animal models for Alzheimer's, Parkinson's and Huntington's disease. Bayer's laboratory for contrast media research has performed the contrast media administrations and afterwards the prepared animals were transferred for behavioral testing.

Two studies are envisaged. Study 1 comprises a test battery of different behavioral tests to investigate for any behavioral abnormalities in a hypothesis free approach. The investigated dosage is similar to the dosages in our previous experiments. Study 2 addresses a potential dose dependency for selected tests from Study 1.

Study 1:

For motor function, the following are conducted:

- SHIRPA (SmithKline Beecham, Harwell, Imperial College and Royal London Hospital phenotype assessment - a primary screening cascade to look for gross abnormalities);
- Open Field Test (observes new environment exploration behavior);
- PhenoMaster (observation of the animal over 72h in its home cage);
- CatWalk (gait analysis – animals walk through a tunnel and gait is observed which is a sensitive motor function test);
- Rota Rod (animals have to keep balance on rotating rod which tests for feet coordination).

For cognitive function, the following will be conducted:

- Acoustic startle response / pre pulse inhibition (tests for a startle reaction upon sudden noise and its modulation by a second noise pre-pulse which tests sensory motor gating);
- Operant Wall (animals have to learn to follow visual queues in order to receive a food reward).
- Stair case paw reaching test (animals need to reach for food pellets placed on a stair case using their fore paws; alterations in voluntary movement precision can be observed)

All individual animals in the study will be analyzed in all tests. 1.8 mmol Gd/kg bw has been administered 8x (4 days per week) i.v. to healthy Wistar rats (WI:Crl (Han)). Behavioral testing has been initiated at 4 weeks after last administration. The different assays have been performed over a period of 5 weeks:

Week 5: SHIRPA, open field, CatWalk

Week 6: Rota Rod

Week 7: ASR/PPI, PhenoMaster

Week 8+9: operant wall

Week 12: ASR/PPI

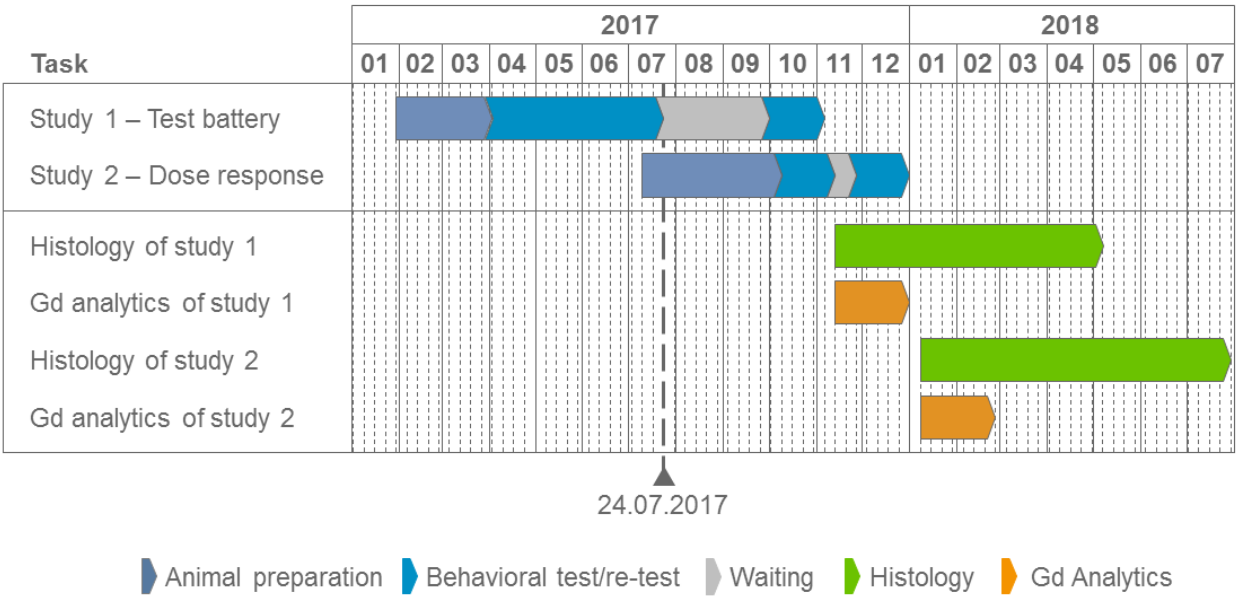
This study has been initiated in January 2017 after approval by local animal ethics authority and is ongoing.

Study 2:

The test battery described above will be followed by a dose response study for selected behavioral tests in order to identify potential effects unrelated to Gd-presence in brain tissue. This study was initiated in July 2017 and is ongoing.

The timeframe of ongoing behavioral studies is illustrated below (**Figure 16**).

Figure 16: Study Timelines for study on neurological changes



3.5 Parameters for the stability of GBCAs

Paramagnetic MR contrast media generally consist of a Gd^{3+} ion bonded to an organic moiety (ligand) to form a gadolinium chelate complex (GBCA). This complex is essential for biologic use, since naked metal ions have known toxicities *in vivo*.

The likelihood of a Gd-chelate to release Gd^{3+} ions depends in particular on the chelate's chemical structure and respective *in vivo* stability. Based on their chemical structures, GBCAs may be divided into two groups: linear chelates and macrocyclic chelates. The dissociation of Gd^{3+} from its ligand is an equilibrium process. It is defined by two distinct and independent parameters (a) thermodynamic stability and (b) kinetics (**Rofsky, 2008**).

a.) Thermodynamic stability

The thermodynamic stability constants describe the equilibrium between concentrations of the Gd-complex on the one hand, and concentrations of free Gd^{3+} and free ligand on the other (**Table 2**).

The thermodynamic stability constant is the *in vitro* measure of affinity between a Gd^{3+} ion and each organic ligand that comprises the metal-ligand complex or chelate. The conditional stability

constant is also an *in vitro* measure of the affinity between Gd^{3+} and each organic ligand measured at a pH of 7.4 under nearly physiologic conditions.

The complex stability of linear (open-chain) chelates is primarily characterized by their thermodynamic (log K valid at pH 14) and conditional complex stability (log K_{cond} calculated for pH 7.4 with use of the protonation constants of the ligand).

Table 2: Characteristics of linear GBCAs with regard to formulation and thermodynamic stability

Trade name	Excess ligand in formulation	Thermodynamic stability	
		log K_{therm}	log K_{cond}
Omniscan	5 %	16.9	14.9
OptiMARK	10 %	16.8	15.0
Magnevist	0.2 %	22.5	18.4
MultiHance	0 %	22.6	18.4
Primovist	0.5 %	23.5	18.7

The constants are influenced by the charge status of the ligand, i.e. an ionic ligand achieves stronger binding to the Gd^{3+} cation than a ligand that results in a neutral chelate upon binding to the Gd^{3+} ion. Non-ionic linear chelates are characterized by lower complex stability and high excess of free ligand in the formulation. Ionic linear chelates are characterized by higher complex stability and low excess of free ligand in the formulation.

b.) Kinetic stability

The kinetic inertia of a GBCA is characterized by its dissociation rate, which expresses how fast the equilibrium is reached and thus how fast Gd3+ is released from a Gd-complex. This question is based on chemical kinetics/ kinetic stability and not thermodynamic stability.

The adequate parameter describing this kinetic process is the dissociation half-life, which describes the time needed for the de-complexation of half of the Gd-complexes in solution.

The thermodynamic stability constants are negligible for macrocyclic GBCAs. Due to their extremely long dissociation half-life (extrapolated > 1,000 years at pH 7.4) an equilibrium is essentially never reached *in vivo* (**Table 3**).

Table 3: Overview of Dissociation Half-Lives (T1/2), determined at different conditions, illustrating the Kinetic Inertias of GBCAs at pH 1 and at higher pH in the absence of a biological matrix.

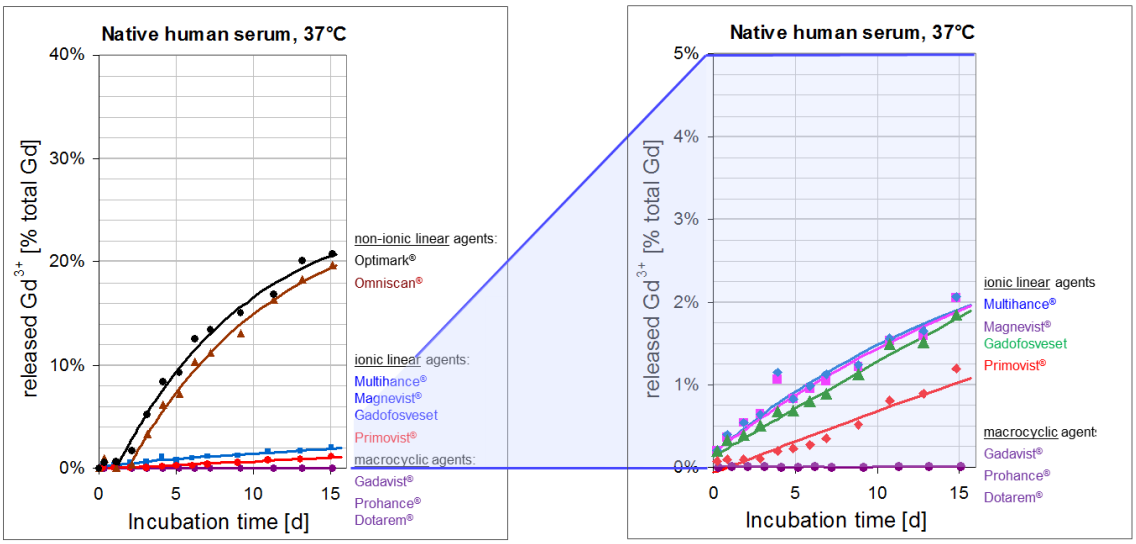
Class	Trade name	$T_{1/2}$, pH 1	Reference	$T_{1/2}$, pH>5	Reference
Linear	Not specified	<5 s (25°C)	Port, 2008	5–7 d (pH 7.4, 25 C)	Sarka, 2002
Macrocyclic	Gadavist	7.9h (37°C)	Port, 2008	65 yr (pH 5.3, 25°C)	Toth, 1996
	ProHance	2.0h (37°C)	Port, 2008	36 yr (pH 5.3, 25°C)	Toth, 1996
	Dotarem	26.4h (37°C)	Port, 2008	37 yr (pH 5.3, 37°C)	Toth, 1994

The values were taken from the cited references or were calculated from the given rate constants. Because of the differing conditions, the values are not directly comparable, but they give an impression of the marked differences between the dissociation rates of macrocyclic and linear GBCAs.

Macrocyclic chelates differ from linear chelates regarding the kinetics of complexation and de-complexation. Significant activation energy is necessary to both generate and dissociate the Gd-complexes.

Due to high kinetic stability, macrocyclic agents are basically inert under physiological conditions. No release of Gd could be measured over 15 days *in vitro* incubation (**Figure 17**). Any differences in dissociation half-life *in vitro* are extremely unlikely to result in relevant differences *in vivo* and the extrapolated dissociation half-lives at physiological conditions for all three macrocyclic GBCAs are similar.

Figure 17: GBCA Stability: Stability of GBCAs, as measured by Gd^{3+} ion release during 15-day *in vitro* incubation in human serum at 37 °C. Gadovist, Dotarem and ProHance are macrocyclic GBCAs, the other agents shown are linear GBCAs (Frenzel et al., 2008)



For the linear non-ionic GBCAs Omniscan and Optimark the lowest stability (release up to 20%) have been demonstrated. The ionic-linear GBCAs Magnevist and MultiHance are more stable: up to 2% of the Gd was released over 15 days (**Frenzel, 2008**). Among the ionic-linear Gd-chelates, Primovist shows the highest thermodynamic stability constant which resulted in 1% Gd-release over 15 days. The differences in the stability of the Gd chelates were also considered in the NSF referral procedure in which all the macrocyclic GBCAs (including Gadovist) have been classified as low risk agents.

3.6 Non-clinical safety data obtained in routine (regulatory requirement) systemic toxicity studies conducted in the past with Bayer's GBCAs

In the context of the non-clinical development of the three GBCAs marketed by Bayer (Magnevist, Gadavist and Eovist) numerous systemic toxicity studies after repeated i.v. dosing (usually daily up to 4 weeks) were conducted in rats and dogs to support the approval of these contrast agents by Health Authorities worldwide. All studies performed included (besides other routine toxicological endpoints) investigations that allowed for the identification of potential substance-related effects indicating neurotoxicity.

In none of the pivotal repeat-dose toxicity studies conducted in rats and dogs could any signs of neurotoxicity based on clinical observations (with focus on changes in the behavior of treated animals) or changes/alterations in routine patho-morphological investigations of the brain (including cerebrum, cerebellum, medulla oblongata and spinal cord) be identified, even at high multiples of the human diagnostic dose and after long-lasting observation periods.

This conclusion is supported by the fact, that studies conducted in 2014 using neonatal to juvenile rats clearly indicated (according to the newly introduced and concomitantly performed Gd analysis in selected tissues), that brains of treated animals were relevantly exposed to Gd.

Moreover, a relevant brain exposure with Gd can retrospectively also be assumed for the adult rats used in the other pivotal repeat-dose toxicity studies with the three GBCAs, as in-house research studies (**Lohrke, 2017**) recently demonstrated that repeated high i.v. doses of linear and macrocyclic GBCAs administered to adult rats also resulted in detectable Gd brain concentrations in these animals without showing any morphological alterations.

Therefore, from a non-clinical (toxicological) point of view, all pivotal repeat-dose toxicity studies conducted under Good Laboratory Practice conditions in adult rats and dogs as well as in neonatal to juvenile rats using the three Bayer GBCAs did not provide any evidence that repeated high i.v. doses of Magnevist, Eovist or Gadavist induced any harm to this organ system. Especially, the studies conducted in rats allow for the conclusion that Gd exposure in the brain did not result in any adverse alterations in this organ, as no signs of inflammatory, degenerative or proliferative reactions were noted histologically.

Acute toxicity studies in experimental animals with only a single i.v. or other routes of administration conducted in-house and/or being published are not considered appropriate to conclude on potentially induced neurotoxic effects of GBCAs due to the following reasons:

These studies are usually conducted only in a very limited number of animals per dose group that were treated at unrealistic high dose levels and – in case of an intracisternal injection (directly into the cerebrospinal fluid [liquor]) – at an unrealistic route of administration when compared to the clinical situation/exposure in patients. Moreover, these studies generally lack any information on concrete Gd exposure in the target organ, and – most importantly – they do not provide information on potentially induced morphological alterations, as histological investigations of the brain (including cerebrum, cerebellum, medulla oblongata and spinal cord) are usually not included.

3.7 Future directions in non-clinical research

Bayer's non-clinical research will continue, focusing on four important topics:

1.) Identification of the binding partners of released Gd (macromolecules):

    Protein biochemistry and mass spectrometry-based approach to identify endogenous Gd-binding proteins. Methods will include subcellular fractionation of brain homogenates, electrophoresis under conditions that retain metal-protein interactions and subsequent protein identification with LC-MS/MS based peptide sequencing.

2.) Investigation of the importance of potential local leakages of the BBB, due to brain tumors, regarding the increase in SI and the concentration of Gd in the deep cerebellar nuclei.

3.) Long-term investigations of the Gd concentration and the molecular form of GBCAs in other body tissues, especially bone tissue.

4.) Mechanism of further GBCA distribution from the CSF into the brain tissue and deep compartments specifically to the DN and the GP.

Respective approvals of the local animal welfare committee were applied and technical methods and technologies are currently evaluated and established.

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4. Pharmacovigilance Update

4.1 Pharmacovigilance at Bayer

Patient safety is Bayer's primary concern, and PV is a critical component of Bayer's efforts to ensure the safe and appropriate use of its products. An important part of PV is the detection, evaluation and management of safety signals. A safety signal is information on a new or known adverse event that may or may not be caused by a medicine and requires further investigation and assessment to establish whether or not there is a causal relationship between the medicine and the reported event**.** Signal detection is performed in a qualitative and quantitative manner. Safety signals can be detected from a wide range of sources, including spontaneous reports, clinical studies and scientific literature. External databases such as FAERS and EudraVigilance are additional sources of information.

All emerging safety issues and newly identified signals are presented and discussed within cross-functional Safety Management Teams and brought to medical governance committees for endorsement and guidance. Additionally, because of the nature of the topic of Gd presence in the brain and body, and its unknown clinical relevance, a cross-functional Task Force has been formed to specifically focus on this topic. This team is comprised of individuals with long-term experience and expertise in contrast media from different functions (including Medical, Non-Clinical, Pharmacovigilance, and Regulatory Affairs).

4.2 Bayer's GBCAs

Bayer is the innovator of three GBCAs.

4.2.1 Magnevist (gadopentetate dimeglumine)

Magnevist was the first GBCA approved for contrast-enhanced MRI. Since its launch in 1988, when it revolutionized the field of diagnostic imaging, more than 148.8 million Magnevist-enhanced procedures as of April 30, 2017 have been performed in neonates, infants, children, adolescents, adults, and the elderly. It is the most widely studied GBCA, and over its nearly 30 years of marketing experience, has maintained an overall very favorable safety profile.

Magnevist is a multi-purpose linear GBCA, indicated for cranial and spinal MRI and for whole body MRI (excluding the heart and the breast in the US).

The most frequently observed adverse drug reactions (≥ 0.4%) in > 11,000 patients receiving Magnevist in interventional clinical trials were:

- Various injection site reactions
- Headache
- Nausea

In post-marketing experience, three rare but serious risks were identified:

- Anaphylactoid reactions/anaphylactoid shock
- Acute renal failure/acute kidney injury

- Nephrogenic systemic fibrosis (NSF)

4.2.2 Gadavist (gadobutrol)

Gadavist is a macrocyclic GBCA indicated in adults and children of all ages, including full-term newborns, for contrast enhanced whole body MRI including:

- Cranial and spinal MRI
- MRI of the head and neck region
- MRI of the thoracic space
- MRI of the breast
- MRI of the abdomen (e.g. pancreas, liver and spleen)
- MRI of the pelvis (e.g. prostate, bladder and uterus)
- MRI of the retroperitoneal space (e.g. kidney)
- MRI of the extremities and musculoskeletal system
- Magnetic Resonance Angiography (CE-MRA)
- Cardiac MRI

Indications may vary according to the country of approval/registration. More than 40.8 million administrations of Gadavist have been given worldwide since its first launch in 1998.

Gadobutrol is well tolerated with a safety profile comparable to other multi-purpose MR contrast agents. Evaluation of special populations including pediatric patients, patients with renal or hepatic impairment and patients with cardiovascular disorders did not reveal any specific risks in those populations.

The most frequent (≥0.5%) Adverse Drug Reactions (ADRs) associated with the use of Gadavist in clinical trials of >7,000 patients were headache, nausea, and dizziness.

In post-marketing experience, the following rare but important risks were identified:

- Anaphylactoid reactions
- Acute kidney injury
- NSF

4.2.3 Eovist (gadoxetate disodium)

Gadoxetate disodium was first approved as Primovist in 2004 and was later approved in the US as Eovist in 2008. The 0.25 mmol/mL solution for injection is approved for i.v. administration at a dose of 0.1 mL/kg/BW (0.025 mmol/kg BW) for T1w MRI of the liver. Eovist is selectively taken up by hepatocytes resulting in increased signal intensity in liver tissue. It is eliminated in equal amounts via the renal and hepatobiliary routes.

Eovist has proven effective at detecting small metastases (< 1 cm). In dynamic and delayed imaging, Eovist improves the detection of liver lesions (e.g. number, size, segmental distribution and visualization) and provides additional information regarding classification and characterization of focal liver lesions, thereby increasing diagnostic confidence.

In clinical trials in > 2,400 subjects, the most frequently observed ADRs (≥ 0.5 %) in patients receiving gadoxetate disodium were nausea, headache, feeling hot, blood pressure increased and dizziness.

In post-marketing experience in > 4.3 million patients, anaphylactoid shock has been the most serious adverse drug reaction reported in patients receiving Eovist. The low dosing (1/4 that of other GBCAs) and dual elimination pathways (50% renal and 50% hepatobiliary), and thus relevantly lower exposure (AUC), may be partly responsible for the fact that events such as NSF have not occurred in association with Primovist and acute renal failure has not been plausibly associated.

4.2.4 Overall Adverse Event Profiles of Bayer's GBCAs

As a part of post-marketing surveillance, every adverse drug experience and medication error (both serious and non-serious) reported to the company from any source is captured in a validated electronic Oracle-based database maintained by Bayer's Pharmacovigilance Department. In addition to spontaneously reported events from health care professionals and patients, the database contains cases derived from the scientific literature, cases forwarded from health authorities, serious adverse events (both related and unrelated) from clinical trials, serious and non-serious events (related and unrelated) from observational studies, reports from patient support programs and from active online listening. The database is comprised of various components that fulfill the following functions: storage of adverse event and medication error reports in retrievable and coded fields; performance of database queries; signal validation and tracking; and additional tools to facilitate the retrieval and display of data. Information regarding adverse events, patient history, and laboratory values are coded in retrievable fields using MedDRA, which is up-versioned twice yearly. Concomitant medications are coded using the World Health Organization Drug Dictionary (WHO-DD). All reports received are submitted to regulatory authorities according to the reporting requirements in each country.

Bayer's Pharmacovigilance Department utilizes this collection of adverse event reports to gain a better understanding of a product's overall safety profile once it is on the market and away from the controlled setting of clinical trials. The evaluation of this data can yield valuable safety signals. This can provide insight and real-life evidence as to how the product is being used by consumers and physicians. As useful as the data can be, however, its limitations are equally well recognized, and attempts to utilize such spontaneous reports as a basis for characterization of relative risk can result in flawed and biased conclusions.

Limitations of spontaneous reporting include the following:

- As reporting is voluntary, there is a probability of underreporting, especially for non-serious events
- Reporting customs and regulations may vary by country and region and by individual institutions
- Reporters may have different motivations to report or not report (e.g., whether or not they consider the adverse event to be serious, unexpected and/or related)
- Introduction to new markets may result in a spike in adverse event reporting
- Reports can be stimulated by media coverage or in other ways
- The reports often lack critical details which preclude an assessment

Nevertheless, when looking at the adverse event profiles of Bayer's GBCAs over time, and considering all of the aforementioned limitations, although the products belong to different subclasses (one multi-purpose linear, one macrocyclic, and one liver specific linear agent), the overall reporting rates for all three products are very low and comparable across the three products. Furthermore, the pattern of AE occurrence is well established and is almost exactly the same across the different "types" of GBCAs.

The majority of events are hypersensitivity reactions, most of which are non-serious. For all products, the reporting rates for life-threatening and fatal anaphylactoid reactions are exceedingly low. The highest reporting rates are seen in the Skin and Subcutaneous Disorders SOC (primarily urticaria) and the Gastrointestinal Disorders SOC (primarily nausea and vomiting) for all three products.

In summary, over the company's vast experience over the past three decades the overall reporting rates for all of three of Bayer's GBCAs is low and well within the ranges quoted in literature. Consistent with observations in clinical trials, the majority of events for all three products are non-serious, mild to moderate in intensity and transient. Most of the reported events can be characterized as hypersensitivity reactions. Furthermore, although the three products are distinctly different in their chemical structures (one multi-purpose linear, one macrocyclic and one liver-specific agent), the overall patterns and reporting rates of adverse events seen with each agent are very similar and in some cases identical. The safety profiles of all agents are well-established and the benefit-risk profiles of each agent are positive.

4.3 Nephrogenic Systemic Fibrosis

NSF is a systemic scleromyxedema-like fibrosing disorder that occurs primarily in patients with severe renal impairment. The disorder is typically characterized by fibrosis of the skin and connective tissues; muscles and internal organs may also be affected in some patients. It was first described in the medical literature in 2000, with the first identified case dating back to 1997 (**Cowper, 2000**).

Major signs and symptoms of NSF include marked skin induration, patterned plaques, "cobblestoning" and joint contractures, primarily of the lower and upper extremities.

A possible link between NSF and GBCA administration was first suggested by the Austrian researcher Thomas Grobner in 2006 (**Grobner, 2006a**; **Grobner, 2006b**)**.** Bayer received its first report of NSF associated with Magnevist in July 2006.

Since then, Bayer has received 220 single agent reports of NSF-like symptoms in patients who reportedly received Magnevist, 74 of which, based on currently available information, meet the Cowper/Girardi clinicohistopathological scoring criteria (**Girardi, 2011**), as being diagnostic of (n = 32) or consistent with (n = 42) NSF. These numbers are likely to be overestimated, as Bayer takes a very conservative approach when applying the clinical and histopathological scores. The vast majority of the reports involved high and/or repeated dosing in patients on dialysis. Although Magnevist is marketed in more than 100 countries, and was the market leader with more than 50% of all sales during the critical time period, these reports originated from only four countries, with the vast majority (> 90%) originating from the United States and most of these received as a result of the litigation process in the US. Other countries and regions that had high Magnevist usage during the critical time period in which NSF emerged had very few or no

single agent reports of NSF with Magnevist (e.g., only 6 in Europe from 1988 – 2017 and none in Japan). Notably, during the critical time period when NSF emerged (prior to 2006), Magnevist and Omniscan completely dominated the market – MultiHance had only been recently launched and had little opportunity to have any "unconfounded" reports, and Eovist, Gadavist, and Dotarem were not even approved or available in the US.

Overall, in 34 reports of NSF-like symptoms involving Magnevist and other products received by Bayer, Gd was measured in the skin and/or other tissues and organs of patients. This is discussed further under the section on Gd presence in the body.

For Gadovist, to date, only three single agent reports diagnostic of (n = 2) or consistent with (n = 1) NSF have been received, all from Europe.

For Eovist, no reports of NSF have been confirmed from any source. The reasons for this likely include the lower overall usage and later market entry of Eovist, the low dose/low systemic exposure to Gd, the dual (renal and hepatobiliary) elimination pathways, and less usage in the severely renally impaired population.

Risk minimization efforts by the manufacturers, radiologists, and regulatory authorities (including the addition of a black box warning by the FDA and the elimination of high and repeated dosing in the high-risk severely renally impaired population by clinicians) were immediately successful in reducing and then virtually eliminating new occurrences of this disorder.

From evaluation of all available information, NSF is a rare disorder, and its etiology is likely to be multifactorial. Although the severely renally impaired population has been identified as being primarily at risk, and the leading theory of causation involves the partial dechelation of certain GBCAs, clearly this is not the only factor, as most severely renally impaired patients do not get NSF, despite being on dialysis and receiving multiple high doses of even the least stable agents. Additionally, there are NSF reports associated with even the most stable macrocyclic agents.

4.4 New Potential Risk Identified for all GBCAs: Presence of Gadolinium in the Brain and Body

In 2014, articles were published by researchers in Japan and Italy noting an increase in signal intensity in certain areas of the brain (specifically the dentate nucleus and globus pallidus) on the unenhanced scans of patients who had received multiple contrast-enhanced MRIs.

The **Kanda (2014)** and **Errante (2014)** studies suggested a correlation between the number of GBCA administrations and increased signal intensity on T1w unenhanced imaging (**Kanda, 2014**; **Errante, 2014**). However, both studies had many acknowledged limitations, including the lack of pathologic correlation with imaging studies, lack of dosing information, the retrospective nature of the studies, and the small or inadequate control groups. No adverse events were specifically noted in the patients with increased signal intensity in the dentate nucleus or globus pallidus compared to those who did not have this increased signal intensity.

The publications by **Kanda (2014**) and **Errante (2014)** triggered an intense effort by the Pharmacovigilance Department to investigate this topic in order to try to answer the important clinical questions:

- Is increased SI always indicative of Gd presence in the brain or can there be other reasons for it?
- If the increased signal intensity serves as a surrogate for the presence of Gd in the brain, what is its clinical relevance?
- What kind of clinical manifestations could be anticipated from dysfunction of the DN and GP, since those are the areas most frequently manifesting the increased SI?
- Is there a limit of toxicity that can be defined?
- What about the traces of Gd that have been measured in other organs and tissues?
- If any adverse effects are associated, are certain patient populations at increased risk?
- What risk mitigation measures can be put in place to ensure that health care providers are aware of the emerging data so that individual benefit-risk decisions can be made that are in the best interests of the individual patient?

This search for any potential adverse effects, including subtle and possibly late-occurring effects, is being pursued in a multidirectional manner and has been ongoing for approximately three years with no confirmed conclusions.

In addition to ongoing review of the clinical literature, the following searches of the PV database (not limited to GBCAs) are being pursued on an ongoing basis:

- Weekly ongoing monitoring of case reports of special interest
- Ongoing review of all adverse events reported in association with GBCAs, with initial focus on Nervous System Disorders, and prospective and retrospective review of long-term chronic more generalized symptoms.
- Monthly qualitative review of reports of Gd presence in any organ with any product with or without clinical symptoms
- Monthly quantitative signal detection based on defined computerized algorithms and proportional reporting ratios.
- Targeted signal detection review of Bayer's ~300,000 reports in MS patients (assumed to have received multiple contrast-enhanced MRIs) by body system
- Re-review of NSF radiology reports and medical records to search for increased signal intensity, presence of Gd and/or non-NSF but possibly Gd-related symptoms
- Review of reports of intrathecal administration
- Ongoing review of GBCA administration during pregnancy and associated fetal and childhood outcomes

- Periodic and cumulative review in the context of annual periodic benefit-risk evaluation reports

4.4.1 Clinical Relevance of the Presence of Gadolinium in the Brain

As early as 2001, increased signal intensity was observed and Gd presence was detected in the CSF of renally impaired patients (**Rai, 2001**), with speculation that the point of entry could be the choroid plexus of the brain or the ciliary body of the eye, both of which have fenestrated capillary endothelia, or the circumventricular organs in the brain, which normally lack tight junctions in the capillary endothelium. No adverse events were associated with this phenomenon – the author rather considered it a "diagnostic pitfall" that could potentially lead to confusion or misdiagnosis.

Other earlier publications of increased signal intensity and/or reported persistence of Gd in the CSF or subarachnoid spaces included the following:

- **Ong, 2007**: This publication described a 66-year-old woman with diabetic nephropathy who presented with symptoms suggestive of a stroke and received Magnevist for evaluation. The following day, strikingly increased signal intensity was seen diffusely in the subarachnoid spaces on the unenhanced T1w images. The CSF signal hyperintensity was consequently attributed to the probable persistence of Gd in the CSF. Authors commented that the presence of Gd in the CSF resulting in high signal intensity on MR imaging is a rare phenomenon that has been described in a number of patients with renal impairment, brain tumors, intracranial or spinal infection, and acute stroke. It has been postulated that this is due to the breakdown of the blood-brain barrier from intracranial pathology, and that the rate of accumulation in the CSF is proportional to the size of the area of disruption.
- **Yildiz, 2013**: This publication described increased signal intensity of the subarachnoid spaces (SAS) and cystic metastatic lesions in a patient in which authors theorized that blood-brain barrier disruption and abnormal plasma clearance due to mild renal functional impairment might have resulted in accumulation of Gd in cystic metastases and SAS on T1-weighted and FLAIR images. They suggested that two administrations of GBCAs (15 mL each, for a total of 30 mL) within approximately 30 hours also might have prolonged the clearance of the Gd from the plasma and resulted in continued hyperintensity up to 160 hours on FLAIR images.

Although no adverse events were described in the above-referenced publications, a 2005 article by **Maramatton et al. (2005)** reported Gd-induced encephalopathy in a 57-year-old renally impaired female who was hospitalized with left hemiparesis, acute difficulty in walking and hypertensive crisis following a 40 day fast. Over the next seven days, the patient underwent MRI and MRA examinations of the brain, receiving a total of 60 – 80 mL gadolinium-based contrast media. The specific GBCAs were unknown; authors considered the event more likely an effect of Gd itself rather than a specific Gd chelate complex. The patient's mental status progressively declined during the week Gd was administered. MRI showed multiple subcortical hyperintensities (multiple lacunar infarcts) without evidence of a posterior encephalopathy syndrome. On day 14, the patient underwent a non-contrast MRI which showed a marked reduction in the CSF hyperintensity from previous MRIs. On review of earlier MRI films, it was noted that the CSF had become increasingly hyperintense with successive MRI scans.

White matter lesions remained unchanged. As the CSF did not indicate an infectious process, subarachnoid hemorrhage, or elevated proteins, Gd diffusion in the CSF was considered. To evaluate this possible diagnosis, on day 14, serum Gd level was checked and found to be 28,591 ng/mL. Authors hypothesized that Gd retention due to renal failure led to Gd -induced encephalopathy. In a second publication about this case (**Hui, 2009**), authors stated that the patient recovered after dialysis.

In most of the recent publications, the increased SI is predominantly observed in the DN and the GP. However, even with the greatly increased scrutiny on the topic of Gd presence in the brain and other organs over the last three years, most publications still only describe increased signal intensity in the brain; very few describe associated clinical symptoms.

Other literature reports in the PV database describing increased SI in the brain include:

- **Roberts et al. (2017**) used laser ablation ICP-MS (LA-ICP-MS) to demonstrate the distribution of Gd presence throughout the cerebellum in a deceased 17 year old patient with normal renal function who presented to the hospital in status epilepticus, the proximal cause of death. Prior to death, the patient underwent 4 MRI scans with GBCA administration (two with Magnevist and two with an unspecified GBCA, probably Magnevist or Omniscan). The 4 GBCA doses were administered over a 10-week period with the last dose administered 84 days before death. It was not specified why the patient was having this series of MRIs. On MRI obtained prior to the last dose of GBCA (i.e., after 3 scans), no hyperintense signal was noted within the dentate nucleus, which previous studies suggest becomes visible only after approximately 5 GBCA administrations. Despite the lack of T1w hyperintensity within the DN on MRI, heavy presence of Gd was found within the DN on the corresponding LA-ICP-MS Gd distribution map, in addition to Gd presence throughout the cerebellar cortex. Interestingly, higher levels of Gd presence were noted within the folia depths, which may be related to cerebellar microvasculature as arterial ramifications are present within the bases of the folia, a site that demonstrates selective vulnerability during ischemic states. Using ICP-MS, total Gd concentration within the dentate/peridentate white matter was 1.01 mg/g, similar to previous reports. For example, McDonald, 2015 reported 0.1–58.8 mg/g of Gd within the dentate nuclei of patients receiving 4–29 doses of a linear GBCA.

***Bayer's comment**: This case demonstrates that Gd may be present despite the lack of signal hyperintensity. In this case, it is unknown why the patient was having this succession of MRIs in the months preceding his death. Authors did not attribute either the status epilepticus or the death to the presence of Gd. In agreement with the authors, "the potential clinical significance of Gd presence within the brain is unknown; further research is needed to identify any long-term clinical consequences (e.g., possibly subtle signs/symptoms of dentate nucleus dysfunction.)"*

Meanwhile, **Quattrochi et al.** (**2015**) who noted an increased signal intensity in the DN of patients with meningioma who had received 6 or more administrations of Omniscan, specifically noted that to date, they had not received any reports of neurologically significant complaints that could be attributed to the hyperintensity of the dentate nucleus (**Quattrocchi, 2015**).

In summary, with the exception of Maramatton et al. (**Maramattom, 2005**), this review of literature which reports adverse events in association with SI increase or Gd presence in the brain did not confirm an association of Gd administration with the reported symptoms.

4.4.1.1 Reports in MedDRA SOC "Nervous System Disorders" with Bayer's GBCAs

Most reports of increased SI occur in the DN and GP. For this reason, Bayer PV's initial focus was on adverse events occurring in the Nervous System Disorders SOC and especially on events that could potentially result from damage to the DN or GP.

The cerebellum is involved in the following functions:

- Maintenance of balance and posture
- Coordination of voluntary movements
- Motor learning
- Certain cognitive functions

The cerebellum consists of two major parts: the deep cerebellar nuclei (DCN) and the cerebellar cortex. The DCN are the sole output structures of the cerebellum. Encasing the DCN is the cerebellar cortex, which contains almost all of the neurons in the cerebellum.

In general, patients with cerebellar damage may display uncoordinated voluntary movements and problems maintaining balance and posture. These disorders include ataxia, dysmetria, dysdiadochokinesia, intention tremors, and delay in initiating movements. Patients with cerebellar damage may also demonstrate subtle cognitive deficits, such as an impaired ability to estimate time intervals.

The DN is the largest of the cerebellar nuclei. The DN receives input from the lateral hemispheres. Its name derives from its extensive connections with the cerebral cortex, via the pontine nuclei (afferents) and the ventrolateral thalamus (efferents). The DN is responsible for the planning, timing, initiation and control of voluntary movements and contains complex input and output channels that are also involved in nonmotor function, such as cognition and visuospatial function. The motor and non-motor functions are inexorably linked by these complex processes. Evidence for a motor and non-motor domain in the human dentate nucleus was also demonstrated by **Kuper et al**. (**2011**).

The GP is a paired structure in the brain and is one of the nuclei that make up the basal ganglia. The globus pallidus has a primarily inhibitory action which balances the excitatory action of the cerebellum. These two systems are designed to work in harmony with each other to allow people to move smoothly, with even, controlled movements. Imbalances can result in tremors, jerks, and other movement problems, as seen in some people with progressive neurological disorders such as Parkinson's disease.

The initial focus of PV was on events occurring in the Nervous Systems Disorders SOC and these events are still under evaluation. After review of all reports of movement disorders and/or cognitive dysfunction associated with each of Bayer's GBCAs, no permanent neurological disorders or brain damage of any kind could be confirmed for any of the GBCAs. In general,

such events occurred in association with acute, sometimes hypersensitivity, reactions to GBCAs, were of mild to moderate intensity and were transient or self-limiting. Furthermore, the reporting rates for such reactions are very similar for Bayer's three distinctly different GBCAs. The nature of the reported reactions is also similar across the products. For all three products, the two most commonly reported events in the Nervous System disorders SOC were dizziness and headache. This was followed by paresthesia for both Magnevist and Gadavist and by tremor for Eovist.

After almost 30 years on the market and more than 148.8 million administrations, ataxia has only been reported in three Magnevist cases. Two of these occurred in association with unapproved intrathecal administration (20 mL and 10 mL intrathecal respectively). In the third sparsely documented case from 1993, a 26-year-old female experienced "gait impairment secondary to impaired balance" immediately following a Magnevist enhanced scan. Ataxia was not reported in association with Gadavist or Eovist. Neither Parkinson's disease nor Parkinsonism has been reported for any of Bayer's GBCAs.

To examine an association between Gd administration and Parkinson's disease, a study by **Welk et al. (2016**) retrospectively examined a cohort of almost 250,000 patients and found no significantly increased incidence of Parkinson's disease in patients exposed to GBCAs (1.17%) vs. those who were not exposed to GBCAs (1.16%) (**Welk, 2016**).

Other nervous system events of potential interest are discussed under the category Gd presence in the body and Gd disorders as proposed by **Semelka et al. (2016)**.

A first suggestion of possible dentate nucleus dysfunction associated with GBCA administration was reported in 2017.

Forslin et al. (2017) published the results of a retrospective evaluation of the relationship of multiple GBCAs administrations to the Signal Intensity Index (SII) in the DN and GP and any associations with cognitive function in MS (**Forslin, 2017**). The 18-year longitudinal cohort study included 23 patients with MS who received multiple GBCA administrations (Omniscan, Magnevist and Dotarem) and 23 healthy age and sex-matched controls. Participants underwent comprehensive neuropsychological testing. Patients with MS who received multiple contrast administrations had a higher SII in the DN and GP compared with non-exposed healthy controls. The increased SII in the dentate nucleus in patients with MS was associated with lower verbal fluency scores, which remained significant after correction for several aspects of disease severity. After adjustment for all other factors, this was the only cognitive measurement that remained associated with the increased signal intensity. Authors noted that executive functioning, including verbal fluency, was less frequently affected in MS than other factors such as episodic memory and information processing speed. However, they noted that many areas of the brain are affected by MS, and that the effects of the disease itself on the neuropsychological test results could not be excluded. Authors acknowledge that a major limitation to their study was the fact that they could not find a matched MS group without exposure to GBCAs.

Bayer's comment: *Bayer agrees that the study results would be far more clinically relevant and meaningful if an MS group exposed to GBCAs was matched with an MS group not exposed to GBCAs, rather than with non-exposed healthy controls. In agreement with the authors, these results should be interpreted with caution. The test results are very likely to be confounded by progression of the underlying disease.* ***Chiaravallotti and DeLuca (2008)*** *noted that*

*"Multiple sclerosis (MS) is a progressive disease of the CNS that is characterized by widespread lesions in the brain and spinal cord. MS results in motor, cognitive, and neuropsychiatric symptoms, all of which can occur independently of one another. The common cognitive symptoms include deficits in complex attention, efficiency of information processing, executive functioning, processing speed, and long-term memory." Chiaravallotti and DeLuca also emphasize that cognitive tasks that require a rapid spoken response might be detrimentally affected by impairments in rudimentary oral motor functions in people with MS, and that decreased performance on tasks that require oral responses may be partially caused by dysarthria in MS patients (**Chiaravalloti, 2008**). This may be one of the explanations for the result observed by Forslin. However, the results of the Forslin study emphasize the need for further studies on potential subtle or long-term effects of GBCA administration.*

One other publication referenced a decrease in executive function:

Miller et al. (2015) described a child diagnosed with rhabdomyosarcoma at age 5 by contrast-enhanced MRI who over the course of 12 years received 35 additional scans with Magnevist (**Miller, 2015**). The patient had undergone radiation, surgery and chemotherapy for his underlying and recurrent diseases. At the age of 21, neuropsychological testing revealed "difficulties with executive functioning", a well-recognized late effect of the patient's radiation therapy, surgery, chemotherapy and underlying conditions. The authors specifically attributed only the increased signal intensity to GBCA administration.

***Bayer's comment**: In Bayer's opinions, this patient's difficulties with executive functioning are clearly confounded by the underlying diseases and treatments. The radiation therapy he received could also have influenced the increased signal intensity. Both the Forslin and Miller articles show a direct and significant confounding by the underlying diseases and treatments. However, since both articles reference an effect on executive functioning, they might provide a "hint" of what to look for neurologically when searching for subtle or long-term effects of Gd presence in the DN, and Bayer continues to investigate this possibility.*

"Executive function" is a complex concept that is defined in different ways by different clinical disciplines. **Koziol et al. (2012)** propose a definition that places executive function within a model of continuous sensorimotor interaction with the environment encompassed in the broader definition of "cognition" and focuses on the cerebellum's critical role in those processes. Executive functioning" (EF) can include problem solving, planning, concept formation, strategy development and implementation, and controlling attention and working memory; action (or movement) and goal-directed behavior are inherent in the concepts and definitions of cognition and EF (**Koziol, 2012**).

A search for the MedDRA Preferred Term (PT) "Executive dysfunction" in Bayer's large PV database, which contains information on hundreds of products and many hundreds of thousands of cases, retrieved only six cases, including the one described above in Miller et al. The other five cases all involved different products, none of which were assessed as being causally linked to the development of the events. Several of the patients in the cases had undergone MRI examinations, all apparently after the onset of symptoms.

Since Bayer also markets a multiple sclerosis drug, Betaseron, its safety database contains almost 300,000 reports containing approximately 900,000 events involving multiple sclerosis patients. Assuming that the majority of these patients have likely received multiple contrast-enhanced

MRIs, Bayer PV, in association with a neurologist familiar with Betaseron as well as with the variable course of MS, has undertaken a search of these reports for events in any body system that seem unusual or inconsistent with the variable spectrum of MS which theoretically could have been caused or exacerbated by the presence of Gd in various organs. No reports of executive dysfunction were reported in this patient population; however, there were a very small number of reports coded to the MedDRA Preferred Term (PT): "Slow speech", which could be indicative of a decrease in verbal fluency as noted by Forslin. In a few of these reports, patients also noted a disturbance in attention or differences in their cognitive functioning. However, as stated by Forslin et al., and confirmed by other experts in the field, such effects could very well be a manifestation of disease progression or dysarthria. Furthermore, the reports did not specifically mention any GBCA administration, and the number of reports was too small to conclude that the events could be attributable to probable GBCA administration rather than the underlying disease.

The occurrence of Parkinson's disease and parkinsonism [SMQ Parkinson-like events] were also reviewed in this MS population. Search retrieved a total of 18 cases coded to PT Parkinson's disease and 4 cases coded to Parkinsonism (Total: 22 cases). Results suggest that multiple sclerosis itself can lead to parkinsonism when a demyelinating lesion is strategically located within the basal ganglia and therefore responsible for extrapyramidal symptoms. Idiopathic or autoimmune mechanisms for development of Parkinson's disease are also suggested.

Events affecting other SOCs in MS patients, based on Bayer's safety database, are addressed in the section on Gd in the body.

Other nervous system disorders reported in the literature in patients with increased signal intensity in the brain include the following:

Barbieri et al. (2016) described three patients with impaired renal function and vascular calcification (including two NSF patients) who received 5-9 administrations of both linear and macrocyclic GBCAs and developed increased signal intensity in the brain (**Barbieri, 2016**).

The authors reported that all patients suffered from "transient signs of neurological disorders of unspecified cause" including episodes of acute confusional states and impaired vigilance.

In one of these reports**,** a 52-year-old female with chronic kidney disease requiring kidney transplantation and dialysis received 8 GBCA administrations between 1996 and Dec 2005: two with Omniscan (in 1996 and 2001), three with ProHance (in 2001, 2002 and Apr 2005), two with Magnevist (in 2001 and 2002), and one with Gadovist in Dec 2005. She was diagnosed with NSF in Dec 2005. In Dec 2005, she experienced episodes of confusion and impaired consciousness. She received a total of 0.68 mmol/kg of linear agents and 0.58 mmol/kg of macrocyclics. At an unspecified time point, increased signal intensity was observed in the DN and GP.

In the other report involving Bayer products, a 65-year-old female with polycystic kidney disease and calcific uremic arteriopathy received one dose of Omniscan in 1999, followed by five administrations of ProHance and one administration of Gadovist between 2001 and 2006, with the last GBCA (ProHance) given in Mar 2006. The patient received a total of 0.27 mmol/kg of the linear agent Omniscan and 0.95 mmol/kg of the macrocyclic agents Gadovist and ProHance. Increased signal intensity in the DN and GP was observed in 2003, 2005 and 2010. In 2010, the patient was evaluated for impaired vigilance and episodes of acute confusional state.

***Bayer's comment**: Bayer agrees with the author that the transient neurological disorders could not be causally linked to the GBCA administrations or to the increased signal intensity. Authors also could not exclude the underlying vascular calcification or minerals supplemented during dialysis as additional causes for the observed signal hyperintensities, and this should serve as a reminder that Gd presence might not be the only reason for increased signal intensity in the brain. As all of the patients were exposed to multiple products, including both linear and macrocyclic GBCAs, no conclusions about individual products or about linear vs. macrocyclic GBCAs could be made.*

In summary, no clear causal association can be confirmed between the few published reports of neurological disorders and increased signal intensity or possible presence of Gd in the brain.

4.4.2 Clinical relevance of gadolinium presence in areas of the body other than brain

The presence of Gd has been described in tissues and body fluids other than the brain.

In 2004, Bittle described the case of a 39-year-old female who suffered traumatic injuries after being thrown from and stamped on by a horse (**Bittle, 2004**). She had multiple fractures of her ribs, pelvis and left humerus, and a deep laceration of her left elbow. The patient underwent diagnostic imaging including an MRI of the head with Magnevist (dose not reported). She had declining hemoglobin, which was attributed to blood loss from her fractured pelvis, and blood transfusions with packed red cells were initiated. Four months later, she underwent a second MRI with Magnevist to assess the pelvic fracture for soft tissue sequestration of blood. Immediately following the MRI, she experienced exacerbation of anemia requiring increased amounts of PRBC infusions to maintain her hemoglobin level. Five months after the accident, a 24-hour urine heavy metal screen to rule out lead intoxication was positive for arsenic. Lab results from two independent laboratories showed arsenic at 118 mcg and 128 mcg, respectively (normal range 0 - 62 mcg). She was referred for arsenic chelation therapy. A provoked 24-hour urine heavy metal screen with NaMgEDTA induced the release of 202 mcg of Gd, 404 times the normal threshold of 0.5 mcg, with an accompanying provoked release of zinc and manganese. After five weeks, her condition stabilized. Eighteen months after her injury, a third MRI with Magnevist was performed at another facility without recurrence of anemia. Authors theorized that arsenic was released from stored deposits in inflamed bone and that dissociated Gd3+ ions could be released from metal-ligand complexes like Gd-DTPA (gadopentetate dimeglumine) where they can precipitate as salts in serum or can be deposited in tissues by transmetallation.

In 2010, Greenberg described the occurrence of prolonged urinary Gd excretion in a 55-year-old male who developed progressive numbness in his hands and feet and gait ataxia due to zinc toxicity (**Greenburg, 2010**). Unenhanced MRI of the cervical and thoracic spine showed signal hyperintensity on T2-weighted images within the spinal cord. During the course of the patient's evaluation, he underwent two unspecified Magnevist-enhanced MR procedures. The patient was treated over a six-month period with oral chelation therapy and with intravenous chelation infusions. Two years later, the patient learned that a denture cream (Super Poligrip; GlaxoSmithKline Consumer Healthcare, Brentford, England) he had been using for at least 10 years contained large amounts of zinc (28-34 mg of zinc per gram of cream). 24-hour urine

collections intended to monitor zinc removal simultaneously measured levels of 34 other elements, including Gd. At 241 days, after receiving the initial dose of Magnevist during the first enhanced MR imaging examination and before starting chelation therapy, urine Gd excretion was 0.8 µg/day, a level interpreted by some laboratories (>0.5 µg/d) as elevated. At 29 days after undergoing his second enhanced MR imaging, after starting chelation therapy, Gd excretion was 89 µg/d. Urine Gd excretion continued during chelation therapy; at 166 days after the second Gd exposure, 34 µg/d of Gd ion was being excreted. Most Gd was excreted during the 154-day period of regular chelation treatment, although 869 days later, after an additional 2 years of intermittent chelation treatment, 0.6 µg/d of Gd was present in urine. The estimated total amount of Gd excreted (the area under the curve) was 9283 µg (9.3 mg), which is equal to approximately 0.59% of the amount received during the second injection. Because urine Gd measurements did not begin until 17 days after chelation therapy was started, extrapolation predicts that approximately 89 µg/d was excreted during that period, for an estimated total excretion of 0.69% of the amount received during the second injection. Authors theorized that gadolinium-zinc transmetallation could occur in patients with massive zinc overload syndromes, in which excess zinc in the body might displace free Gd ions from their chelates.

Roberts et al. (2016) described a patient with normal renal function and a history of an aggressive brain tumor treated with resection, chemotherapy and radiation (**Roberts, 2016**). Over the course of 11 years, between the ages of 19 and 30, he had undergone 61 contrast-enhanced MRIs. The patient was potentially exposed to multiple contrast agents, including MultiHance, Magnevist, Omniscan and ProHance, all of which were used at the institution at the time. Likely the highest level of exposure was to MultiHance which was the agent most used for adult brain tumor imaging. The last MRI was performed 8 months before the skin biopsy. The patient also had a history of a seizure disorder, and had significant cognitive and developmental delays with visual impairment bilaterally. The patient had no skin issues, but did suffer from joint contractures of unknown etiology. A skin biopsy of the right forearm and right shin showed no signs of NSF but revealed high levels of gadolinium, including intact MultiHance and Magnevist. Authors stated that they used a mild extraction technique in order to keep any potential ligand-GBCA complex within the sample intact. There was no co-localization with significant levels of phosphorus in the tissue sample. Authors recommended that caution be used in patients receiving large cumulative doses of GBCAs.

Bayer's comment: *The etiology of the patient's joint contractures were unknown but could potentially be related to his severe underlying conditions. His seizure disorder, cognitive and developmental disorders were likely a result of his aggressive brain tumor and subsequent resection, radiation and chemotherapy. The case illustrates that high levels of gadolinium may be retained in the skin of patients with normal renal function without resulting in NSF or other symptoms. Additionally, the method of extraction used might impact the nature of the gadolinium species found.*

Bayer is utilizing its long-term experience with multiple sclerosis patients and its large safety database containing reports from multiple sclerosis patients for general signal detection, assuming that most or all of these patients have received multiple GBCAs. Thus far, no signals have been detected in any SOC that could potentially be the result of Gd administration. Evaluation of these cases included, for example:

- Any cases involving presence of metals: The only element that has been specifically identified is mercury.
- Unusual skin disorders, including those mimicking NSF, such as sclerotic skin plaques: No indication of any cutaneous disorders potentially attributable to Gd presence.
- Renal disorders: No indication of any unusual events possibly attributable to Gd presence in the kidneys.
- Additionally, no signals were detected in any other body system, or for any other event, including rheumatic disorders, other musculoskeletal disorders, and disease progression.

4.4.2.1 Presence of gadolinium in patients with NSF-like symptoms

A search of Bayer's reports of NSF/NSF-like symptoms involving Magnevist, Gadavist and unspecified GBCAs (there are no reports of NSF/NSF-like symptoms associated with Eovist) resulted in 34 reports in which Gd/GBCAs were detected in various organs of the body by various methods, either on biopsy or on autopsy. In 29 of the patients, Gd was detected in the skin, and was detected in other organs/other parts of the body as indicated in **Table 4**.

Table 4: Reports of gadolinium/GBCA detection in patients with NSF/NSF-like symptoms

Organ/Part of Body	***Number of Patients***
Skin	29
Heart	4
Liver	4
Lung	4
Kidney	3
Muscle	3
Adrenal gland	1
All body tissue NOS	1
Blood	1
Cecum	1
Colon	1
Hair	1
Ileum	1
Lymph node	1
Nails	1
Plasma	1
Thyroid	1
Toxic elements screen	1

Patients in the 34 reports received from 1 to 19 GBCA administrations. Products administered in the reports in which gadolinium was detected included Magnevist, Magnevist Pharmacy Bulk Package, Dotarem, MultiHance, Omniscan, OptiMARK, ProHance, and unknown GBCAs. Four of the patients had normal renal function or mild to moderate renal dysfunction; the remaining 30 had severe chronic or acute renal impairment.

Of the patients with normal renal function or mild to moderate renal dysfunction:

- One elderly gentleman with moderate renal dysfunction developed skin tightening, tethering and hyperpigmentation, flexion contractures and reticulated eczema craquele. Skin biopsy showed only a subtle increase in fibroblasts and no other features of NSF. Differential diagnosis was eczematous dermatitis.
- A middle-aged female with no renal dysfunction received only one administration of a GBCA and developed fibrotic changes, skin hyperpigmentation, skin thickening with peau d'orange appearance, indurated plaques, and flexion contractures. Her skin biopsy showed increased dermal cellularity, CD34+ cells in a tram-track pattern and septal thickening. As her renal function was normal, the clinicohistopathological score was only "suggestive of NSF." Successive skin biopsies definitively diagnosed eosinophilic fasciitis (Shulman's syndrome).
- A middle-aged female with chronic kidney disease III/moderate renal impairment reportedly developed NSF after receiving one GBCA administration on an unknown date. While the reporter stated that the case was "biopsy proven", no clinical signs or symptoms were reported and no biopsy was provided. In follow-up with the hospital, they stated that they were unaware of such a case.
- A middle-aged female with renal insufficiency per legal complaint but no renal disease per medical records claimed to have received 24 contrast-enhanced MRIs with various products, although only eight could be found in the medical records. She developed thickened skin with an orange-peel texture, and mottled hypo- and hyperpigmentation. Skin biopsy showed increased fibroblasts, thickened septa and mildly increased CD34 expression. Elastic fibers were altered, decreasing the histopathological score by 1. Overall diagnosis was "suggestive of NSF." Differential diagnoses included erythema nodosum, lichenoid drug eruption, inflamed seborrheic keratosis, mild spongiotic dermatitis, venous stasis dermatitis, and pseudoxanthoma elasticum.

No gadolinium-associated plaques and no events clearly attributable to gadolinium administration were reported in the patients with normal renal function who reported NSF-like symptoms.

At least eight of the patients with detectable Gd levels (in the skin, heart, and/or lungs) were considered unlikely to actually have NSF or were determined to not have NSF. For example, one patient with end stage renal disease whose skin biopsy samples demonstrated relatively high amounts (479 and 448 ppm, dry weight, formalin fixed) of Gd by ICP-MS had NSF-like clinical features (hardening of skin, peau d'orange, woody edema, and hyperpigmented areas), but a biopsy score inconsistent with NSF. As no differential diagnoses were provided for this patient, it cannot be completely excluded that the symptoms were attributable to Gd. In another patient with end-stage renal disease who received 9 GBCA administrations, the legal complaint alleged without medical confirmation that "high levels of Gd were found in her heart tissue."
The patient's clinical signs and symptoms included "fibrotic changes" NOS and contractures. Her skin was also reportedly found to contain 232.9 and 135.4 ppm Gd (dry weight basis) in two separate skin samples. Yet, this patient's skin biopsy was "not at all supportive" of a diagnosis of NSF. Histopathology was near normal, showing none of the features of NSF. In yet another patient with ESRD who received 16 GBCA administrations and developed skin induration, contractures and erythematous plaques, dry tissue specimens revealed 50-60 mcg/g Gd

("normal" range reported as < 0.5 mcg/g), yet skin biopsy diagnosed epidermal spongiosis with focal dermal fibrosis and failed to diagnose NSF.

For several other patients, a diagnosis of NSF could not be confirmed because biopsy results were not provided.

Ten of the patients had clinicohistopathological scores diagnostic of NSF and had gadolinium detected in their skin (8 patients) as well as (in one patient each) the kidney, adrenal gland, lymph node, thyroid, liver, lung, heart, muscle, colon, ileum, cecum and "all body tissue" NOS.

In summary, traces of gadolinium were found by various methods in the skin and other organs of patients with either normal renal function or renal impairment who reported having NSF-like symptoms, whether or not they had a confirmed diagnosis of NSF. No specific presence of gadolinium in the brain was reported. Measurements had been taken from one month to seven years after the last GBCA administration and the timeframe was unknown in some patients. The clinical significance of this gadolinium presence, especially in organs and tissues other than the skin, is unknown. As gadolinium was detected in patients with and without NSF, it cannot be concluded with certainty that the gadolinium presence led to development of NSF. In one report in which NSF was not diagnosed, association of the reported symptoms with GBCA administration could not be excluded, as no differential diagnoses were provided. There were no reports of "gadolinium-associated plaques" in patients who were not diagnosed with NSF. These 34 reports are compiled in a table attached in **Appendix 1**

In addition, reports of gadolinium presence in the bodies of patients with reported NSF have been derived from the literature.

Researchers such as **Caravan and Koreishi** (**Caravan, 2009; Koreishi, 2009**) have reported on gadolinium quantification in the autopsies of patients who reportedly had NSF. Three of these cases involving Bayer GBCAs are included in the list of cases in **Appendix 1** . Another researcher (**Sanyal, 2011**) analyzed the tissues of a deceased 36 year old patient with NSF for the presence of Gd. The patient had received repeated contrast-enhanced scans with Omniscan (total dose 65 mL or 0.5 mmol/kg bw). Gd deposits were detected in her skin, liver, lungs, intestinal wall (ileum), kidney, lymph node, skeletal muscle, dura mater and cerebellum, all associated with calcium and phosphorus. The predominant site of the Gd deposits was in the vessel walls. The authors noted that although Gd was suspected to trigger the systemic fibrosis seen in NSF, Gd was not detected in all fibrotic areas but was frequently detected in the vessel walls, where the calcification in renal failure occurs. Authors theorized that inflammatory and fibrotic effects could possibly develop at sites remote from the Gd deposits, mediated by soluble growth factors and cytokines, such as osteopontin and MCP-1 that have been reported to be upregulated in nephritis and nephropathy. They further acknowledged however that the relative causal contributions of NSF and the underlying chronic renal failure itself to the systemic manifestations seen in NSF patients remains unclear, as non-specific tissue fibrosis and calcification are frequent findings in the tissues of patients with chronic renal failure who do not have NSF. Gd presence was also documented in this patient's brain parenchyma.

Bayer's comment*: In this case, even in an NSF patient with end stage renal disease, in whom gadolinium deposits were detected throughout most of the body, the deposits were not necessarily located in fibrotic areas, making it unclear whether the deposits themselves were causative of the fibrosis. Gd was frequently detected in the vessel walls. The patient also had*

*Gd deposits in the brain, which were not associated with any pathologic changes in the cerebellum, and the authors could find no instances of CNS pathology in other NSF patients. In the study by **McDonald et al. (2015)** also, where Gd was detected in the epithelial walls and in the neural tissue interstitium, there were no detectable histologic differences between the contrast-enhanced group and the control group.*

Birka et al. (2015) presented the combined use of elemental bioimaging and speciation analysis as a novel means for diagnosing NSF. They described the case of a 23 year old female who received Magnevist in 2002 and ProHance in 2005 and developed NSF-like symptoms beginning in 2011. Using ICP-MS, in 2013, Gd concentrations ranging from 3.02 to 4.58 mg/kg were detected in the skin biopsy samples (**Birka, 2015**). Speciation analysis showed the presence of ProHance, reportedly intact 8 years after its administration.

***Bayer´s comment:** A diagnosis of NSF can only be made on the basis of specific clinical and histopathological criteria. While the authors claim to have developed a novel way of diagnosing NSF, no conventional biopsy results were provided to verify the findings. The presence of ProHance in the patient's body eight years after its administration suggests that traces of gadolinium (both linear and macrocyclic) may remain in patients' bodies longer than previously suspected.*

4.4.2.2 Gadolinium Disorders proposed by Semelka and Burke et al. 2016

In 2016, **Semelka et al. (2016**) proposed that there are four major Gd disorders: acute adverse events, NSF, "gadolinium storage condition" (presence of Gd in brain, bone or tissue), and "gadolinium deposition disease" (a name they assigned to patients with normal renal function who experience persistent symptoms not attributable to other causes which they associate with gadolinium presence). Such symptoms may include pain in the lower arms and legs, persistent headache, bone and joint pain, clouded mentation, subcutaneous soft tissue thickening, tightness of hands and feet, and excruciating pain.

The same group of researchers then went on to publish several other articles on this same topic, describing the same group of patients, including the following:

- **Burke et al. (2016)** reported about an anonymous online survey of 50 patients with normal renal function who attributed certain chronic symptoms to "gadolinium toxicity". The most common symptoms included bone/joint pain, skin changes, headache, and vision and hearing changes. The patients received from 1 to 23 administrations of GBCAs and had samples of their urine, blood, skin, hair, thyroid and breast biopsy tested for the presence of Gd. Results of these tests were not provided. Authors hypothesized that patients may have a genetic abnormality in metabolizing heavy metals. They suggested that a possible treatment for "gadolinium deposition disease" may be de-chelation and immune modulation therapy (**Burke, 2016**).
- **Semelka et al. (2016**): Authors reported on four patients with normal renal function who developed chronic symptoms attributed to persistence of gadolinium in the body**.** One case described a 43-year-old female patient who underwent four MRI scans within a 2-month period to rule out MS (OptiMARK 7 mL [0.1 mmol/kg), liver cancer (Eovist 0.025 mmol/kg) and breast cancer (Gadavist 0.1 mmol/kg, and 0.1 mmol/kg). The shortest interval between MRI scans was 5 days. Immediately after the fourth MRI with

Gadavist, the patient noted markedly decreased ability to sense bladder fullness and voiding. The next day, non-pitting swelling began in the injection site and ipsilateral hand, and progressed over several weeks to involve the face, arms, hands, feet, legs, and torso, accompanied by areas of nodular and linear subcutaneous abnormal firmness, except in the face and hands. Swelling and subcutaneous lesions were preceded by tingling neurological pain. The hands and feet continue to ache, more so when pressure is applied, as in standing or walking. Other symptoms include tightening of the skin in the face, neck, hands, feet, and calves; "crawling sensation" beneath the skin in the first month; diminished temperature perception in the hands and feet; diminished memory; fatigue; and subjective muscle weakness. On physical examination by the coordinating physician approximately 3.5 months after the last MRI, the patient showed subcutaneous lesions, skin tightness, and shiny appearance of skin over fingers.

Total Gd amounts in 24-hour urine collections were performed at the Mayo Clinic: 82 μg/24 hours (28 days after the last MRI), 15 μg/24 hours (48 days after), 13 μg/24 hours (56 days after), 7.6 μg/24 hours (69 days after), 0.8 μg/24 hours (74 days after), and 3.3 μg/24 hours (103 days after) (reference values, 0.0-0.4 μg/24 hours; therapeutic trials of high-potency antihistamines, imatinib 100 mg/d for 2 weeks, and prednisone (20 mg/d tapered to zero) failed to stop symptom progression, and symptoms continued at the time of the report.

Another case involved a 29-year-old female with history of medullary sponge kidney who underwent an MR study to evaluate suspected complex renal cysts seen on a previous ultrasound examination. Within 24 hours of receiving Magnevist 20 mL (0.1 mmol/kg). she developed flu-like body aches and paresthesia, which progressed over a period of days into intense feelings of burning and sharp pins and needles affecting the central torso, arms, and legs, all with a similar severity. These symptoms gradually diminished in intensity; the patient still, however, reports sporadic episodes of burning sensations affecting the limbs and torso. The patient also reported experiencing the onset of clouded mentation, severe headaches, and arthralgia soon after the MRI. To date, clouded mentation, severe and frequent headaches, and arthralgia persist to varying degrees. No physical examination findings were seen on examination. Gadolinium was present in serum at 0.7 ng/mL (normal range, <0.5 ng/mL) and in a 24-hour urine sample at 18 μg/24 hours (normal range, 0-0.4 μg/24 hours), both performed at the Mayo Clinic (reference values, 0.0–0.4 μg/specimen) at 31 and 33 days after GBCA administration, respectively.

***Bayer's comment**: Bayer is committed to further exploring the hypotheses proposed by Semelka et al. and to attempt to identify patient populations that may be more susceptible to such effects. The PV department continues to evaluate such reports received by the company and to actively search for evidence of a causal association between the reported symptoms and gadolinium administration. Unlike NSF, which has distinct clinical features and histopathological criteria by which a diagnosis can be made, Semelka et al. presents "gadolinium deposition disease" as a distinct clinical entity, but does not provide parameters by which such a collection of symptoms can be definitively diagnosed, and how such symptoms can be distinguished from other chronic and/or inflammatory conditions that have been long established. In order to avoid having all chronic symptoms, inflammatory symptoms and cutaneous events misclassified as related to gadolinium administration in patients who have received MRIs, it would be important that in order to further characterize*

these symptoms, the same type of scientific discipline and guidelines have to be established as were established for NSF, including:

- *What clinical features define it?*
- *Are there any histopathological criteria or biomarkers that can help diagnose it?*
- *Is a specific patient population at risk for it?*
- *What other conditions should be ruled out before concluding that gadolinium administration was the cause of the symptoms?*

Bayer has received approximately 100 reports of increased SI and/or presence of gadolinium in the body, most of which are not associated with any clinical symptoms. Those in which clinical symptoms are also reported can be found in a table attached in **Appendix 2**

As noted in the table (**Appendix 2**), Bayer has received < 20 reports in which increased gadolinium levels have been reported in association with clinical symptoms for both linear and macrocyclic agents (including Magnevist, Gadavist, Omniscan, OptiMARK, Dotarem, Eovist and unknown GBCAs). The patients received from 1 to 75 administrations of GBCAs. No pattern could be clearly identified when looking at the reported events or the patient population reporting them.

Additionally, Bayer is conducting a retrospective review (dating back to 1988) of all cases in which chronic symptoms were reported. Some of the more recent reports received, in which chronic or persistent symptoms were attributed to Gd, but no evidence of Gd presence was provided are included in **Appendix 3**.

The PV department continues to evaluate such reports and to actively search for evidence of a causal association between the reported symptoms and gadolinium administration. To date, however, Bayer has not identified a clear "index case" that can validate a signal for a potential causal association between the reported constellation of symptoms and gadolinium administration. The cases lack relevant details, many are not medically confirmed, and some do not provide evidence of gadolinium presence in the body. Bayer has developed a targeted follow-up questionnaire in order to request specific additional information to better understand these reports.

In addition to review of the PV database, Bayer is exploring options to conduct several epidemiological studies in large claims databases in order to develop and test various hypotheses and hopefully to gain further insight on the clinical relevance of Gd presence in the body.

4.4.3 Other Ongoing Pharmacovigilance Activities

4.4.3.1 Monthly quantitative signal detection screening

Based on defined algorithms and statistical methods to detect disproportionality of product-event combinations, this computerized screening is applied to all data and all products in the Bayer safety database on a monthly basis using Empirica Signal. No potential signals have been detected based on this method.

4.4.3.2 Re-Review of Bayer's reports of NSF

The massive amounts of data that Bayer received regarding reports of NSF are being reevaluated to look for:

- Reports of possible gadolinium-related symptoms in patients ultimately not diagnosed with NSF, including "gadolinium deposition disease"-like symptoms and subclinical or lesser forms of NSF (e.g., "gadolinium-associated plaques.")
- Increased SI
- Presence of Gd measured in any tissues or organs

4.4.3.3 Reports of Intrathecal Administration

To date, reports have been received for both Magnevist and Gadavist showing sometimes severe but generally reversible neurological effects (seizures, coma, etc.), when the GBCAs were administered undiluted by this unapproved route of administration.

Literature reports of studies conducted in animals and humans with intrathecal GBCA (primarily Magnevist) administration demonstrated a dose-related response to intrathecal administration, with general overall tolerability demonstrated when used at very low doses and transient neurological effects noted at higher concentrations. The low dose was often diluted with normal saline or with cerebrospinal fluid.

Post-marketing reports demonstrate that neurological effects can occur when GBCAs are administered intrathecally, often inadvertently and at large (e.g. 15-20 mL) doses.
The neurological effects were reported at doses of 4.5 – 20 mL for Magnevist and at 2 to 3 mL for Gadavist. Where the information could be obtained, these effects are generally reversible. As similar neurological effects have been noted when high osmolar iodinated contrast agents reach the brain, the contributory osmotic effect cannot be excluded. When used at low doses (e.g. < 2 mL), intentionally or unintentionally, no adverse events or only mild transient events such as headache were recorded.

4.4.3.4 Ongoing review of use of GBCAs during pregnancy and associated outcomes

One retrospective study **Ray et al**. (**2016**) was published in September 2016 in the Journal of the American Medical Association (**Ray, 2016]** .

The goal of the study was to provide clinicians with more data about the long-term safety for the child exposed to MRI in the first trimester of pregnancy or to gadolinium at any time during pregnancy. The authors concluded that "Exposure to MRI during the first trimester of pregnancy, compared with non-exposure, was not associated with increased risk of harm to the fetus or in early childhood. Gadolinium MRI at any time during pregnancy was associated with an increased risk of a broad set of rheumatological, inflammatory, or infiltrative skin conditions and risk of stillbirth or neonatal death. The study may not have been able to detect rare adverse outcomes."

As a result of this publication in May 2017 FDA issued a DARRTS Tracked Safety Issue (TSI) regarding concerns about pregnancy outcomes following GBCA administrations.

Bayer's comment: *The study has significant limitations. As pointed out by the authors, confounding by the underlying medical conditions of women undergoing MRI during pregnancy*

compared to pregnant women without such examinations may play a major role. The underlying medical condition of the patient could be the cause of the adverse pregnancy outcome, rather than the MRI examination which was done to evaluate this underlying condition.

In three decades of experience with gadolinium-based contrast media, no evidence of fetal toxicity or adverse clinical neonatal outcomes has emerged in the literature or in post marketing experience. Bayer has received no reports of stillbirths, and no reports of rheumatological, inflammatory or cutaneous conditions in children who were exposed to GBCAs in utero.

4.4.3.5 Overdoses

In clinical trials, each of Bayer's GBCAs was tested at doses in multiples of the approved dose, with no toxicity and no increase in adverse effects identified compared to the standard dose.

In post-marketing experience, overdoses (mostly occurring in the context of medication errors without harm) are evaluated and included in periodic benefit-risk evaluation reports (PBRERs). Bayer also receives and evaluates reports of overdose from the Poison Control Center. To date, no specific adverse clinical effects of overdoses have been reported, even at doses many times in excess of the approved dose. For example, a group of infants was overdosed at 10 times the prescribed dose due to medication error, with no adverse events reported. It should be noted, however, that dose-dependent events such as acute renal failure and nephrogenic systemic fibrosis have primarily occurred after multiple high dose administrations of contrast media.

4.5 Summary of Post-Marketing Data Received

In summary, post marketing data evaluation is ongoing through routine PV qualitative and quantitative signal detection which includes but is not limited to literature review, database review of Individual Case Safety Reports (ICSRs) for all GBCAs as well as for other products, and computerized screening of the database according to defined algorithms.

To date, after three years of evaluation, there is no clear signal of any specific event, other than NSF, that can be confirmed to be plausibly associated with traces of Gd in the body. Bayer continues to evaluate the Gd associated disorders proposed by Semelka and other chronic or inflammatory symptoms reported in association with its GBCAs. Bayer plans to further search for any such type of signal via epidemiological studies in large longitudinal healthcare databases.

5. Considerations about Clinical Studies / Epidemiology

5.1 Introduction

The ongoing efforts to identify any signals from PV data that would allow hypothesizing on the potential clinical relevance of the presence of Gd in the brain and body have not revealed any such signal to date. However, because of the well-known inherent limitations in PV data, such data may not be sufficient to identify those signals, other options and approaches need to be considered. In the following section, a potential path forward is outlined and discussed.

5.2. Challenges when considering studies

Generally, all the options that from Bayer's perspective can be considered -- be it a prospective study, prospective observational studies, retrospective studies, or the generation of data from large databases -- face some fundamental challenges, as illustrated by the following examples:

- Lack of a clearly identified definitive endpoint or even a reasonable hypothesis for such an endpoint.
- Multiple confounding factors. Especially when considering any potential effects related to presence of Gd in the brain (but also other organs) a number of diseases and also medications may present with symptoms that could be difficult to distinguish from potential effects related to the presence of Gd.
- The lack of knowledge about any potential dose effect and clinical threshold
- The lack of knowledge if the various GBCAs or at least the classes of linear and macrocyclic agents may lead to different clinical effects, if any
- An adequate control group allowing for multiple comparisons (as we need to look at multiple outcomes) is needed.
  From a statistical point of view multiple comparisons are vulnerable for generating chance results.

5.3 Explorative signal detection in large epidemiological healthcare databases

In particular because of the need to have a sample size sufficient to detect subtle signals and to allow for multiple comparisons Bayer focusses its thinking on large database analysis as the next step.

1) Screening for signals

This step should primarily serve as a screening tool and allow for identification of signals that then can be further evaluated. At the same time and as appropriate some hypothesis testing for specific predefined conditions (e.g. for Parkinsonism, fibrotic and inflammatory disorders) could be done as well.

In the US and in several other countries, large longitudinal healthcare databases are available, which, beside demographic information, contain patient-level data on procedures performed (such as contrast-enhanced MRI) and information on in- and out-patient diagnoses as well as ambulatory drug prescriptions. As such, a large population could be identified retrospectively

which has undergone multiple contrast-enhanced MRI, with considerable pre-exposure confounder information (e.g. comorbidities) and all recorded diagnoses after the MRI procedure(s) captured.

The frequency of any new diagnosis made could then be compared with the frequency observed in a comparable population to find possible signals in the contrast-enhanced MRI exposed population. Considerable limitations arise from the fact that type and dose of contrast agent used are typically not recorded, and most patients, especially those undergoing multiple MRI procedures (e.g. for common indications like CNS imaging, abdominal MRI, MR angiography) suffer from severe underlying conditions which will probably impact on the type and frequency of subsequently recorded diagnosis. Thus, lots of false signals in this population are expected to be generated ("confounding by indication"). To avoid that, one would prefer a cohort of patients, which are per se relatively healthy, but nevertheless undergoing multiple contrast-enhanced MRI procedures, such as women undergoing breast MRI examination, but not having breast cancer (e.g. for breast cancer screening in high risk groups or further lesion characterization in rare cases where mammography and ultrasound are inconclusive) or patients with benign pancreatic tumors (e.g. IPMN) undergoing monitoring with contrast-enhanced MRI.

Minimization of random errors
To minimize the risk of random error / random false positive signals such an analysis should be performed in several different databases. A true signal should be consistent across multiple databases, while a random false positive would not be expected to appear repeatedly in these analyses.

Minimization of systematic errors
Systematic errors (selection bias / confounding by indication / channeling) should be minimized with the use of an appropriate exposure group (see above) appropriate controls and adjustment by important covariates (e.g. age, comorbidity). Another feature would be to analyze dose-response, i.e., a population with the same indication exposed to fewer MRI procedures.

2) Evaluation of signals
Any potential signals identified reproducibly in the screening studies above need to be interpreted appropriately as data-derived hypotheses. Thus, the next step would be to perform another study in a different setting, this time designed specifically to test the hypotheses generated above.

5.4 Additional clinical research activities

One of the limitations of the existing retrospective imaging studies evaluating the effect of the various GBCAs on SI increase in the brain is the lack of standardization as far as region of interest (ROI) placement and quantitative measurements as well as variation of sequences is concerned.

In order to better control and standardize the assessment Bayer has developed software in cooperation with MINT Labs that allows for automatic segmentation of the brain, selection of comparable ROIs and correction for varying imaging parameters. First studies are ongoing validating this new tool.

6. Change of labels / Risk mitigation

In its May 22, 2017 Drug Safety Communication, FDA noted that the manufacturer of OptiMARK had updated its label and that the agency is in the process of reviewing the labels of other GBCAs to determine if changes are needed. Bayer has reviewed the recent labeling changes to OptiMARK and is open to considering a similar approach for the labels of its GBCAs in the US.

Relatedly, Bayer reviewed all available safety and efficacy data and concluded that the benefit/risk balance of all GBCAs remained favorable for the vast majority of patients. Therefore, Bayer recommends that the labels for GBCAs be updated as outlined below.

Bayer recommended a class approach regarding changes to the respective labels; specifically addressing the differences between linear and macrocyclic GBCAs.

6.1 Class approach: Linear GBCAs

After repeated use of linear GBCAs, increased signal intensity and higher Gd concentrations in the body were reported. Therefore, Bayer suggests describing the phenomena around Gd presence in the body in more depth and detail in section 5, *Warnings and precautions* as the very last precautionary statement (*section 5.6 Gadolinium presence in the body*) or alternatively, given the theoretical nature of the risk, the FDA may consider including a similar wording in section 12.3 *Pharmacokinetics*.

Although the updates for linear GBCAs should be made as a class approach, the wording needs to be adapted to the individual product to reflect differences in indication or dose.

6.1.1 Wording for class of multi-purpose linear GBCAs (e.g. Magnevist)

As outlined above, Bayer considers that the benefit/risk balance for linear GBCAs including Magnevist remains positive for the vast majority of patients. Therefore, suggestions for updating the PI are made.

> **Gadolinium presence in the body**
> Gadolinium-containing contrast agents (GBCAs) are either linear or macrocyclic in their chemical structure. Magnevist is a linear GBCA. Studies have shown that after exposure to intravenous GBCAs traces of gadolinium can be detected in parts of the body including the brain. Higher concentrations were detected for linear GBCAs. Repeated administrations of linear GBCAs can cause signal intensity increases in the brain on T1-weighted MRI, particularly in the dentate nucleus, globus pallidus, and thalamus. Signal intensity increases of this type have not been confirmed with macrocyclic GBCAs. Signal intensity increases and non-clinical data suggest that traces of gadolinium may be released from linear GBCAs. No histopathological changes in the brain have been detected in an animal model. Evidence on the clinical consequences of Gd in the brain is limited; no adverse clinical consequences have been confirmed. Nevertheless, the possible diagnostic advantages of using Magnevist in patients who will require repeated scans should be weighed against the potential risk of gadolinium presence in the brain and other tissues.

Bayer considers the following points to be important:

- Appropriately describe the phenomena of SI increase and Gd presence in the brain, mainly the GP and the DN and in other areas in the body after repeated iv use and its associated risk
- Only traces of Gd have been detected after repeated intravenous dose of linear GBCAs
- Higher tissue concentrations of Gd for linear GBCAs compared to macrocyclics
- No adverse clinical consequences have been confirmed
- No histopathological changes detected in an animal model
- The term "Gd presence in the body" is used as it is not entirely clear whether and how the traces of Gd are eliminated from the body

6.1.2 Wording for liver specific Eovist

Gadolinium presence in the body
Gadolinium-containing contrast agents (GBCAs) are either linear or macrocyclic in their chemical structure. Liver specific Gadoxetic acid is a linear GBCA administered at a lower dose (one quarter the dose) compared to multi-purpose linear GBCAs. Studies have shown that after exposure to GBCAs traces of gadolinium have been detected in parts of the body including the brain. Higher concentrations were detected for linear GBCAs. Repeated administrations of linear GBCAs can cause signal increases in the brain on T1-weighted MRI, particularly in the dentate nucleus, globus pallidus, and thalamus. Signal intensity increases of this type have not been confirmed with macrocyclic GBCAs. Signal intensity increases and non-clinical data suggest that traces of gadolinium are released from linear GBCAs. No histopathological changes in the brain have been detected in an animal model. Evidence on the clinical consequences of Gd in the brain is limited; no adverse clinical consequences have been confirmed. Nevertheless, the possible diagnostic advantages of using gadoxetic acid in patients who will require repeated scans should be weighed against the potential risk of gadolinium presence in the brain and other tissues.

Important points:

- Appropriately describe the phenomena of SI increase and Gd presence in the body after repeated i.v. use
- Eovist is used only at 25% of the dose of multi-purpose linear agents, therefore represent substantially lower plasma Gd concentrations and systemic burden compared to other linear GBCAs
- Only traces of Gd have been detected in the body after repeated i.v. use of GBCAs
- No adverse clinical consequences have been confirmed
- No histopathological changes detected in an animal model
- The term "Gd presence in the body" is used as it is not entirely clear whether and how the traces of Gd are eliminated from the body over time

6.2 Sub-class approach Macrocyclic GBCAs

The PIs of all macrocyclic GBCAs should inform healthcare professionals about reported presence of Gd in the body. As this is not associated with a signal intensity increase or any known risk and as there may be a slow wash out effect, Bayer suggests including the following statement for all macrocyclic GBCAs in the PI section 12.3 *Pharmacokinetics.*

> **Gadolinium presence in the body**
> Studies have shown that after repeated exposure to all gadolinium-containing contrast agents (GBCAs) traces of gadolinium may be detected in areas of the body such as brain and bone. Higher gadolinium concentrations were detected for linear GBCAs. The clinical relevance of these findings is not known.

Important points:

- Healthcare providers using macrocyclic GBCAs need to be informed about reported presence of Gd in the body after repeated use
- Only traces of Gd have been detected after repeated i.v. dose of macrocyclic GBCAs
- For linear GBCAs, higher concentrations have been detected
- There is an unknown clinical relevance regarding Gd presence in the body

All other sections of the PI already appropriately address safe and effective use of macrocyclic agents.

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7. Overall Summary of Bayer Position

7.1 Benefit / Risk Profile

- The efficacy and benefits of GBCAs have been conclusively demonstrated in clinical trials and post-marketing experience over three decades
- All GBCAs have a very favorable overall safety profile
- Increased SI has been confirmed for all multi-purpose linear GBCAs when approximately five or more injections are administered with full dose (0.1 mmol Gd/kg BW)
- For the ¼ dose liver-specific GBCA Eovist a SI increase seems to occur only after a substantially higher number of administrations (approximately 20 administrations)
- For macrocyclic agents like Gadavist no visible SI has been observed as of today, even after a high number of administrations
- The clinically observed SI is considered to be a surrogate for traces of Gd in the brain and the different *in vivo* behavior of linear agents compared to macrocyclic GBCAs is based on their differences in stability
    - All GBCAs can enter the brain most likely via a physiological leakage on the level of the blood-CSF barrier (choroid plexus)
    - In brain tissue some Gd from linear GBCAs may bind to macromolecules. The relative portion of Gd bound to macromolecules increased from day 3 to day 24 for all three linear agents. Most likely this is due to a partial de-chelation of linear GBCAs.
        - The newly formed Gd macromolecules may have a much higher relaxivity than the intact chelate, which could partially explain the visible SI increase for linear agents only.
        - All multi-purpose linear GBCAs behave in the same manner in this respect.
- It appears from the first long-term, non-clinical studies that the small traces of Gd after repeated administrations of macrocyclic GBCAs may be slowly eliminated from the brain tissue. This could not be shown for linear agents over the observation period of one year.
- Most importantly, however, despite the different stability of macrocyclic and linear GBCAs and their subsequent different behavior, no adverse health effects have been confirmed to be associated with the presence of Gd in the brain or other organs for any of the agents (with the exception of NSF).

Given the proven diagnostic benefit of all the compounds in their approved indications and a generally very favorable overall safety profile, Bayer affirms a positive benefit-risk profile for all its marketed products, namely in the US Gadavist, Eovist and Magnevist i.v. in the vast majority of patients. To date, no evidence has been identified that traces of Gd in the brain are harmful. The potential risks can be addressed via label updates and communication to healthcare providers.

7.2 Conclusions

Traces of Gd may be detectable in the brain and other organs for prolonged periods of time with all GBCAs, both linear and macrocyclic. There is no confirmed evidence that the presence of trace amounts of Gd in the brain or other parts of the body (with the exception of NSF) is clinically relevant. The benefits of GBCAs are clearly documented in controlled clinical trials and confirmed by decades of clinical experience with more than 450 million administrations worldwide.

GBCAs help physicians answer critical medical questions in the diagnosis and monitoring of disease. These agents address important medical needs that in certain conditions may not be easily replaced by another imaging modality. These facts support a positive benefit-risk profile for all GBCAs in the vast majority of patients when used in accordance with approved labeling.

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Appendix 1 - Gadolinium Presence in Body – NSF Reports

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
1	59 yo female Renal cell carcinoma s/p bilateral nephrectomy HD since 1999	**Total GBCA administrations = 13** **Total cumulative dose = 1.1 mmol/kg + 5 unknown doses** **Dates and doses of GBCA administrations:** 21 Jun 1995: Magnevist (dose unk); 26 May 2003: Omniscan 0.26 mL/kg (0.13 mmol/kg); 05 Jun 2003, 23 Oct 2003, 30 Oct 2003 and 18 Mar 2004: Dotarem (doses unk); 10 Nov 2004: Magnevist 0.27 mL/kg (0.14 mmol/kg); 17 Nov 2004: Omniscan 0.27 mL/kg (0.14 mmol/kg); 17 Dec 2004: Magnevist 0.28 mL/kg (0.14 mmol/kg); 21 Feb 2005: Magnevist 0.29 mL/kg (0.15 mmol/kg); 30 May 2005: Magnevist 0.30 mL/kg (0.15 mmol/kg); 04 Feb 2006: Omniscan 0.27 mL/kg (0.14 mmol/kg); 20 Jul 2006: Omniscan 0.26 mL/kg (0.13 mmol/kg)	Oct 2006: Whole blood: 36.30 ng/mL (control < 0.10 ng/mL); Oct 2006: Plasma: 11.60 ng/mL (control < 0.10 ng/mL); Jan 2007: Whole blood: 1.80 ng/mL; Jan 2007: Plasma: 2.60 ng/mL; Sep 2006: Hair: 0.03-1.09 ng/mg; Jan 2007: Nails: 1.23 ng/mg (control 0.16 - 26.2 ng/mg) Note: Control group consisted of 100 subjects who had not been exposed to gadolinium	Cutaneous infiltration with "pig-skin" aspect spreading progressively to upper limbs, progressive brownish violescent color of skin, extensive fibrosis of limbs Biopsy results not provided Score = 3, unknown: Not assessable
2	66 yo Caucasian female Chronic severe renal failure since 1995	**Total GBCA administrations = 4** **Total cumulative dose = 40 mL (wt unknown) + 4 unknown doses** **Dates and doses of GBCA administrations:** May 1998, Jun 2004, 15 Nov 2005: Magnevist (dose unk); 16 Feb 2007: Magnevist 40 mL for MRI and MRA	10 Oct 2007 (Skin biopsy): Further examination by mass spectroscopy detected Gd in 2 tissue samples (tissue 1: 0.43 ng/g; tissue 2: 465 ng/g)	Induration Biopsy showed irregularly widened collagen bundles with clefts, abundant CD34+ fibrocytes which at times extended into the subcutaneous tissue. Septal involvement Score = 3, 4 = NSF

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
3	64 yo Caucasian male ESRD/PD and HD	**Total GBCA administrations = 2** **Total cumulative dose = 0.21 mmol/kg + 1 unknown dose** **Dates and doses of GBCA administrations:** 12 Jul 1999: Magnevist 40 mL (0.21 mmol/kg); 04 Feb 2005: Magnevist (dose unk)	25 Jun 2007: Liver biopsy: Mass spectrometry demonstrated 10.0 ppm gadolinium in tissue on dry weight basis	Tethering, thickening of skin, brawny hyperpigmentation, peau d'orange around umbilicus, flexion contractures of elbows and knees, skin of belly hard, firm lumps on calves, hyperpigmented plaque, yellow scleral plaques Biopsy showed sparse interstitial increase in fibroblasts, septal collagen and fibrosis Score = 4, 2 = Consistent with NSF
4	43 yo Caucasian female ESRD/CVVH, HD, PD	**Total GBCA administrations = 10** **Total cumulative dose =** Pt was initially reported to have received a cumulative dose of 158 mL (0.88 mmol/kg) Gd prior to NSF diagnosis. In a subsequent literature article (**Koreishi, 2009**), it was stated that patient had received 198 mL (1.1 mmol/kg) **Dates and doses of GBCA administrations:** 10 Feb 1999, 17 Feb 1999, 04 Mar 1999, 20 Mar 1999, 28 Mar 1999, 04 May 1999: Magnevist (dose unk); 18 Jun 1999: Unknown GBCA; 25 Jan 2000 Magnevist (dose unk); 12 Dec 2002: Unknown GBCA; 18 Jun 2004: Magnevist (dose unk).	Post mortem examination by ICP-MS: Gd detected in all tissue samples analyzed. Skin (left lateral thigh): 29.5 ppm; right kidney + adrenal gland: 488.2 ppm; left kidney + adrenal gland: 585.4 ppm; Skin (right thigh): 130.2 ppm; Skin (left thigh): 36.7 ppm; Lymph node: 184.5 ppm; left thyroid + liver: 56.3 ppm; lung (right middle lobe): 145.5 ppm; Heart (left ventricle): 544.7 ppm	Hard, wooden skin; induration; flexion contractures, tense edema extending to hips, skin dry and thick; joint contractures of elbows and knees CD34+ cells in the dermis; fibroblastic expansion of deep reticular dermis and fibrous septae; elastic fibers paralleled collagen fibers Score = 4, 3 = NSF

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
5	31 yo male ESRD since childhood/HD, PD, multiple KTPs, HD	**Total GBCA administrations = 3** **Total cumulative dose = 0.68 mmol/kg** **+ 2 unknown doses** **Dates and doses of GBCA administrations:** 29 Apr 2003: Magnevist 60 mL (0.68 mmol/kg); 14 May 2003: Omniscan (dose unk); 31 Jul 2003: Magnevist (dose unk)	02 Oct 2003: Skin biopsy (right proximal forearm): Per legal complaint, biopsy contained 511.1 ppm gadolinium	Thickened skin, raised rash with cobblestone-like appearance, tethered skin, flexion contractures, joint contractures Biopsy report not provided Score = 4, unknown = Not assessable
6	32 yo Middle Eastern male ESRD secondary to Meningococcemia/HD	**Total GBCA administrations = 5** **Total cumulative dose = 0.52 mmol/kg** **Dates and doses of GBCA administrations:** 16 Dec 2003: Magnevist 0.1 mmol/kg; 18 Dec 2003: Magnevist0.1 mmol/kg; 15 Jan 2004: Magnevist 0.1 mmol/kg; 25 Feb2004: Magnevist 0.1 mmol/kg; 06 Jul 2004: Magnevist 17 mL (0.12 mmol/kg)	2006 NOS: Tissue samples analyzed for determination of Gd using SEMS/EDS, which provides quantification of Gd in tissue along with its distributional analysis. Concentration was calculated as counts per second divided by the total scanned area (cps/mm^2). 19 Mar 2004 Skin biopsy (left forearm): 833 cps/mm^2. 01 Nov 2004: Skin biopsy (Scar, left arm): 1195 cps/mm^2. 14 Mar 2007: Skin biopsy (left forearm): 550 cps/mm^2.	Burning pain in skin; fibrotic violaceous plaques; depressed plaques; indurated skin; severe flexion contractures; joint contractures Biopsy showed dense infiltrate of spindled cells, histiocytes, and multi-nucleated giant cells throughout the pannicular septae and extending into the fascia, with expansion of the pannicular septae; CD34+ in dermis, Consultation: There is a deep dermal and sc infiltrate of

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
				spindled to epithelioid cells expanding the sc septa and encroaching on sc fat lobules. The nuclei generally stream parallel to collagen and elastic fibers. In some areas, distinct bundles of collagen are noted with interposed spindle cells Score: 4, 3 = NSF
7	71 yo Black female ESRD/HD since 01Mar 2000	**Total GBCA administrations = 7** **Total cumulative dose = 0.32 mmol/kg = 4 unknown doses** **Dates and doses of GBCA administrations:** 09 Sep 2003: Magnevist 19 mL (0.11 mmol/kg); 17 Oct 2005, 31 Jan 2006, 03 May 2006 and 06 Jun 2006: Magnevist (doses unknown); 18 Dec 2006: Magnevist 20 mL (0.12 mmol/kg); 05 May 2007: Magnevist 15 mL (0.09 mmol/kg)	18 Jul 2007 and 01 Jan 2008: Skin biopsy: Gd quantification using ICP-MS and appropriate controls demonstrated 7 ppm Gd in the tissue.	Debilitating and worsening fibrotic changes to her body, peau d'orange, firm woody induration, shiny erythema, muscle contractures, constrained mobility Widened subcuticular septa with increased number of fibrocytic cells and fibrosis; multinucleated giant cells Score = 3, 2 = Consistent with NSF
8	61 yo Caucasian male ESRD/HD since Jun 2008	**Total GBCA administrations = 2** **Total cumulative dose = 0.14 to 0.19 mmol/kgs**	01 Jan 2008: Lower leg: Specimens demonstrated 479 and 448 ppm Gd by ICP-MS	Hardening of skin, peau d'orange, woody edema, hyperpigmented areas, contractures

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
		Dates and doses of GBCA administrations: 11 Jun 2004: Magnevist 10 or 20 mL; 05 Apr 2006: Magnevist 20 mL (0.09 mmol/kg)		Biopsy showed dermal fibrosis, increased fibroblasts Score = 4, 1 = Inconsistent with NSF
9	40 yo Caucasian female ESRD/ HD, PD since 1994	**Total GBCA administrations = 9** **Total cumulative dose = 120 mL (wt unknown) + 3 unknown doses** **Dates and doses of GBCA administrations:** 27 Nov 2002: Omniscan 20 mL (wt unk); 18 May 2005: Omniscan 20 mL; 21 May 2005 and 23 May 2005: Unknown GBCAs (doses unk); 03 Sep 2005: Magnevist 20 mL; 18 Oct 2005: Unknown GBCA (dose unk); 28 Oct 2005: Omniscan 20 mL; 28 Oct 2005: Omniscan 20 mL; 25 Jan 2006: Omniscan 20 mL	June 2007 per legal complaint: "High level of gadolinium found in her heart tissue". Dermatology consultation report indicated the deposition of gadolinium in a donated tissue sample from the patient. Testing was reported to reveal significant gadolinium in the vast majority of her tissues. The patient's skin contained 232.9 and 135.4 ppm gadolinium (dry weight basis) in two separate skin samples tested. The anatomic location of these skin samples was never specified in the materials that accompanied the tissue donation.	Fibrotic changes, contractures Skin biopsy showed CD34+ fibrocytes Skin biopsy was not at all supportive of a diagnosis of NSF. Histopathology was near normal showing none of the features of NSF. Score = 3, 1 = Not NSF

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
10	59 yo Hispanic male ESRD/HD since Jun 2000	**Total GBCA administrations = 19** **Total cumulative dose = 0.42 mmol/kg = 15 unknown doses** **Dates and doses of GBCA administrations:** [8/5/2017 5:44 PM] Achim Hermann: 14 Feb 2001, 17 Apr 2001: Unknown GBCAs; 31 Jul 2001: Magnevist (dose unk); 22 Oct 2001 and 08 Nov 2001: Unknown GBCAs; 20 Dec 2002: Magnevist 30 mL (0.11 mmol/kg); 22 Dec 2002, 23 Dec 2002, 26 Dec 2002 and 30 Dec 2002: Unknown GBCAs; 17 Jan 2003: Magnevist 25 mL (0.09 mmol/kg); 05 Mar 2003: Unknown GBCA; 26 Mar 2003: Magnevist 30 mL (0.11 mmol/kg); 05 Jun 2003: Magnevist (dose unk); 07 Jul 2003: Unknown GBCA; 20 Aug 2003: Magnevist (dose unk); 24 Aug 2003: Unknown GBCA; 08 Dec 2003: Magnevist (dose unk); 23 Mar 2005: Magnevist 27 mL (0.1 mmol/kg)	17 Aug 2007 (distal right arm): Gd quantification by ICP-MS demonstrated 15 ppm Gd	Joint contractures Increased fibrocytes with some increased fibrosis in the dermis and upper subcutis; suggestion of a single widened subcuticular septum Score = 3, 2 = Consistent with NSF
11	76 yo male ESRD/HD	**Total GBCA administrations = 3** **Total cumulative dose = 49.5 mmol** **Dates and doses of GBCA administrations:** Within 2 weeks, patient underwent 3 MR scans with Magnevist, 2 of which were MRAs	Feb 2007: Electron spectroscopic imaging and electron energy loss spectroscopic analyses revealed deposition of Gd in irregular small aggregates of 100 to 1000 nm that adhered to cell profiles and collagen fibers of the connective tissue, forming a perivascular "Gd deposit zone" in the skin.	No clinical symptoms suggestive of NSF Biopsy showed fibrosis of corium and subcutis; perivascular inflammatory infiltrates; CD34 and CD68+ Score 0, 2 = Alternative diagnosis

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
12	66 yo Caucasian male ESRD/HD since Mar 2004	**Total GBCA administrations = 2** **Total cumulative dose = 0.22 mmol/kg** **Dates and doses of GBCA administrations:** 11 Mar 2004: Omniscan 30 mL (0.22 mmol/kg); Date unknown: Omniscan (dose unk)	08 May 2007: Skin biopsy (left inner thigh): Contained 44.2 ppm gadolinium	indurated lesions, large indurated woody mildly erythematous plaques on thigh, cobblestoned appearance of skin on back Dermal thickening with diffuse proliferation of fibroblasts and thickened collagen bundles. Diffusely positive for CD34 Score = 4, 3 = NSF
13	52 yo Hispanic male ESRD/ HD, PD and KTP since 2002	**Total GBCA administrations = 6** **Total cumulative dose = Unknown** **Dates and doses of GBCA administrations:** 05 Nov 1997, 29 Jan 2001, 30 Jan 2003: Unknown GBCAs; 30 Dec 2004 Magnevist (dose unk); 23 Aug 2005: Magnevist (dose unk)	11 Sep 2007 Skin biopsy (left elbow): 167 ppm Gd found by ICP-MS	Joint contractures; skin thickening and hardening Increased fibrocytes, septal involvement Score = 4, 2 = Consistent with NSF
14	45 yo Caucasian female ESRD/HD, KTP, HD since 1988	**Total GBCA administrations = 9** **Total cumulative dose = 365 - 382 mL** **(2.4 - 2.5 mmol/kg): Discrepancy in medical records** **Dates and doses of GBCA administrations:** 27 Jan 2004: Magnevist 40 mL (0.26 mmol/kg) for fistulogram and angiography; 19 Apr 2004: Magnevist 50 mL (0.33 mmol/kg) for fistulogram and angioplasty; 13 Sep 2004: Magnevist 15 mL (0.1 mmol/kg) for	30 May 2007: Left calf and thigh: Gd quantification using ICP-MS demonstrated 445 ppm Gd in the calf and 475 ppm Gd in the thigh.	Skin thickened, indurated and bound down Biopsy showed diffuse infiltration and intercalation of fibrocytes in the dermis and septa of the subcutis, with widening of the sc septa Score = 3, 2 = Consistent with NSF

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
		fistulogram; 10 Dec 2004: Magnevist (dose unk) for fistulogram and angioplasty; 18 Feb 2005: Magnevist 30 mL (0.2 mmol/kg) for fistulogram and angioplasty; 25 Oct 2005: Magnevist 33-35 mL (0.22 - 0.23 mmol/kg) for fistulogram, central venogram and multiple fistula angioplasties; 23 Mar 2006: Magnevist 42 mL (0.28 mmol/kg) for fistulogram and angioplasty; 20 Jun 2006: Magnevist 30 mL (0.2 mmol/kg) for fistulogram and angioplasty; 12 Oct 2006: Magnevist 70-80 mL (0.46-0.53 mmol/kg) for fistulogram and angioplasty		
15	60 yo Caucasian male ESRD/HD since Sep 2005	**Total GBCA administrations = 10** **Total cumulative dose = 0.47 mmol/kg** **+ 4 unknown doses** **Dates and doses of GBCA administrations:** 20 Sep 2005: Unknown GBCA; 08 Dec 2005: Omniscan 20 mL (0.09 mmol/kg); 23 Dec 2005: Unknown GBCA; 07 Mar 2006: Magnevist 20 mL (0.09 mol/kg); 29 Mar 2006: Magnevist 20 mL (0.09 mmol/kg); 25 Jul 2006: Magnevist or ProHance 20 mL (0.09 mmol/kg); 26 Jul 2006: Magnevist or ProHance (dose unk); 04 Mar 2007: Omniscan 20 mL (0.09 mmol/kg);	05 Apr 2007 (thigh): Addendum to biopsy report noted that tissue demonstrated 166.8 ppm Gd on a dry weight basis	Progressive thickening and hardening of the skin of the lower extremities, progressive contractures of hands and LEs, tight edema, skin taut, shiny and thickened; flexion contracture of hands Increased numbers of fibroblasts within the dermis and extending into the septae of the underlying subcutaneous tissues. The collagen bundles are irregularly thickened and

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
		07 May 2007 and 24 May 2007: Unknown GBCAs		haphazardly organized. The primary pathologic process is that of a hypercellular dermis with fibrotic and hypercellular subcutis with widened and fibrotic septae. The spindled, infiltrating cells appear to be slightly stellate fibroblasts and are associated with thickened collagen bundles which are haphazardly organized. Score = 4, 3 = NSF
16	92 yo Caucasian male CKD 3-4/ Not on dialysis	**Total GBCA administrations = 2** **Total cumulative dose = 0.1 to 0.2 mmol/kg + 1 unknown dose** **Dates and doses of GBCA administrations:** 2003: Unknown GBCA; 31 Jul 2008: Magnevist PBP 20-40 mL (0.1 - 0.21 mmol/kg)	03 Dec 2008 (left leg): 27 ppm Gd detected in tissue. Diagnostic changes of NSF not seen.	Skin tightening and tethering; difficulty extending knees; skin hyperpigmentation; dermal fibrosis; itching; yellow scleral plaques; flexion contractures; reticulated eczema craquele Biopsy showed subtle increase in dermal myofibroblasts / fibroblasts Score = 4, 1 = Inconsistent with NSF
17	40 yo Caucasian female ESRD since Nov 2005/	**Total GBCA administrations = 4** **Total cumulative dose = 80 mL (wt unknown)**	Autopsy report 13 Jun 2007: A "Professor of NSF Research" found high levels of gadolinium in all body tissue sent to him. No further information provided.	Fibrosis, skin induration, contractures Slight increase in dermal fibrocytes in papillary and reticular dermis; fibrocytes

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
	HD, PD	**Dates and doses of GBCA administrations:** 20 Dec 2005: Magnevist 15 mL (wt unk) 19 Jun 2006: Magnevist 15 mL 27 Jun 2006: OptiMARK 10 mL 01 Dec 2006: Magnevist 40 mL		appear to stain positively for CD34; haphazardly arranged fibroblasts and collagen bundles throughout the reticular dermis and superficial sc septae; spindled cells are CD34+ and there are admixed CD68+ macrophages. The changes are subtle but appear to represent NFD Score = 4, 4 = NSF
18	59 yo Caucasian female/ ESRD/HD since Apr 2002	**Total GBCA administrations = 7** **Total cumulative dose = 0.16 mmol/kg + 6 unknown doses** **Dates and doses of GBCA administrations:** 22 Dec 2002: Unknown GBCA; 10 Jan 2003 Magnevist (dose unk); 03 Apr 2004: Magnevist 40 mL (0.16 mmol/kg); Apr 2004: Unknown GBCA; 06 May 2004: Magnevist (dose unk); 15 Sep 2005: Magnevist (dose unk); 24 Sep 2005: Unknown GBCA	Dec 2008 - Jan 2009: Skin biopsy sample contained 54 ppm Gd	Skin thickening and discoloration; fibrotic changes; limited mobility; legs hardened; hard muscles; wooden feeling of thighs and calves; induration; difficulty extending knee and elbow; bilateral flexion contractures of fingers; elbow contracture; hardened brawny edema of LEs; yellow scleral plaques in left eye; cobblestoned appearance to thighs Deep dermal and subcutaneous fibrosis with mildly increased fibroblasts Score = 4, 1 = Inconsistent with NSF

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
19	72 yo Black female Stage 5 CKD/ Dialysis NOS	**Total GBCA administrations = 2** **Total cumulative dose 35 mL** **Dates and doses of GBCA administrations:** Magnevist: 2 doses over 14 months	Post mortem analysis: Gd was quantifiable in all tissues assayed; the highest concentration was in the kidney	At autopsy, skin of her lower extremities demonstrated extensive fibrosis of the dermis and underlying subcutaneous tissue. Also extensive fibrosis involving the pericardium, heart, lungs, diaphragm, thyroid and dura mater, including the surrounding spinal cord Proliferation of fibroblasts. Dense fibrosis was associated with calcification and scattered lymphocytic and histocytic infiltrates with multinucleated giant cells Score = 3, 1 = Not NSF
20	60 yo Caucasian female Stage 5 CKD/ Dialysis status not specified	**Total GBCA administrations = 7** **Total cumulative dose 130 mL** **Dates and doses of GBCA administrations:** Magnevist: 7 doses over 16 months	Post mortem analysis: Gd was quantifiable in all tissues assayed	No clinical signs/ symptoms reported. Subcutaneous septal fibrosis, with an increased number of fibroblasts and scattered multinucleated giant cells Score = Unknown, 2 = Not assessable
21	51 yo female No renal dysfunction/ not on dialysis	**Total GBCA administrations = 1** **Total cumulative dose 0.09 mmol/kg**	Skin biopsy: 6 ppm gadolinium detected	Fibrotic changes, hyperpigmentation, unable to close hands or extend elbow, skin

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
		Dates and doses of GBCA administrations: 21 Apr 1999: Magnevist 13 mL (0.09 mmol/kg)		thickening, indurated plaques, peau d'orange, flexion contractures Slightly increased dermal cellularity, septal thickening, CD34 in tram track pattern Score = 4, 2 = Suggestive of NSF Successive skin biopsies diagnosed eosinophilic fasciitis (Shulman's syndrome)
22	63 yo female/ CKD III; moderate renal impairment	**Total GBCA administrations = 1** **Total cumulative dose : Unknown** **Dates and doses of GBCA administrations:** date unknown: Magnevist (dose unk)	Low amounts of Gd were detected in the skin lesions; 6 ppm.	No clinical signs/ symptoms reported. No biopsy provided. Score = Unknown, Unknown = Not assessable Reporter stated case was biopsy proven; however. hospital stated they are unaware of any new cases of NSF at their facility
23	53 yo Hispanic male ESRD/HD	**Total GBCA administrations = 16** **Total cumulative dose: 0.93 mmol/kg**	26 Aug 2009: The blocks were analyzed using scanning electron microscopy and energy dispersive x-ray spectroscopy (SEM/EDS).	Thick, hard, leathery skin; limitations of flexion and extension, contractures; hyperpigmentation, muscle

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
	since 2003	**+ 6 unknown doses** **Dates and doses of GBCA administrations:** 13 Sep 2002: Omniscan (dose unk); 21 May 2003: Omniscan 15 mL (0.08 mmol/kg); 23 Jun 2003: Omniscan (dose unk); 05 Sep 2003: Omniscan 20 mL (0.1 mmol/kg); 06 Sep 2003: Omniscan 20 mL (0.1 mmol/kg); Sep 2003 NOS: Unknown GBCA; 25 Jun 2004: OptiMARK 20 mL (0.1 mmol/kg); 21 Oct 2004: Omniscan 20 mL (0.1 mmol/kg); 25 Nov 2004: Omniscan 15 mL (0.08 mmol/kg); 11 Mar 2005: Magnevist 18 mL (0.09 mmol/kg); 16 Jun 2005: Omniscan 15 mL (0.08 mmol/kg); 04 Jul 2005: Omniscan (dose unk); 05 Jul 2005: Unknown GBCA15 mL (0.08 mmol/kg); 09 Aug 2005: Omniscan 15 mL (0.08 mmol/kg); 27 Sep 2005: Omniscan 15 mL (0.08 mmol/kg); 04 Oct 2005: Omniscan 20 mL (0.1 mmol/kg)	Gadolinium- containing deposits were readily detected in biopsies of 05 Nov 2008 and 31 Mar 2009. No Gd was detected in biopsy sample from 19 Nov 2008. Deposits containing Gd also contained phosphorus, calcium & sodium. Similar deposits of calcium, phosphorus & sodium were noted in the 19 Nov 2008 biopsy in which Gd was not detected, and these deposits also contained abundant iron.	contracture Dense dermal fibrosis and fibroblastic proliferation, extending into the subcutis. Thick, horizontal bands of collagen separate fatty lobules in the subcutis. Abundant hemosiderin is present within spindly histiocytes in the deep dermis (iron stain). There is some mild degree of loss and fragmentation of elastic fibers in the deep reticular dermis with an elastic tissue stain. By immunohistochemistry, CD34 positive stromal cells are not increased in the dermis. In contrast, CD68 labels many of the spindly fibrohistiocytic cells in the dermis. Score = 4, 3 = NSF
24	65 yo Caucasian female ESRD/HD since 1979	**Total GBCA administrations = 8** **Total cumulative dose: 0.08 mmol/kg** **+ 7 unknown doses** **Dates and doses of GBCA administrations:** 27 Jun 1996: Magnevist (dose unk); 29 Sep 1998: Omniscan (dose unk); 11 Jul 2002, 05 Nov 2002, 15 Nov 2002, 16 Mar 2004, 02 Jan 2006: Magnevist (dose unk);	12 Jul 2007: 22.6 ppm Gd in tissue	Joint ROM decreased, thickening skin, skin hyperpigmentation, pingueculae, joint contractures, peau d'orange, woody induration, skin tethering Slightly fibrotic reticular dermis; focal fibrosis of septae noted in subcutaneous

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
		06 Apr 2006: Omniscan 10 mL (0.08 mmol/kg)		tissue, with slightly increased number of fibrocytes Score = 4, 2 = Consistent with NSF
25	55 yo Caucasian male ESRD/HD, PD, KTP	**Total GBCA administrations = 1** **Total cumulative dose: Unknown** **Dates and doses of GBCA administrations:** 02 Nov 2006: Magnevist (dose unk)	25 Nov 2008: Skin biopsy (left dorsal hand): Gadolinium identified by special studies. Letter dated 01 Apr 2009 indicated "Gadolinium was present in both biopsy specimens. The findings were 170.2 ppm in the first biopsy and 55.8 ppm in the second biopsy	Thick skin, joint ROM decreased, hardened skin, claw-like hands Increased CD34+ cells in dermis; increased spindle cells, collagen, mucin and elastic fibers extending through the subcutis along the septa of fatty lobules; Score = 3, 3 = NSF
26	58 yo female ("other" race/ethnicity) ESRD/HD, PD, since May 2004	**Total GBCA administrations = 2** **Total cumulative dose: Unknown** **Dates and doses of GBCA administrations:** 01 Sep 2004 and 04 May 2005: Magnevist (dose unk)	14 May 2008: 14 ppm Gd in tissue	Skin hardening and induration, contractures Biopsy report not provided Score = 4, unknown = Not assessable
27	57 yo Caucasian female Renal insufficiency per lawsuit; no renal disease per medical records	**Total GBCA administrations =** Patient states she had 24; 8 found in medical records **Total cumulative dose: Unknown** **Dates and doses of GBCA administrations:** 20 Apr 2001, 25 Mar 2002, 20 May 2002, 04 Jan 2003: Unknown GBCAs; 14 Jan 2003: ProHance 15 mL; 09 Jul 2004: Magnevist (dose unk); 24 May 2006: Unknown GBCA;	09 Sep 2008: Toxic elements screen: Gd level 0.245 (units not provided; normal range <0.019. Patient also had elevated levels of mercury and cadmium	Thickened skin, orange peel texture, mottled hypo and hyperpigmentation Increased fibroblasts, thickened septa, altered elastic fibers (-1), CD34 expression mildly increased Score = 3, 2 = Suggestive of

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
		20 Oct 2006: ProHance (dose unk)		NSF
28	52 yo Caucasian female ESRD/CCPD, HD since 2003	**Total GBCA administrations =** 4 **Total cumulative dose: 0.27 mmol/kg + 1 unknown dose** **Dates and doses of GBCA administrations:** 13 Sep 2003: Magnevist (dose unk); 11 May 2007: ProHance 20 mL (0.07 mmol/kg); 09 Nov 2007: ProHance 40 mL (0.13 mmol/kg); 04 Mar 2008: MultiHance 20 mL (0.07 mmol/kg)	17 Jul 2008: Muscle biopsy: Gd Gadolinium Quantitation on punch biopsy of muscle 18.0 mcg/g of dry weight. Tissue normal range = < than 0.5 mcg/g of dry weight. Dry weight = 12.844 mg; Wet weight = 44.821 mg. 07 Aug 2008: Office Visit: Patient is 21 days status post punch biopsy of the left mid-calf and evaluation of gadolinium levels. The gadolinium levels are measured at 18 mcg/gram of dry weight which is noted to be 36 times the normal range.	Skin thickening, woody induration, brownish hyperpigmentation, limited ROM, knee contractures focal ossification within the subcutis and prominent fibrosis of the deep dermis and subcutis Score 4, 3 = NSF
29	33 yo Caucasian female ESRD/HD x 23 years	**Total GBCA administrations =** 6 **Total cumulative dose: 0.53 mmol/kg + 2 unknown dose** **Dates and doses of GBCA administrations:** 07 Sep 2001: Magnevist 12 mL (0.13 mmol/kg); 10 Sep 2001: Omniscan 12 mL (0.13 mmol/kg); 18 Sep 2001: Unknown GBCA 15 mL (0.16 mmol/kg): 27 Sep 2001: Unknown GBCA 10 mL (0.11 mmol/kg); 29 Nov 2001 and 27 Feb2002: Unknown GBCAs	09 May 2007 (left arm): Gd quantification via ICP-MS demonstrated 318 ppm Gd in tissue of inferior arm and 337 ppm Gd in tissue of superior arm. 01 Jan 2008 (left arm): Gd quantification via ICP-MS demonstrated 337 ppm Gd in tissue.	Thickened skin, significant tightness of skin over hands, decreased flexion and extension of hands, induration on upper back, patchy hyperpigmentation, induration of hands and forearms, patch of "salt and pepper" pigmentation on thigh, contractures of hands, stiffening of the fingers and skin, contractures of the fingers, depressed hypopigmented areas on the back with surrounding hyperpigmentation, slightly erythematous urticarial

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
				plaques on legs, erythematous papules, bumps and scar tissue similar to burns, contractures of arms, skin tough and leathery to touch, scattered erythematous plaques with a dense woody induration and irregular borders Dense fibrosis with intercalation of fibrocytic cells between and amongst collagen bundles extending throughout the dermis and subcuticular septae, which are widened. Score = 4, 2 = Consistent with NSF
30	76 yo Caucasian female ESRD since 1983/ HD since 1985	**Total GBCA administrations =** 2 **Total cumulative dose: Unknown** **Dates and doses of GBCA administrations:** 06 Jan 2004: Magnevist (dose unk); 20 Aug 2005: Magnevist (dose unk)	26 Jan 2012: Autopsy performed. The examination of the autopsy tissue was able to detect Gd in the skin and lung. Gadolinium was noted to be easily detected in the skin, and only with difficulty in the lung. Gadolinium deposits were not detected in the heart, aorta, and liver. All the Gd deposits detected contained Gd in association with phosphorous and calcium and were confirmed to represent insoluble deposits of Gd phosphate (confirming release of free Gd from	Skin induration Biopsy only showed fibrocyte proliferation Score = 3, 1 = Not NSF. However, autopsy report stated that the pathology of the sampling of skin at the time of autopsy was noted to support the diagnosis of NSF.

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
			the Gd contrast agents).	
31	49 yo Black female ESRD/HD, KTP, HD, KTP since Jul 1997	**Total GBCA administrations =** 3 **Total cumulative dose: 0.41 mmol/kg** **Dates and doses of GBCA administrations:** 24 Mar 2006: Magnevist 20 mL (0.14 mmol/kg); 19 May 2006: Magnevist 40 mL (0.27 mmol/kg); Date unknown: Magnevist 19 mL	17 Sep 2009 (left anterior leg): Mass spectrometric analysis of deparaffinized tissue demonstrated 68.7 ppm Gd	Indurated plaques Haphazardly arranged fibroblasts and collagen bundles throughout the reticular dermis and subcutaneous septa Score = 3, 2 = Consistent with NSF
32	44 yo Caucasian male ESRD/HD, KTP, HD since Mar 1998	**Total GBCA administrations =** 16 **Total cumulative dose: 1.04 mmol/kg** **+ 7 unknown doses** **Dates and doses of GBCA administrations:** 12 Nov 2004: Magnevist 15 mL (0.09 mmol/kg); 24 Jan 2005: Omniscan 20 mL (0.12 mmol/kg); 28 Jan 2005: Omniscan 20 mL (0.12 mmol/kg); 28 Feb 2005: Omniscan 20 mL (0.12 mmol/kg) 28 Mar 2005: Omniscan 20 mL (0.12 mmol/kg); 25 Apr 2005: Omniscan 20 mL (0.12 mmol/kg); 17 Jun 2005: Magnevist 20 mL (0.12 mmol/kg); 08 Aug 2005, 31 Oct 2005, 30 Jan 2006: Magnevist (doses unk); 22 Apr 2006: Omniscan 20 mL (0.12 mmol/kg); 24 Jul 2006: Magnevist (dose unk); 13 Nov 2006: Magnevist 17 mL (0.1 mmol/kg);	22 Feb 2010 Skin biopsy (left forearm). Addendum report dated 23 Apr 2010: A dry tissue specimen was sent for gadolinium testing which revealed 50 mcg/g (normal range < 0.5). 21 Apr 2010 note stated that tissue specimen contained 60 mcg/g gadolinium (normal range < 0.5 mcg/g)	Skin induration, contractures, erythematous plaques Mild increase in fibroblastic appearing cells in the dermis, without significant CD34 staining. Skin biopsy was inconsistent with NSF. Findings were not specific. Final diagnosis was epidermal spongiosis with focal dermal fibrosis. Score = 4, 1 = Inconsistent with NSF

	Patient demographics/ Renal status	GBCA administrations	Evidence of gadolinium presence	Signs/ Symptoms NSF Diagnosis
		12 Feb 2007, 04 Jun 2007, 10 Sep 2007: Unknown GBCAs (doses unk)		
33	58 yo male Septic acute kidney failure	**Total GBCA administrations =** 5 **Total cumulative dose: 100 mL (wt unknown)** **Dates and doses of GBCA administrations:** As reported: About 100 mL Omniscan and Magnevist in 5 sessions within 3 months (Feb - Apr 2006)	The concentration of gadolinium in fibrotic skin decreased from 354,200 µg/kg in 2006 and 9130 µg/kg in 2007 to ≤70 µg/kg in 2008.	Indurated plaques, papules Increased fibroblasts 3, 1 = Not NSF
34	52 yo male Chronic kidney disease, diabetic nephropathy Dialysis status not provided	**Total GBCA administrations =** Unknown ("Repeated exposure') **Total cumulative dose: Unknown** **Dates and doses of GBCA administrations:** Unknown GBCAs on unknown dates at unknown doses	Gadolinium quantification performed on gastrointestinal specimens: 154.0 ppm in the skin, 22.8 ppm in the colon, and 7.0 ppm in the ileum/cecum/colonic specimens	Skin had a diffuse woody peau d'orange appearance, with tightening of the palmar fascia bilaterally at both hands with all fingers "slightly drawn in" (hand contractures) Increased proliferation of CD34+ dermal fibroblast-like cells with dermal mucin deposition, thickened collagen, and thickened fibrous septae. Score = 4, 4 = NSF

HD= Hemodialysis; PD = Peritoneal dialysis; CVVHD = Continuous veno-venous hemodialysis; CCPD = Continuous Cycling Peritoneal Dialysis;
ESRD = End stage renal disease; CKD = Chronic kidney disease; s/p = status post; KTP = Kidney transplant; ppm = parts per million; sc = subcutaneous;
LEs = lower extremities; ROM= Range of motion

Appendix 2 - Reports of Increased Signal Intensity/Elevated or Persistent Levels of Gadolinium with Clinical Symptoms

	Pt age/ gender/ Relevant History	Products/ no. of procedures/dose	Reported symptoms	Evidence of Gd presence
1.	71/M – ESRD	06 Aug 2008: "large" dose of Magnevist for MRI of neck and thorax	06 Aug 2008 (night of the MRI): Decreased mental function, mentation slowed, decreased ambulation ability, decreased appetite, decreasing PO intake. 09 Aug 2008: Weakness. Significant improvement in symptoms after 3 dialysis sessions.	09 Aug 2008: Unenhanced images of the brain showed diffuse enhancement of the sulci; inconclusive as to whether this was indicative of gadolinium retention or extracerebral hemorrhage.
2.	68/F	02 Feb 2009: 15 mL Magnevist for MRI of foot	03 Feb 2009: felt weak, lethargic, and light-headed, with vomiting, fever, tremors, swollen legs, diarrhea and difficulty urinating. Patient still felt weak and lethargic 2 months post injection. 1 year later still had weakness in legs and pain in hips Patient reported in 2011 that she was "getting better" and "still has to do exercise"	2 months post injection, consumer sent nail clippings to a toxicology lab and was informed that she has 2.8 mcg Gd in her body. Patient later sent her urine for evaluation and it came back as "0.4 and 0.5" which she reported was "dangerous".

	Pt age/ gender/ Relevant History	Products/ no. of procedures/dose	Reported symptoms	Evidence of Gd presence
3.	55/F	06 May 2008: Magnevist 17 mL for unspecified indication	4 days after Magnevist injection: facial and eye swelling. On unspecified dates: broken nose, low platelets, rheumatoid arthritis, and Raynaud's phenomenon	Consumer reports that her Gadolinium level was 500 times the "allowed" limit and that Magnevist never cleared her body.
4.	55/M	Apr 2013: Magnevist 18 mL IV for MRI of brain to evaluate migraine	Bilateral pedal and lower extremity paresthesia persisting 8 months after Magnevist injection with ongoing urinary excretion of Gd	Gadolinium excretion documented as follows: 4 mos post exposure 2.6 mcg/d; 5 months post exposure 2.1 mcg/d; 8 months post exposure 1.2 mcg/d
5.	42/F – Epilepsy, astrocytoma, brain tumors treated with resection, radiation and chemotherapy	75 CE-MRIs with Magnevist and Omniscan (reportedly each at 0. mmol/kg) from Nov 1996 to 24 Sep 2014: Magnevist (46), Omniscan (29) - cumulative dose 7.5 mmol/kg	Cerebellar atrophy, abnormal gait (limping), dyslalia,	Increased signal intensity in dentate nucleus, globus pallidus, pulvinar, Cerebellar atrophy per physician could be caused by antiepileptic drugs, radiation and contrast material. Gait disturbance was caused by coxalgia. Cerebellar atrophy persisted; could be related to underlying disease
6.	Age unknown/ Female with normal renal function	Magnevist (date and dose unspecified) for unspecified indication	"Diverse complaints" (non-serious) NOS	"Increased" (NOS) gadolinium concentration in urine 3 weeks after Magnevist administration
7.	55/F	On unspecified dates, the consumer received Magnevist (doses unspecified) for seven unspecified MRIs	Post-operative infection, focal dermal fibrosis, fibrosis of bowel, bilateral carotid stenosis, fibromuscular dysplasia	Consumer reports "heavy metal gadolinium in my urine, some in my blood, even more in my skin" 7 years after last GBCA administration

	Pt age/ gender/ Relevant History	Products/ no. of procedures/dose	Reported symptoms	Evidence of Gd presence
8.	Unk/F	Gadovist – 1 "regular dose"	14 days after administration: itching skin, feeling of constriction, shortness of breath. She suspected that she had been given an overdose of Gadovist.	14 days post administration: Level of Gd determined by unspecified means and "it was diagnosed that Gadovist was apparently not eliminated." Second set of blood samples were taken (results unknown at the time of the report).
9.	48/F	Date not provided: Gadovist 7 mL for MRI of the upper abdomen	After administration: Tachycardia, burning skin. A few hrs. after administration: Burning in the thoracic region and the joints, and an intense feeling of anxiety. 5 days after administration: Transient mild proteinuria and markedly increased elimination of Gd in urine (1759 micrograms/L). Several weeks after administration: Nonspecific disturbances mainly in the joints and the cutaneous region above the chest.	5 days after administration: Transient mild proteinuria and markedly increased elimination of Gd in urine (1759 micrograms/L). Two months after administration: Increased elimination of gadolinium (6.8 micrograms/L).
10.	Unk/F	16 Apr 2015: Magnevist 10 mL for lumbar MRI; 20 Apr 2015: Magnevist 10 mL for cervical MRI	"Contaminated", ready to kill herself, affects her lungs and breathing, throat raw, chest burning, burning in throat, stomach pains, nausea, arms and legs burn, vomiting, constant burning	06 Jun 2015: Hair sample high (NOS) for gadolinium. 06 Aug 2015: Hair sample high (NOS) for gadolinium. Unspecified date: 24-hour urine positive NOS

	Pt age/ gender/ Relevant History	Products/ no. of procedures/dose	Reported symptoms	Evidence of Gd presence
11.	44/F Grade IV glioblastoma multiforme status post resection, chemotherapy and radiation	12 MRIs of the brain with Magnevist from Jun 2010 to Jun 2011 (193 mL or 1.18 mmol/kg + 4 unknown doses)	Toxicity to various agents, contrast media deposition (Patient died from glioblastoma multiforme)	30 Apr 2012 (8 months after death): Kidney: Gd 12 nmol/kg (also reported as 1.9 PPM) Heart: Gd 0.23 nmol/g (also reported as 0.034 PPM) No Gd reported in brain
12.	30/M	21 Jan 2016: Gadavist 10 mL for MRI of the kidney	A few days after Gadavist injection: metallic taste in mouth. A week later: "cognitive impairments,", a feeling of pressure and pain in the head, muscle spasms, muscle pain, rash on face and cheeks, testicular pain, abdominal pain, mental and physical fatigue, concentration severely impaired, occasional tingling sensation in feet. March 2016: All symptoms getting worse.	Feb 2016 (about a month after the MRI): unspecified test showed Gd levels "off the charts" at 77 mcg (normal range 0 - 4 mcg). Approximately March 2016: Urine test showed "massive amount of gadolinium" in his system
13.	Unk/Male	Date(s) unknown: Gadovist (dose[s] not reported) for unspecified indication(s)	Motoric excitability, nervousness	Increased (NOS) gadolinium values in urine

	Pt age/ gender/ Relevant History	Products/ no. of procedures/dose	Reported symptoms	Evidence of Gd presence
14.	38/F	06 May 1999: Unknown GBCA; 06 Oct 2000: Unknown GBCA; 06 Jul 2000: Unknown GBCA; 03 Nov 2009: Magnevist 12 mL (0.09 mmol/kg); 11 Aug 2010: Magnevist 11 mL (0.08 mmol/kg); 16 Jul 2012: Magnevist 15 mL (0.11 mmol/kg); 21 Dec 2012: Gadovist 7.5 mL (0.11 mmol/kg); 08 Nov 2013: Dotarem 10 mL (0.07 mmol/kg); 25 Feb 2015: Dotarem 15 mL (0.11 mmol/kg); 30 Mar 2015: Gadovist 9 mL (0.13 mmol/kg); 05 Aug 2015: Dotarem 10 mL (0.07 mmol/kg); 05 Oct 2015: Dotarem 15 mL (0.11 mmol/kg) 12 CE-MRIs/ Cumulative dose 0.88 mmol/kg + 3 unknown doses. Products received: Unknown GBCAs (3); Magnevist (3); Gadovist (2); Dotarem (4)	Small fiber neuropathy resulting in disability	"Several" urine tests (dates unspecified) detected "accumulation of gadolinium". As reported: Drug level on 28-JUL-2016 was 0.772 and 2.255 mcg/g 18-AUG-2016: 0.943 mcg/g (normal high range<0.230). 28-JUL-2016: Gadolinium value in Basal Urine: Norm High <0.23 mcg/g creatinine; Result: 2.255 mcg/g creatinine (elevated); 18-AUG-2016: Gadolinium value in 24-hour urine collection 3300 ml: Normal High < 0.23 mcg/g creatinine Result: 2.255 mcg/creatinine (elevated).

	Pt age/ gender/ Relevant History	Products/ no. of procedures/dose	Reported symptoms	Evidence of Gd presence
15.	43/F	25 Nov 2015: OptiMARK 7 mL to rule out multiple sclerosis 18 Dec 2015: Eovist to rule out liver cancer 23 Dec 2015: Gadavist to rule out breast cancer 25 Jan 2016: Gadavist to rule out breast cancer	After the 4th MRI: Decreased ability to sense bladder fullness and voiding, non-pitting swelling progressing over several weeks to involve the whole body, tingling neurological pain, aching in hands and feet, skin tightness, "crawling sensation", diminished temperature perception, fatigue, muscular weakness, diminished memory, subcutaneous lesions, shiny appearance of skin over fingers. Events were reported as gadolinium toxicity.	Persistent urine levels of gadolinium 28 days, 48 days, 56 days, 69 days, 74 days, and 103 days after the 4th MRI, decreasing with each subsequent measurement and then increasing again at day 103
16.	60/F	21 Dec 2016: Gadovist 7.5 mL	A few days after Gadovist injection: metallic taste in mouth. A week later: "cognitive impairments,", a feeling of pressure and pain in the head, muscle spasms, muscle pain, rash on face and cheeks, testicular pain, abdominal pain, mental and physical fatigue, concentration severely impaired, occasional tingling sensation in feet. March 2016: All symptoms getting worse.	On 4 Jan 2017: Nontherapeutic agent blood positive result was 3.6 (elevated). On 2 Feb 2017: Nontherapeutic agent blood positive result was 2.8 (elevated). On 04 Jan 2017: analysis showed increase in gadolinium in urine: 127600 mcg/L. On 02 Feb 2017: analysis showed increase in gadolinium in urine: 15000 mcg/L.

	Pt age/ gender/ Relevant History	Products/ no. of procedures/dose	Reported symptoms	Evidence of Gd presence
17.	Unk/F	2013: 3 MRIs with unspecified GBCAs	Gadolinium deposition disease, burning sensations, dysphagia, musculoskeletal disorders, cerebral disorders	Unknown date: Gd levels were "astonishing" and "off the charts" (no values provided).

Appendix 3

Case Reports of symptoms attributed to GBCA administration although no evidence of Gd presence was provided

- A 39-year-old male consumer reported that after receiving 18 mL Magnevist for cardiac MRI in Oct 2014 he had a transient episode of dizziness, after which his legs felt weak and heavy. The following day, after hiking, his legs felt fatigued, sore, and engorged and his calves felt like they had fluid in them and were swollen. He felt like he had run a marathon. The symptoms did not resolve and then every muscle became sorer. His hips and "gluts" and muscles hurt during sleep and while he walked. He went to urgent care on two occasions where all tests were normal.
- In a follow-up report, the consumer attributed the events to "gadolinium deposition disease", although no documentation of Gd presence was provided. He reported additional symptoms including cognitive impairment, neuropathy, paresthesia, left sided weakness, sensation of coldness throughout the body, pain, reduced range of motion, headaches, bowel function disturbances, cyanosis in his feet, itching, burning skin, dry rough skin on his forehead and muscles and joint tightening.
- A 59-year-old female who reportedly "can't clear heavy metals" out of her system received two pituitary MRIs in Jan-2009 and Feb-2009 with unspecified contrast reactions. She was not sure if she received Magnevist or other GBCAs at those times. In Jul 2009, she received Magnevist (dose not specified) for a pituitary MRI. Since that time, she reportedly experienced scleral icterus, film on eyes, "yellow on eyes", "skin burns internally", skin is cold to the touch, spine has burning chills, headaches, blurred vision, right arm and shoulder pain, right arm and shoulder weakness, trouble sleeping, shortness of breath, joint aches pain, needles and pins and itching. Her sister informed her that she may have heavy metal poisoning. No evidence of Gd or other heavy metals was provided. Events were ongoing at the time of the report.
- In a case obtained via Active Online Listening, a female consumer of unspecified age with a family history of breast cancer reported that after a few contrast-enhanced MRIs for breast cancer screening, she began noticing some "strange cognitive effects". She began missing meetings. Over the next several years she had additional MRIs. The math skills that were crucial to her job as finance manager started deteriorating. She eventually wound up on disability. She stopped worrying about cancer and started worrying about imaging drugs. The two GBCAs she reportedly received are Magnevist and Omniscan. No further information could be obtained.
- A 71-year-old male consumer reported that he received Gadavist (dose unknown) in Oct 2015 for an MRI of the head, and felt "headaches hot" during the injection. Afterwards, he experienced headaches starting at his right ear and going around the back of his head. He reportedly could barely get out of bed and headaches have affected his concentration. He went to the emergency room in Oct 2015 and reported that his head did not feel right – his whole head felt like it had been "bombarded with something". The consumer reported having no previous history of headaches. Events persisted. Since OCT-2015 he has been experiencing a constant, dull, pressure headache in the back of the head to the right side; the headaches are sometimes dull and at other times sharp

pains. He does not seem to concentrate as well, and gets more tired. His head starts to pound and he "feels like the dye is still present" in his head. The consumer reported it felt like Gadavist had adhered to his brain, was causing continuing effects and was destroying his cognitive ability. Starting in March 2016, almost every morning he was awakened from sleep, feeling faint, head throbbing, very warm and blood pressure increased. The episodes felt like contrast dye being administered--warm, head throbbing and needing to go to the bathroom very often. The episodes last for about 4 hours; afterwards he is very weak, feels awful and is hardly able to function. He has to force himself out of bed and normal living became very difficult. He thought Gadavist was in or around the cerebellum, and felt like it was also deteriorating his balance. It created tension and affected his concentration and mental abilities. The headache/pressure was getting stronger. It caused daily suffering. The consumer stated there was no other cause for what he was experiencing except Gadavist

- A 54-year-old male consumer received Gadovist in May 2016 for MRI of small bowel and experienced crippling pain/agony, and thought some of it had been injected into the tissue outside of the vein. Three days post injection, he developed cramps in his legs and his toes curled up in spasm, along with deep aching in hips, knees, and feet, and difficulty in walking. This lasted for nearly two weeks. Two weeks from injection in Jun 2016, he "could feel the shape of small bowel in a burning sensation", then had very black stools followed by a day of light grey stools accompanied by aching in his small bowel. Subsequently, the muscles in both hands collapsed and have not gone back to what they were. At times the skin feels very tight as though someone was crushing his hands. His feet feel like this also and both hands and feet have lost mass. All joints in his body seem to have lost the tissue that cushions the joints. His muscles have wasted all over his body and he lost 5 kg weight in 3 months, all of which is muscle mass and cushioning tissue. He stated he resembles someone who has been in a concentration camp. Along with these symptoms, he had headaches every day since the injection, sometimes absolute agony at the base of the skull at the back, along with endless loud tinnitus. He stated he was at times falling into some kind of mental collapse. It was difficult to think properly and tasks were "very difficult cognitively". The most recent thing he had noticed is that the cartilage around his nostrils was disappearing and deep crevices were appearing around his nose. The skin on his hands now resembles a 90-year-old, and has small gold colored flecks in it. All that is left of his feet is bones, tendons and skin; they resemble a skeleton with skin on. He was getting no help at all from his GP or the radiology department that treated him. Events were ongoing and apparently getting worse. He believes gadolinium was fixing itself into his bones and tissues. He also had tightening of the skin, painful joints (making him almost unable to use his hands or walk) and bone pain.

- A spontaneous case report was received from an attorney on behalf of a plaintiff/plaintiff's family, referring to a female of unspecified age who received an unspecified gadolinium-based contrast agent (GBCA) and experienced "gadolinium deposition disease", manifested by extreme cognitive impairment, difficult mentation, headaches, burning sensation on her face, heaviness in her calf, pain in her calf, shooting nerve pain in her toes, blisters on her skin and nausea. No information was provided on

plaintiff's medical history, concurrent conditions or concomitant medications. No further information was reported. Outcome for the events was not reported.

- An attorney reported on behalf of a plaintiff/plaintiff's family, that a female of unspecified age received an unspecified GBCA and experienced "gadolinium deposition disease", manifested by cognitive impairment, headaches, excessive hair loss, vision disturbances, prominent left sided weakness, muscle weakness, loss of range of motion, difficulty walking, difficulty extending arms, legs, hands and feet, sharp bone and joint pain, shortness of breath/dyspnea, chronic nausea and vomiting. No information was given on plaintiff's medical history, concurrent conditions or concomitant medications. Treatment details were not provided.
- A lawyer reported the occurrence of "Gadolinium Deposition Disease" in a female patient who received Gadavist (dose unknown) for MR angiography and MRI. On an unknown date, the patient received Gadavist and subsequently developed "Gadolinium Deposition Disease" with symptoms including cognitive disorder, mental impairment, neurological symptoms NOS, headache, hypoesthesia, hyperkeratosis, hemiparesis, injury NOS, pain and emotional distress.
- A consumer reported that she was once a very healthy active 39-year-old female who is now "struggling between life and death." In Jan 2015, she received Gadavist 7 mL IV for an unspecified indication. Beginning in Jan 2015, she experienced "lots and lots of cognitive impairment," dyspnea/shortness of breath, "high agitation," and unspecified other side effects. The patient stated that no one knows how to treat her and that she received "standard treatment," including anti-inflammatory treatment, treatment for chronic pain, steroids, pain killers, antidepressants, "everything". Events were ongoing at the time of the report (2.5 years after Gadavist administration).
- A female of unspecified age reported the occurrence of being "very sick" with gadolinium deposition disease after receiving unspecified GBCAs for unknown indications.
- A female patient reported that she has had several MRIs with unspecified contrast since Oct 2012, the most recent one in June 2017. Since Oct 2012, and still ongoing today, she reports that she has developed many serious adverse effects since she had the MRIs. She stated that "They are ongoing, daily and constant. I live with them, I cry daily and I am now very suicidal". No evidence of gadolinium presence in the body was provided.