LIFE FROM INSIDE

Bracco Diagnostics Inc.

MultiHance®
(gadobenate dimeglumine) injection, 529 mg/mL

ProHance®
(gadoteridol) injection, 279.3 mg/mL

Medical Imaging Drugs Advisory Committee
Safety of Gadolinium Based Contrast Agents
08 September 2017

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ADVISORY COMMITTEE BRIEFING MATERIALS
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Executive Summary

The use of gadolinium-based contrast agents (GBCAs) has been part of standard clinical practice to provide treatment guidance for almost three decades in more than 300 million patients to enhance the quality of magnetic resonance (MR) images and improve the diagnostic performance of magnetic resonance imaging (MRI) and MR angiography (MRA). In many instances, GBCA-enhanced MRI or MRA provide diagnostic information that would otherwise be unavailable. As such, their use is routine and a crucial component in the diagnosis and follow-up of patients with serious medical conditions, including cancer, vascular pathology, and infection (see Section 1.3).

While GBCAs provide crucial, life-saving medical information, each time an individual GBCA-enhanced MRI or MRA study is considered, it is important to consider its clinical benefit against the potential risk deriving from its use. In the last few years, GBCAs have been almost exclusively grouped as linear or macrocyclic agents. This grouping leads to an emphasis on one, and only one, of the physicochemical characteristics of these agents, i.e., the stability of the GBCA, and there is a tendency to consider all linear GBCAs or all macrocyclic GBCAs as identical and interchangeable, and that data on one GBCA of that "stability class" are considered relevant to all other agents of the same class. This approach is incorrect, as differences within a class should be assessed as attentively as differences between classes. Importantly, not only the stability, but also the GBCA pharmacodynamics (relaxivity) and pharmacokinetics contribute significantly to the benefit-risk profile of each individual GBCA.

Known risks associated with exposure to GBCAs include the possible occurrence of acute reactions and nephrogenic systemic fibrosis (NSF). Acute (or immediate-type) adverse reactions are those occurring within 1 hour of injection of a GBCA, and most of them are hypersensitivity reactions and are unpredictable. Patients at risk for acute hypersensitivity reactions are those with a history of a previous acute reaction to a GBCA, asthma, or other allergic disorders. To minimize the occurrence and severity of hypersensitivity reactions, warnings and precautions specifically related to the risk of serious hypersensitivity reactions are given in the prescribing information of all approved GBCAs, including those of MultiHance and ProHance (see Section 4.3.1).

NSF is the most serious clinical syndrome associated with Gd retention in body tissues to date. It has been observed only in patients with acute or chronic severe renal insufficiency (eGFR <30 mL/min/1.73 m^2), end-stage renal disease, or with acute renal insufficiency of any severity due to hepatorenal syndrome or in the perioperative liver transplantation period ("high-risk patients"). The available evidence on NSF with the individual GBCAs will be provided at Section 3.2.2.1 of this document. It is important here to note that:

- Monitoring of NSF based on spontaneous reporting of adverse drug reactions and literature case reports may lead to underreporting of NSF cases. Missing or inconclusive information in spontaneously reported cases is also a limitation of the monitoring of NSF, as for some reports it may not be possible to confirm or exclude a diagnosis of NSF using the clinicopathological criteria developed by a group of experts in the disease.

- Clinical data deriving from studies of individual GBCAs in high-risk patients, with prolonged and extensive follow-up and systematic collection of all the clinical and dermopathological information that is needed to make a diagnosis of NSF, overcome the limitations of spontaneous reporting of NSF cases and provide better information on NSF risk deriving from exposure to individual GBCAs, providing the patient population is large enough to draw meaningful conclusions.
- The number of high-risk patients in the MultiHance clinical studies (N=8,486) was more than twice the number of high-risk patients in all the studies of the other available GBCAs, and more than nine times higher than that of the high-risk patients in all the studies of the macrocyclic GBCAs Dotarem, Gadavist and ProHance. No NSF cases were observed following single-agent exposure to MultiHance. No NSF cases were observed in the clinical studies of Dotarem, Eovist, Gadavist, and ProHance, while the rate of new cases of NSF was 2.4% for Magnevist and 4.7% for Omniscan.

Important and effective risk minimization measures were introduced by the United Sates Food and Drug Administration (US FDA) in 2010, and, to Bracco's knowledge, no new cases of NSF have been reported since.

Recently, residual Gd has been found within the brain tissue of patients exposed to GBCAs, particularly in instances in which patients have undergone multiple MRI examinations with GBCAs. However, levels of Gd in brain tissue appear to be much lower than those observed in body tissues. The aim of this document is to provide evidence to address four fundamental questions related to possible retention of Gd complexes in both brain and body tissues following exposure to GBCAs.

The first question is whether exposure to GBCAs is associated with retention of Gd complexes in brain and extra-cerebral (body) tissues, and, if so, whether there are differences among individual GBCAs. Data from experimental animal studies and clinical investigations are reported in Section 2 of this document. Results from these studies can be summarized as follows:

- Exposure to GBCAs is associated with retention of Gd complexes in brain and body tissues. Gd retention was observed in every patient exposed to either linear or macrocyclic GBCAs, and may be dependent on the total cumulative dose given to a patient.
- Gd levels measured in the brain are only an extremely small fraction of the injected dose, and are markedly lower than those measured in body tissues, even if there seems to be a correlation between levels in brain and levels in body tissues.
- Clearance of retained Gd complexes over time has been observed for all GBCAs both in adult and juvenile animals, and from both brain and body tissues. However, Gd complexes have been observed in human patients months or even years after exposure to either linear or macrocyclic GBCAs.
- Clinical data are too limited to evaluate whether patient characteristics, including underlying diseases and renal function, may affect Gd retention in brain and/or body tissues. Impairment of renal function did not seem to influence Gd retention and clearance in mice models, while opposite results were found in rat models;

- As far as brain Gd retention is concerned, the available evidence shows clear differences among the individual agents:
  - The intravenous administration of the macrocyclic GBCA ProHance is associated with the lowest levels of Gd concentrations in brain tissues both in animals and man, often at the limit of quantitation by ICP-MS, and lower than those measured after administration of the other macrocyclic GBCAs Dotarem and Gadavist;
  - Animal studies showed that all the macrocyclic GBCAs, Dotarem and Gadavist included, caused significantly lower levels of Gd retention compared with the linear GBCAs. However, first and limited data from post-mortem, tissue-sample studies in man do not show a clear demarcation between Gd levels retained after exposure to the macrocyclic agent Gadavist and those following the administration of linear GBCAs;
  - Among the linear GBCAs, Omniscan was shown to be associated with the highest brain Gd levels, whereas the lowest Gd levels were observed following administration of MultiHance;
  - Post-mortem, tissue-samples studies showed the highest brain concentration of elemental Gd in the DN and GP, with a majority of elemental Gd deposits found within the endothelial wall, and a smaller fraction identified in the tissue interstitium. Of note, following repeated doses of the GBCA Omniscan, Gd deposits were detected within the nucleus of a neuronal cell in 2 patients;
  - Soluble Gd complexes seem to be cleared more efficiently than insoluble complexes. However, Gd complexes have been observed in the brain of human patients months, or even years after exposure to either linear or macrocyclic GBCAs.
- As for retention in body tissues:
  - Animal studies showed that administration of Omniscan or Magnevist was associated with the highest Gd levels retained in skin, bone, liver and kidney. Data obtained in humans do not allow the drawing of firm conclusions about possible differences among the various GBCAs.
  - The distribution of Gd deposits in body tissues in man is inhomogeneous and seems to be higher in areas of inflammation, increased cellularity, or lesional areas in patients with NSF. In a patient with normal renal function, deposition of Gd was associated with the connective tissue septations of the subcutaneous adipose tissue and increased CD34 immunoreactivity.
- More, and possibly larger studies in humans aimed at determining levels of elemental Gd in brain and body tissues are needed to understand possible differences among the various GBCAs approved and used in the United States. Also, additional clinical studies are needed to determine the chemical form of the Gd complexes retained in human tissues, as well as retention over time.

The second fundamental question is whether long-term Gd retention in tissues is associated with damage of the involved tissues. The available evidence (see Section 3) shows that:

- No sign of neurotoxicity was ever detected in any of the experimental animal studies or post-mortem, tissue-sample studies in man, following exposure to MultiHance, ProHance or any other GBCA.
- Omniscan and OptiMARK were shown to be associated with proinflammatory and profibrotic effects, as well as with skin lesions partially resembling NSF in healthy and renally-impaired animals. Similar skin lesions or proinflammatory and profibrotic effects were not observed following exposure to the other GBCAs;
- In human patients, NSF, a disease that resembles scleroderma and eosinophilic fasciitis clinically and scleromyxedema histopathologically, has been associated with exposure to GBCAs in patients with end-stage renal disease or eGFR <30 mL/min/1.73 m^2, and/or during the perioperative liver transplantation period. Besides NSF, eosinophilic, collagenous, round, or ovoid sclerotic bodies, in various stages of calcification, were observed in patients 3.5 to 5 years after exposure to GBCAs ("gadolinium-associated plaques").

The third question is whether there is evidence of clinically relevant harm to patients (see Section 3). The simple answer is that, besides NSF, there is currently no evidence suggesting that brain or body retention of Gd complexes is associated with harmful effects and potential interaction with disease processes:

- The experimental animal studies aimed at determining the potential correlation between Gd retention in brain tissues and neurological changes did not detect any neurological disorders, behavioral abnormalities, physiological dysfunctions, and any other signs of nervous system toxicity, both in adult and juvenile animals.
- Two population studies seem to exclude an association between exposure to GBCAs and an effect on overall neurologic or neurocognitive performance;
- Case reports of non-NSF, delayed adverse events do not provide sufficient evidence of any additional risk resulting from Gd retention in body tissues.

The final question is if the available evidence on Gd retention in tissues changes the benefit-risk balance of MultiHance and ProHance (see Section 4). The answer is no: the benefit-risk balance of both products is unchanged, because:

- The effectiveness and benefit provided by the two agents has been properly demonstrated before and after their approval by the US FDA;
- There is no evidence of new, additional risk resulting from Gd retention in brain and body tissues. To date, NSF is the most significant clinical syndrome associated with retention of Gd complexes in body tissues, and there are no medically-confirmed, single-agent, unconfounded cases of NSF reported in association with the administration of MultiHance, and only one case following the administration of ProHance. No cases of NSF were observed with either MultiHance or ProHance in clinical investigations carried out in high-risk patients exposed to the agents. Of note, for NSF, important and effective risk

minimization measures were introduced in 2010 (*FDA, 2010*), and, to Bracco's knowledge, no new cases of NSF have been reported since.

Even if there is currently no evidence that, besides NSF, Gd retention in tissues is associated with harm to patients, in order to minimize the risk of Gd retention, the prescribing information for all GBCAs (including MultiHance and ProHance) should contain clear, unambiguous wording to make the health care professionals utilizing the products aware and to reduce exposure.

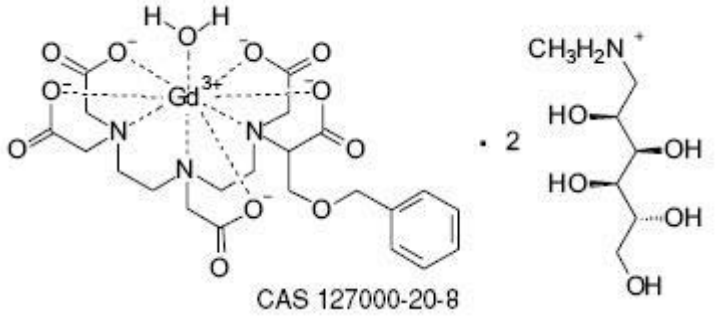
Also, even if Bracco is not aware of any report of visible hyperintensity in deep brain areas negatively affecting the interpretation of brain MR images following the administration of MultiHance, ProHance or other GBCAs, further precautions in the prescribing information of the agent would help to increase awareness and avoid any possible, even if unlikely, effect of T1 shortening on image interpretation. If fully informed, radiologists observing T1 hyperintensity on unenhanced images would be reminded of the importance of gathering information on the patients that would make them understand the possible cause of the abnormal T1 shortening seen on the images.

Finally, there are still important knowledge gaps on Gd retention and its possible association with harmful effects and potential interaction with disease processes. Accordingly, the collection of additional information through new, well designed animal and clinical studies is warranted.

1 Introduction

1.1 MultiHance and ProHance: Chemical Names, Structures and Approved Indications

MultiHance is a gadolinium-based contrast agent (GBCA). Its active ingredient, gadobenate dimeglumine, is chemically designated as (4RS)-[4-carboxy-5,8,11-tris(carboxymethyl)- 1-phenyl-2-oxa-5,8,11-triazatridecan-13-oato(5-)] gadolinate(2-) dihydrogen compound with 1-deoxy-1-(methylamino)-D-glucitol (1:2) with a molecular weight of 1058.2 and an empirical formula of $C_{22}H_{28}GdN_3O_{11} \bullet 2C_7H_{17}NO_5$. The structural formula is as follows:

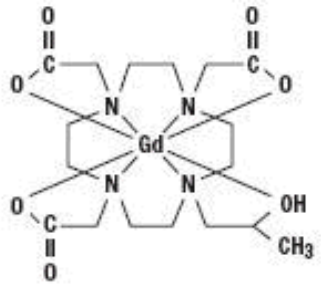


CAS 127000-20-8

MultiHance is currently indicated for use in:

- Magnetic resonance imaging (MRI) of the central nervous system (CNS) in adults and children over 2 years of age to visualize lesions with abnormal blood brain barrier or abnormal vascularity of the brain, spine, and associated tissues.
- Magnetic resonance angiography (MRA) to evaluate adults with known or suspected renal or aorto-ilio-femoral occlusive vascular disease.

ProHance is also a GBCA. Its active ingredient is gadoteridol is the gadolinium complex of 10-(2-hydroxy-propyl)-1,4,7,10- tetraazacyclododecane-1,4,7-triacetic acid with a molecular weight of 558.7, an empirical formula of $C_{17}H_{29}N_4O_7Gd$ and has the following structural formula:



ProHance is indicated for use in:

- MRI of the CNS in adults and children over 2 years of age to visualize lesions with abnormal vascularity in the brain (intracranial lesions), spine and associated tissues.
- MRI in adults to visualize lesions in the head and neck.

1.2 Properties of Gadolinium-based Contrast Agents

The process of MRI is based upon the ability to induce and detect the resonance of small magnetic fields, or moments, of some atomic nuclei which have the inherent properties of spin and charge (*Doyle et al., 1992*). The most abundant of the naturally occurring spinning nuclei in the human body are the hydrogen nuclei, or protons, found in water and lipids. As any charge in motion, protons generate a moment, which behaves with all of the characteristics of a magnet. When placed into an external, strong magnetic field (ranging usually from 1.0 to 3.0 Tesla), these small magnets align with the axes of the applied field. If a pulse of specific radiofrequency energy is introduced, the protons will absorb this energy and re-orient themselves against the external magnetic field. Once the radiofrequency pulse is discontinued, the "excited" nuclei re-align themselves with the axes of the external magnetic field and, in doing so, release the absorbed energy as a detectable characteristic radiofrequency signal. This process is called "relaxation". Measured relaxation times, termed T1 (spin-lattice relaxation) and T2 (spin-spin relaxation), characterize the rate of relaxation of the excited protons.

Gadolinium (Gd) is a metal ion with a large magnetic moment, which shortens both relaxation times, T1 and T2. Due to its seven unpaired electrons, the Gd ion shows the strongest effect of all elements on the T1 relaxation times. In its free ionic form (Gd3+), it can compete with calcium ions (Ca2+) and become toxic in biological systems; therefore, Gd3+ must be chelated to an organic ligand to be used in patients. (*Sherry et al., 2009*). Commercially available GBCAs contain Gd chelated to ligands of different chemical structure. Chelation of Gd by appropriate ligands dramatically reduces its acute toxicity (*Weinmann, 1984; Lauffer et al., 1987; Bussi et al., 2007*).

All GBCAs are nine-coordinate complexes in which the ligand occupies eight binding sites at the metal center (Gd) and the ninth coordination site is occupied by a water molecule for proton relaxation, from which derive the MR signal-enhancing properties of the compound (*Caravan et al., 1999*).

The chemical structure of the ligand affects the stability, relaxivity, and pharmacokinetics of the GBCA.

- *Stability* refers to how tightly the Gd ion is bound to the chelating molecule and how likely it is to dissociate. When this happens, the slowly released Gd3+ are immediately picked up by a variety of competing anions and, to a lesser extent, to macromolecules in the circulating blood and in tissues. In general, GBCAs with a macrocyclic structure of their ligand show lower and slower rates of Gd3+ dissociation compared with GBCAs with linear, or "open-chain" ligands. While the macrocyclic GBCAs (Dotarem, Gadavist and ProHance) all show similar dissociation constants, significant differences can be observed among the linear GBCAs, with MultiHance more stable than the linear Magnevist, and markedly more stable than the linear agents Omniscan and OptiMARK (*Laurent et al, 2006; Idee et al., 2009*).
- *Relaxivity* is a measure of the potency of a GBCA to increase the relaxation rates of surrounding water protons (*Laurent et al., 2005*). The proton relaxation rate is what provides the signal in MRI: the higher the relaxation rate, the higher the signal intensity

(SI) on T1-weighted images and the higher the contrast-enhancing efficacy of the GBCA (*Bleicher et al., 2008*).

All available GBCAs show similar r1 relaxivity values in water (*Laurent et al, 2006*). The presence of macromolecules like human serum albumin has no significant effect on the relaxivity of the linear GBCAs Magnevist, Omniscan or OptiMARK, or the macrocyclic agents Dotarem, Gadavist or ProHance, but markedly increases the relaxivity of MultiHance because of a non-covalent interaction between the active ingredient of the agent (gadobenic acid) with the macromolecules, resulting in slower molecular tumbling rates, longer rotational MR correlation times and faster relaxation rate of surrounding water protons. (*Laurent et al., 2006*). *In vivo*, the resulting increase in the relaxivity of MultiHance depends on the relative amounts of gadobenic acid not interacting with macromolecules and of gadobenic acid bound to macromolecules. Therefore, the resulting relaxivity depends on the concentration of macromolecules in the medium: the higher the concentration, the higher the relaxivity of MultiHance, the greater its effect on SI at a given concentration of gadobenic acid in the medium. (*Giesel et al, 2006*).

- Finally, the chemical structure of the ligand of each individual GBCA also affects the *pharmacokinetics* of these medical imaging agents. Most of the available GBCAs (Dotarem, Gadavist, Magnevist, Omniscan, OptiMARK and ProHance) have an initial short intravascular distribution and then diffuse into the extracellular space throughout the body without any tissue or organ specificity ("extracellular-fluid" or "general-purpose" agents). The active ingredient of MultiHance (gadobenic acid) possesses an aromatic (benzyloxymethyl) group on the contrast-effective molecule, whereas the macrocyclic agents do not. (*Hao et al, 2012*) This aromatic group on gadobenic acid not only provides increased relaxivity relative to that of the pure extra-cellular GBCAs, due to a transient interaction of the gadobenic acid molecule with macromolecules in the medium, but also determines the unique and specific pharmacokinetics of MultiHance.

  Following its intravenous injection, 95-97% of the injected dose has an initial short intravascular distribution and then diffuses into the extracellular space throughout the body. Gadobenic acid is also actively transported into functioning hepatocytes; therefore, 3-5% of the injected dose (8-10% in patients with renal impairment) is excreted into bile.

In the last few years, GBCAs have been almost exclusively grouped as linear or macrocyclic agents. This grouping leads to an emphasis on one, and only one, of the physicochemical characteristics of these agents, i.e., the stability of the GBCA, while also the GBCA pharmacodynamics (relaxivity) and pharmacokinetics do contribute to the benefit-risk balance of each individual agent. Moreover, there is a tendency to consider all linear GBCAs or all macrocyclic GBCAs as identical and interchangeable, and that data on one GBCA of that "stability class" are considered relevant to all other agents of the same class. This approach is incorrect, as differences within a class should be assessed as attentively as differences between classes.

1.3 Problem Statement

GBCAs enhance the quality of MR images by altering the magnetic properties of nearby water molecules in the body (*RSNA, 2017*). The use of GBCAs has been part of standard clinical practice to provide treatment guidance for almost three decades in more than 300 million patients by enhancing the diagnostic performance of MRI and MRA in locating and outlining normal (or variants of normal) anatomic structures, in distinguishing between normal and abnormal anatomy, in detecting and assessing disease or pathology in defined clinical settings. (*ACR, 2013; ACR-NASCI-SPR, 2015; ACR-SAR-SPR, 2015; ACR, 2017a; RSNA, 2017*)

While GBCAs provide crucial, life-saving medical information, each time a GBCA-enhanced MRI or MRA study is considered, it is important to consider its clinical benefit against the potential risk deriving from GBCA use (*ACR, 2017a*).

Known risks associated with exposure to GBCAs include the possible occurrence of acute reactions and nephrogenic systemic fibrosis (NSF). Acute (or immediate-type) adverse reactions are those occurring within 1 hour of injection of a GBCA (*ESUR, 2016*). The type, severity and rate of acute adverse reactions is similar for all approved GBCAs (*ESUR, 2016, ACR, 2017b*). Serious acute reactions to GBCAs are rare (approx. 1 every 10,000 exposures) (*ACR, 2017b*). Most of these are hypersensitivity reactions and are unpredictable (*ESUR, 2016, ACR, 2017b*). Patients at risk for acute hypersensitivity reactions are those with a history of a previous acute reaction to a GBCA, asthma, or other allergic disorder (*ESUR, 2016, ACR, 2017b).* To minimize the occurrence and severity of hypersensitivity reactions, warnings and precautions specifically related to the risk of serious hypersensitivity reactions are given in the prescribing information of all approved GBCAs, including those of MultiHance and ProHance.

NSF is the most serious clinical syndrome associated with Gd retention in body tissues to date. It has been observed only in patients with acute or chronic severe renal insufficiency (eGFR <30 mL/min/1.73 m^2), end-stage renal disease, or with acute renal insufficiency of any severity due to hepatorenal syndrome or in the perioperative liver transplantation period (*ACR, 2017c*). The available evidence on NSF with the individual GBCAs will be provided in Section 3.2.2.1 of this document. It is important here to note that important and effective risk minimization measures have been introduced in 2010 (*FDA, 2010*), and, to our knowledge, no new cases of NSF have been reported since that time.

Recently, residual Gd has been found within the brain tissue of patients exposed to GBCAs. The retention of Gd complexes appears to occur preferentially in certain specific areas of the brain. Levels of Gd in brain tissue, however, appear to be much lower than those observed in body tissues. Therefore, this document is aimed at providing evidence to address four fundamental questions related to possible retention of Gd complexes in both brain and body tissues, and any change in the benefit-risk balance of intravenous use of MultiHance and ProHance:

1. Is exposure to GBCAs associated with retention of gadolinium (Gd) complexes in brain and body tissues? If so, is there a difference among the individual GBCAs?
2. Is Gd retention associated with damage of involved tissues?
3. Is there evidence of clinically relevant harm to patients?
4. Does the available evidence change the benefit/risk balance of MultiHance or ProHance?

2 Evidence of Gd Retention in Brain and Body Tissues

2.1 Gd Retention in Brain Tissues

The association between exposure to GBCAs and retention of Gd complexes in brain tissues has been studied in nonclinical and clinical studies aimed at:

a) Directly determining levels of elemental Gd or Gd complexes in brain tissues ("tissue-sample studies"), and

b) Indirectly implying Gd retention in brain tissues by determining change in signal intensity (SI) or R1 relaxation rate on unenhanced MR images of the brain ("imaging studies").

2.1.1 Tissue-Sample Studies

2.1.1.1 Nonclinical, Tissue-Sample Studies

Bracco has reviewed peer-reviewed publications of 13 studies in adult healthy rats, and of 3 studies comparing healthy *vs.* renally impaired rats or mice. Bracco has also conducted one study in juvenile rats (*Study AB21194*) and one study in adult healthy rats, both not published yet (*Study FRCG-03-15)*. Bracco also reviewed important information on Gd retention in brain tissues contained in the PRAC (Pharmacovigilance Risk Assessment Committee) Re-Examination Rapporteur's Assessment Report prepared by the Medical Product Agency of Sweden (Rapporteur Member State) during a Referral Procedure under Article 31 of Directive 20010/83/EC and released on June 28, 2017 (*PRAC Re-Examination Rapporteur's Assessment Report, 2017*).

The cumulative dose injected in adult healthy rats ranged between 6 and 50 mmol/kg. Considering that a dose of 0.6 mmol/kg corresponds to 0.1 mmol/kg in adult humans of 60 kg body weight (FDA *Food and Drug Administration, Guidance for Industry, 2005*), those doses in rats correspond to a total cumulative GBCA dose of 1 to 8.3 mmol/kg, i.e., to 10 to 83 standard 0.1 mmol/kg doses in a human being of 60 kg body weight. Of note, high cumulative doses were administered to the study animals in a short period of time in order for the studies to be completed in a reasonable timeframe. The off-dose period (for adult healthy rats) ranged between 7 days and 52 weeks. Different regions of the brain have been investigated in the different studies.

The most significant results are summarized and discussed in the following sections.

2.1.1.1.1 Levels of Elemental Gd in Brain Tissues Following Exposure to Different GBCAs in Healthy Rats

Most of the nonclinical studies used inductively coupled plasma combined with mass spectrometry (ICP-MS) to determine Gd levels in brain tissues. ICP-MS is a sensitive means of quantifying Gd. However, this is a destructive technique that reports only on the elemental composition and not on form of the Gd, i.e., whether it is still present as intact contrast agent molecule, $GdPO_4$, or other forms.

A comparison of results of levels of elemental Gd can be done only among three studies with similar methodology carried out by Guerbet and Bracco (*Robert et al.2015; Robert et al.2016; Bracco study FRCG-03-15*). Determination of levels of elemental Gd was performed using ICP-MS. In these studies, healthy rats received 20 intravenous injections (4 injections per week for 5 consecutive weeks) of 0.6 mmol Gd/kg, up to a total cumulative dose of 12.0 mmol/kg. A dose of 0.6 mmol/kg in rats corresponds to 0.1 mmol/kg, or 0.2 mL/kg in adult humans of 60 kg body weight (FDA *Food and Drug Administration, Guidance for Industry, 2005*); therefore, a total cumulative dose of 12 mmol/kg in rats corresponds to a total cumulative dose of 2.0 mmol/kg in human beings of 60 kg body weight, i.e., to 20 injections of a standard dose of 0.1 mmol/kg in 5 weeks. An additional control group receiving matched volumes of saline was included. The off-dose period before animal sacrifice was 4 weeks.

The lowest amount of elemental Gd (and close to the limit of quantitation) was observed following repeated doses of ProHance, followed by Gadavist and Dotarem (24%-78% higher than ProHance). Levels of elemental Gd following MultiHance were 3 to 6 times higher than those measured for the macrocyclic GBCAs, depending on the brain area and the macrocyclic agent tested. However, levels of Gd deposition following MultiHance were 60% to 100% lower than those observed following Magnevist, and 3 to 4 times lower than those measured after Omniscan.

Of note, the difference in levels of elemental Gd in brain tissues between MultiHance and Magnevist was not the result of difference in pharmacokinetics in rats between these two GBCAs, as often erroneously mentioned by the Sponsor Bayer in its publications. While it is known that biliary excretion of gadobenic acid (active ingredient of MultiHance) accounts for approximately 50% of plasmatic clearance in rats, this does not result in a lower exposure in this animal species compared to gadopentetic acid (active ingredient of Magnevist). The plasma elimination half-lives of gadobenic acid (MultiHance) and gadopentetic acid (Magnevist), when administered intravenously to rats, are superimposable (19.8 vs 19.6 min following a dose of 0.5 mmol/kg for MultiHance and Magnevist, respectively) (*Lorusso et al. 1999; Weinmann et al. 1984*); therefore, the biliary excretion of MultiHance does not significantly influence animal exposure.

Table A: Gd Concentration in Brain Tissues after Repeated Administrations of GBCAs: Data from Robert et al. 2015, Robert et al. 2016, and Bracco Study FRCG-03-15)

	ProHance	Gadavist	Dotarem			MultiHance		Magnevist		Omniscan		
Brain Area	a	a	a	b	c	a	b	a	b	a	b	c
Cerebellum nmol/g fresh tissue Mean (SD)	0.130 (0.020)	0.161 (0.026)	0.217 (0.027)	0.26 (0.12)	0.27 (0.16)	0.78 (0.23)	1.21 (0.48)	1.12 (0.18)	1.67 (0.17)	2.75 (0.45)	3.66 (0.91)	3.75 (0.18)
Cerebral cortex nmol/g fresh tissue Mean (SD)	0.1276 (0.0032)	0.174 (0.046)	0.227 (0.041)	0.30 (0.08)	nd	0.621 (0.053)	nd	1.42 (0.30)	nd	1.56 (0.31)	2.32 (0.46)	nd
Subcortical brain nmol/g fresh tissue Mean (SD)	0.114 (0.052)	0.147 (0.020)	0.181 (0.025)	0.30 (0.10)	nd	0.686 (0.079)	nd	1.37 (0.34)	nd	2.88 (0.34)	2.47 (0.62)	nd

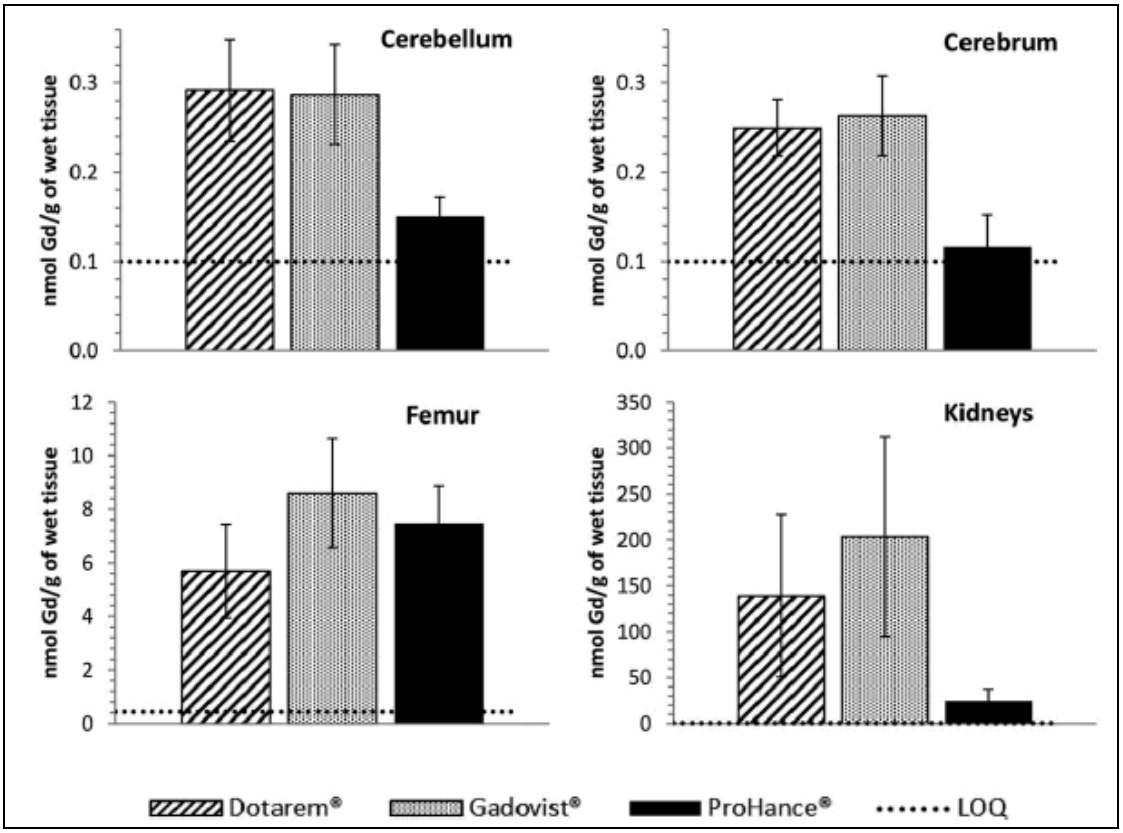
a Final Report of Bracco Study FRCG-03-15 (Ref. 31)
b Robert et al. 2015
c Robert et al. 2016
nd = not determined

The rat model in the three studies described above was used by Bracco in a subsequent study (*Bussi et al, 2017*) aimed at assessing differences in Gd retention among the individual macrocyclic GBCAs. Dotarem, Gadavist, and ProHance were administered to rats 4 times a week for 5 consecutive weeks at a dosage of 0.6 mmol/kg (corresponding to 0.1 mmol/kg, or 0.2 mL/kg in adult humans of 60 kg body weight) for a cumulative dose of 12.0 mmol/kg (240 mL, or 2.0 mmol/kg in adult humans of 60 kg body weight). Animals (15 males/GBCA group and 5 males in control group, injected with saline solution) were sacrificed after an off-dose period of 4 weeks after the last GBCA administration. Measurements of elemental Gd levels were performed using ICP-MS.

In the different cerebral areas (brain, cerebellum), Gd content in animals treated with Dotarem and Gadavist was significantly higher ($P<0.001$) than in animals treated with ProHance. Both in cerebellum and in brain tissues, the Gd content of animals treated with Dotarem and Gadavist was approx. twice higher than the values found following administration of ProHance (Figure 1).

Measurements of elemental Gd were also performed in the femoral bone tissues and in the kidneys of the treated animals. It is noteworthy that retained Gd levels were markedly higher in the extra-cerebral tissues (Figure 1).

Figure 1. Gd Content in Cerebellum, Brain, Femur and Kidney Tissues of Healthy Rats (n=15) Treated with Dotarem, Gadavist or ProHance (Source: Figure 1 in the original publication by Bussi et al. 2017)



The Sponsor Bayer conducted a study in rats to determine the potential correlation of histopathological changes and Gd distribution in rodent brain and skin samples after multiple administrations of either linear (Magnevist, Omniscan) or macrocyclic (Gadavist, ProHance) GBCAs. In addition, the specific distribution of Gd in brain and skin tissue was assessed using highly sensitive laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) (*Lohrke et al, 2017*). Fifty rats were randomly allocated to control and 4 GBCA groups (n = 10 per group). The animals received 20 intravenous injections each at a dose of 2.5 mmol Gd/kg body weight for 5 consecutive days per week over period of 4 weeks, up to a total cumulative dose of 50 mmol/kg, representing more than the 83-fold of the surface-adapted standard 0.1 mmol/kg human dose.

The effect of high intravenous doses of GBCAs on the amount of residual Gd in skin, bone, skeletal muscle, and brain tissue was assessed using ICP-MS. Eight weeks after the last injection, animals administered Magnevist displayed the highest Gd concentrations in the brain (13.1 ± 7.3 nmol Gd/g tissue), followed by Omniscan (11.1 ± 5.1 nmol Gd/g tissue). Brain Gd levels in the animals administered Gadavist were significantly lower (0.7 ± 0.4 nmol Gd/g tissue) but still higher than those after ProHance (0.5 ± 0.2 nmol Gd/g tissue). The overall amount of Gd observed in the brain after linear GBCA administration remained extremely low, with 0.0002%

of the injected dose per gram of tissue. The Gd concentrations in the brain were approximately 100-fold less compared with the skin.

Spatially resolved LA-ICP-MS measurements displayed the highest Gd signals in skin and brain tissue after the administration of Omniscan and Magnevist. According to the concentration scale, which ranged from 0 to 100 mg Gd/kg for skin and 0 to 4 mg Gd/kg for brain, the highest Gd values were observed in the skin and deep cerebellar nuclei of rats administered Omniscan and to a lesser extent, with Magnevist. In addition to the specific localization in the deep cerebellar nuclei, substantial Gd signals could also be visualized in other brain regions such as the granular layer of the cerebellar cortex. In all brains from linear and macrocyclic GBCAs groups, very low and homogeneous, background-like distribution of Gd was observed.

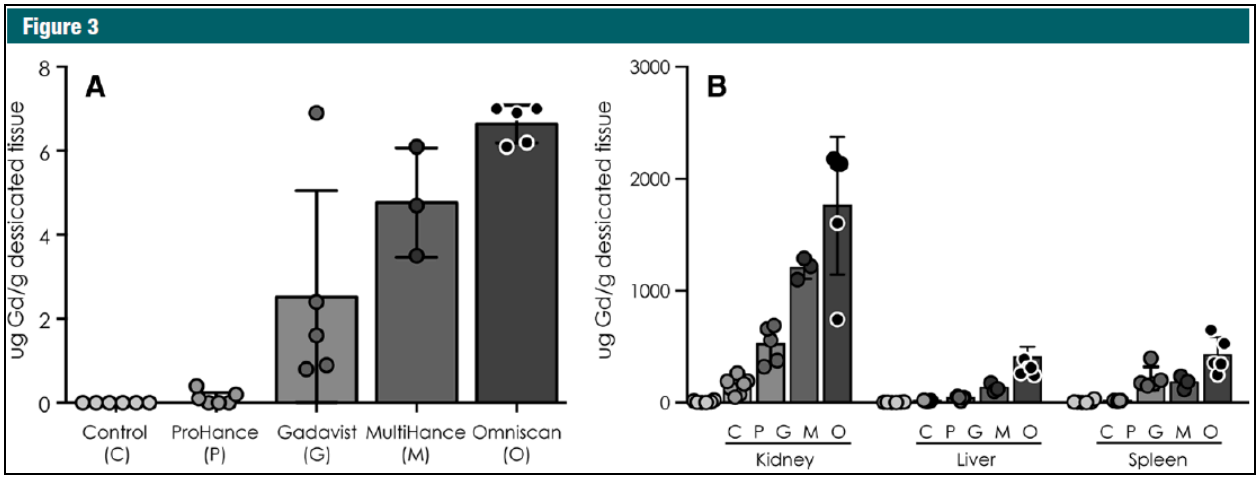
Of interest is the only independent study conducted in a rat model by investigators of the College of Medicine, Mayo Clinic, Rochester, MN, USA and recently published in Radiology (*McDonald et al., 2017a*). Healthy rats received 20 intravenous injections of 2.5 mmol/kg (gadolinium-exposed group) or saline (control group) over a 26-day period. Total cumulative dose was 50 mmol/kg, corresponding to >83 standard 0.1 mmol/kg doses in a human patients of 60 kg body weight. Rat brain and renal, hepatic, and splenic tissues were harvested 7 days after final injection and subjected to ICP-MS for determination of levels of elemental Gd, and transmission electron microscopy (TEM) for quantification and characterization of Gd deposits. Differently from previous studies that assessed Gd levels in the entire cerebellum, the cerebellar dentate nuclei (DN) were carefully extracted by using microdissection for brain tissue samples in this study by McDonald et al.

Levels of elemental Gd in the DN varied significantly with GBCA type (F = 31.2; P, 0.0001), with median concentrations of 0 mg Gd per gram of tissue (95% confidence interval [CI]: 0, 0.2) in ProHance-injected rats, 1.6 mg Gd per gram of tissue (95% CI: 0.9, 4.7) in Gadavist-injected rats, 4.7 mg Gd per gram of tissue (95% CI: 3.5, 6.1) in MultiHance–injected rats, and 6.9 mg Gd per gram of tissue (95% CI: 6.2, 7.0) in Omniscan-injected rats.

Figure 2 shows the original graph from the publication by McDonald et al. reporting Gd levels in the DN of the treated animals. It is noteworthy to observe the large variability in levels of elemental Gd in the animals treated with Gadavist, with one rat showing Gd levels as high as those observed after administration of Omniscan, and higher than those observed after administration of MultiHance.

The study also assessed levels of elemental Gd in other tissues (kidney, liver and spleen). Gd retention in non-neural tissues was markedly and significantly higher compared with neural tissues. Similarly to neural tissues, Gd levels were the lowest and highest following the administration of ProHance and Omniscan, respectively. Of note, similarly to what was observed by Bussi et al. (Bussi et al., 2017), levels of elemental Gd in body tissues were markedly higher than those observed in the DN, despite the DN being the main repository of metals in the cerebellum.

Figure 2. Gd Content in Dentate Nuclei, Liver, Kidney and Spleen of Rats Treated With ProHance, Gadavist, MultiHance or Omniscan (Source: Figure 3 in the original publication by McDonald et al. 2017a)



The study by McDonald et al. also used transmission electron microscopy with energy dispersive x-ray spectroscopy for quantification and characterization of elemental Gd deposits within the DN. Despite a relative increase in electron dense foci in ProHance-treated rats, the characteristic x-ray spectrum of Gd was not detected by using this method. Increasing concentrations of elemental Gd were detected in the DN tissues of rats treated with Gadavist, MultiHance, and Omniscan (Figure 3, from the original publication).

While a majority of these elemental Gd deposits were found within the endothelial wall, a smaller fraction was identified in the tissue interstitium.

Figure 3. Elemental Gd Deposits in the Dentate Nuclei Following Administration of Saline (Control), ProHance, Gadavist, MultiHance and Omniscan (Source: Figure 4 in the original publication by McDonald et al. 2017a)

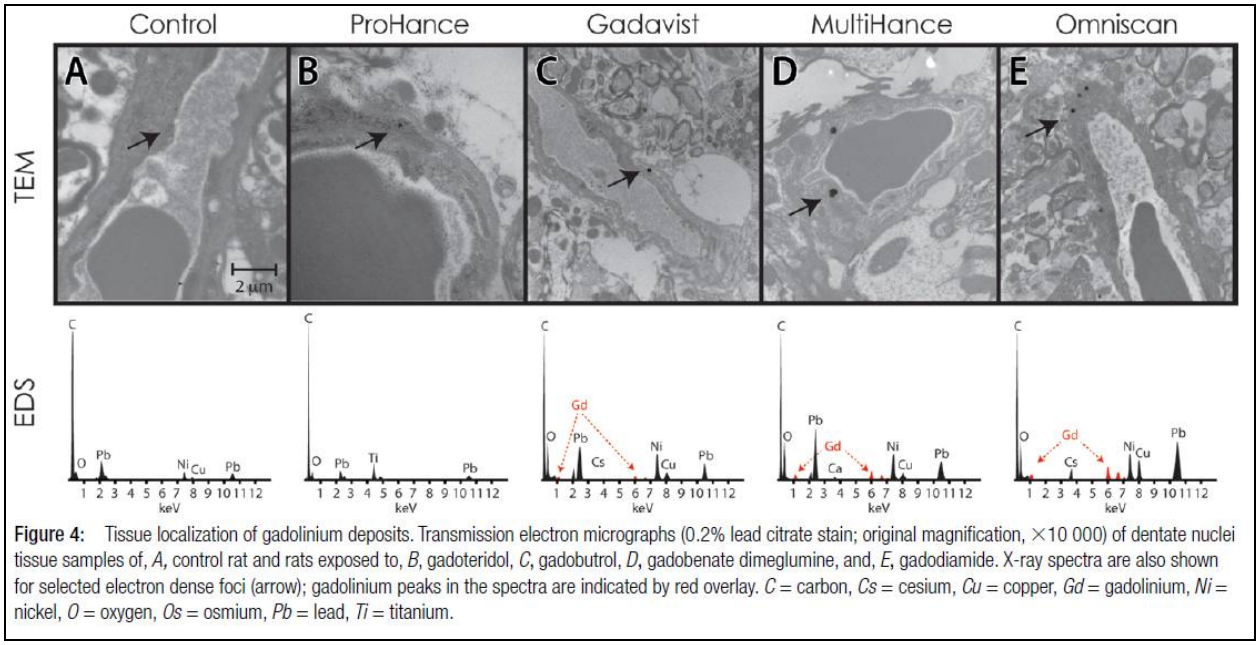


Figure 4: Tissue localization of gadolinium deposits. Transmission electron micrographs (0.2% lead citrate stain; original magnification, ×10 000) of dentate nuclei tissue samples of, *A*, control rat and rats exposed to, *B*, gadoteridol, *C*, gadobutrol, *D*, gadobenate dimeglumine, and, *E*, gadodiamide. X-ray spectra are also shown for selected electron dense foci (arrow); gadolinium peaks in the spectra are indicated by red overlay. *C* = carbon, *Cs* = cesium, *Cu* = copper, *Gd* = gadolinium, *Ni* = nickel, *O* = oxygen, *Os* = osmium, *Pb* = lead, *Ti* = titanium.

2.1.1.1.2 Levels of Elemental Gd in Brain Tissues Following Exposure to Different GBCAs in Animals with Impairment of Renal Function

Guerbet (*Rasschaert et al., Invest Radiol 2017*) performed a study aimed at evaluating the levels of elemental Gd present in the rat brain after repeated dosing with Omniscan or matched volumes of saline in a moderate chronic kidney disease model (subtotally nephrectomized rats or sham-operated rats) vs. healthy rats. The animals received 4 daily injections of 0.6 mmol Gd/kg a week for 5 weeks (cumulative dose of 12 mmol Gd/kg, corresponding to 240 mL, or 2.0 mmol/kg in adult humans of 60 kg body weight) of Omniscan or saline solution. One week after the final injection, the total Gd concentration was determined by ICP-MS in different regions of the brain including the cerebellum. Total Gd concentrations in the brain and total cerebellum correlated with creatinine clearance in both the Omniscan-treated groups, showing that Gd levels were significantly higher in rats with impairment of renal function.

The higher Gd retention observed in rats with impaired renal function is not consistent with data in mice from independent studies. Kartamihardja et al (*Kartamihardja et al., 2016a*) conducted a study in mice to investigate the distribution and clearance of retained Gd in various parts of the brain after intravenous injection of Omniscan and Dotarem in 12 mice with normal renal function and 12 mice with renal failure induced by electrocoagulation of the kidneys. Each animal received a total cumulative dose of 5 mmol/kg of Omniscan and Dotarem. Both groups were divided into two subgroups (n=6 in each subgroup) based on the time-point for sample collection: 3 days (3d) and 45 days (45d) after the last injection. Levels of elemental Gd in each sample were quantified using ICP-MS. In the Omniscan group, the average Gd concentration in

the brain at 3d was similar between normal mice and mice with renal failure. In the Dotarem group, the average Gd concentration at 3d was significantly higher in the normal mice than in mice with renal failure. The average Gd concentration was significantly higher in the Omniscan group than in the Dotarem group at both 3d and 45d after the last dose.

Kartamihardja et al. (*Kartamihardja et al., 2016b*) also conducted a study to assess Gd retention in the brain and other organs. Twenty-six mice with renal failure (induced by electrocoagulation of the kidneys) were randomly divided into 4 groups to be injected with the following injection agents: Omniscan (n=7), Dotarem (n=7), Gd chloride, GdCl3 (n=7), and saline (n = 5). Each animal in the Omniscan and Dotarem groups received 5 mmol/kg of the GBCAs, 5 injections per week for 4 consecutive weeks. The GdCl3 solution was injected at a dose of 0.02 mmol/kg. Mice in the control group received injections of 250 µL saline. All groups were divided into subgroups based on the time-point for sample collection: 3 days (3d; 3 animals per group) and 45 days (45d; all remaining mice) after the last injection. Samples of mouse brain, liver, spleen, kidney, femoral bone and skin were obtained, and levels of elemental Gd in each sample were quantified using ICP-MS. The results showed that the lowest levels of Gd retention were found in the brain, and renal function did not affect brain Gd retention, regardless of the GBCA used.

2.1.1.1.3 Possible Chemical Forms of Gd Complexes Retained in Brain Tissues Following Exposure to Different GBCAs

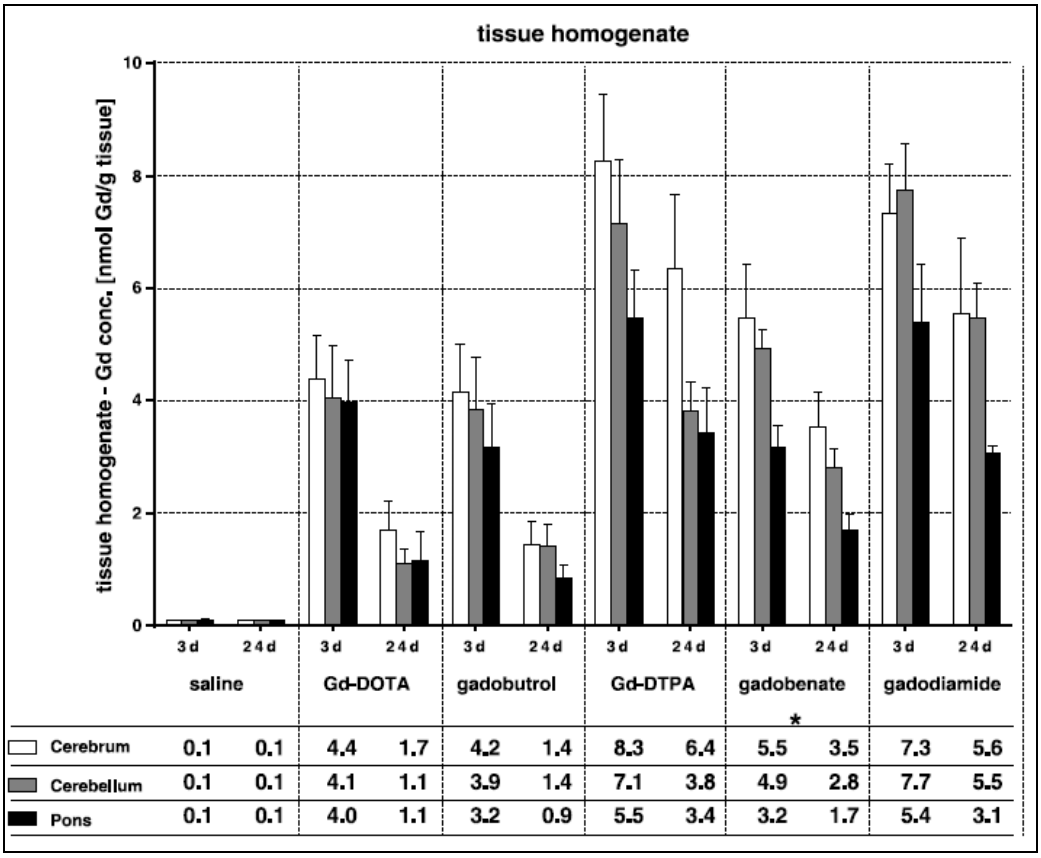
The Sponsor Bayer (*Frenzel at al., 2017*) performed a study in rats to investigate whether the residual Gd is present as intact GBCA or in other chemical forms.

Rats were divided randomly into 6 groups of 10 animals each. They received 10 daily injections of 2.5 mmol/kg of 1 of 5 different GBCAs: Omniscan, Magnevist, MultiHance, Gadavist, and Dotarem, or matched volumes of saline. On days 3 and 24 after the last injection, 5 randomly chosen animals from each group were sacrificed by exsanguination, and their brains were excised and divided into cerebrum, pons, and cerebellum. The brain sections were homogenized by sonication in ice-cold buffer at pH 7.4. Soluble and insoluble fractions were separated by centrifugation, and the soluble fractions were further separated by gel permeation chromatography (GPC). It should be noted that methods used to determine the chemical form of Gd present *in vivo* that use tissue homogenization and/or sonication followed by chromatography are prone to artefact since this destroys normal tissue architecture and exposes GBCA to intracellular components and environments that would not be encountered *in vivo.* The Gd concentration in all tissue fractions and in the GPC eluate was measured by ICP-MS. In a recovery control experiment, all GBCAs were spiked to blank brain tissue and more than 94% recovery of Gd in the tissue fractions was demonstrated by using tissue fractionation and chromatography. Total gadolinium concentrations in the 3 brain sections, cerebrum, cerebellum, and pons were measured 3 and 24 days after the last injection.

Total Gd concentrations are shown in Figure 4 below (from the original publication). At day 3, there were no significant differences between MultiHance and the macrocyclic agents Gadavist and Dotarem, while higher levels were already observed following administration of Magnevist and Omniscan. At day 24, a reduction in Gd levels was observed with all the GBCAs tested, especially with Dotarem and Gadavist. Among the linear GBCAs, the lowest Gd levels were

observed with MultiHance, both at day 3 and day 24. Of note, as mentioned in Section 2.1.1.1.1 of this document, the difference in level of elemental Gd in brain tissues between MultiHance and the GBCAs Magnevist and Omniscan cannot (and should not) be explained as the result of difference in elimination rate between the active ingredient of MultiHance and pure extracellular GBCAs.

Figure 4. Gd Content in the Tissue Homogenates of 3 Rat Brain Sections (Cerebrum, Cerebellum, and Pons), Measured 3 And 24 Days After the Last Injection of Dotarem (Gd-DOTA), Gadavist (gadobutrol), Magnevist (Gd-DTPA), MultiHance (gadobenate) or Omniscan (gadodiamide)
(Source: Figure 3 in the original publication by Frenzel et al., 2017)



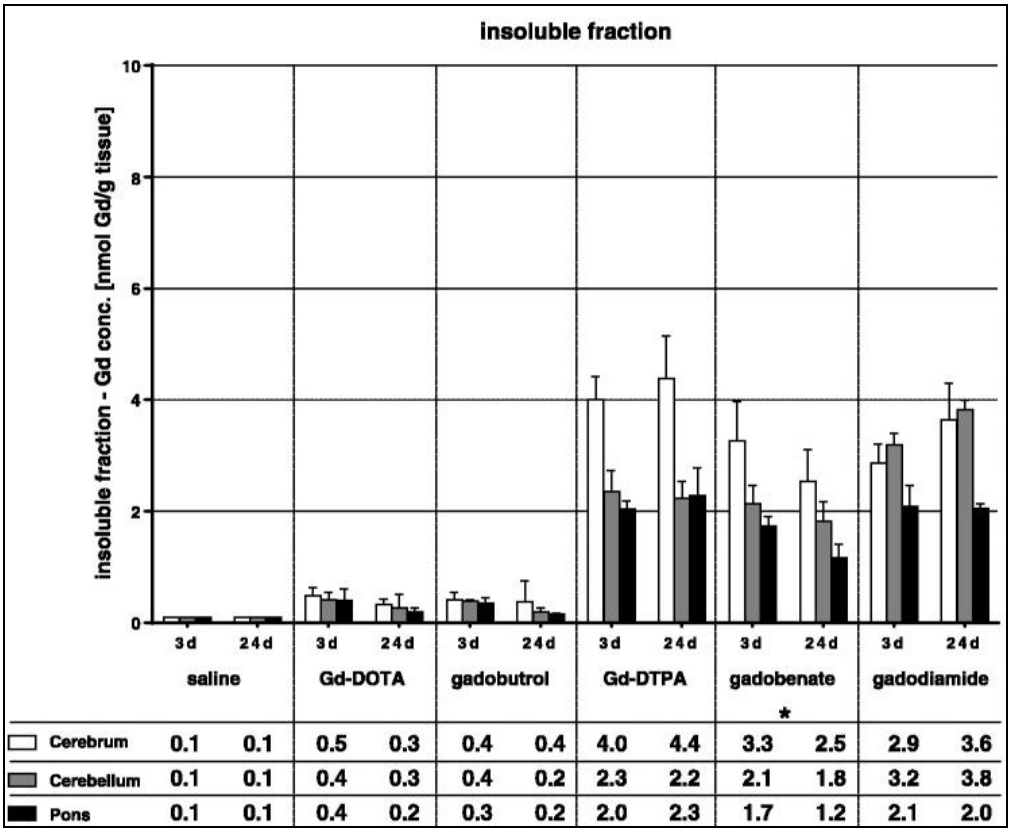
The residual Gd found in the rat brain after repeated administration of the GBCAs was present in at least 3 distinctive forms: soluble small molecules, including the intact GBCA, soluble macromolecules, and to a large extent in insoluble forms.

The insoluble fraction, most likely showing formation of insoluble Gd complexes like Gd phosphates, carbonates or citrates, was higher after administration of Magnevist, MultiHance, and Omniscan (Figure 5 below).

A decrease in the concentration of insoluble complexes from day 3 to day 24 post-injection was observed for the macrocyclics Dotarem and Gadavist and for the linear MultiHance (in all brain

areas), but not for the linear GBCAs Magnevist or Omniscan (in all brain areas), for which no change or even an increase in insoluble forms was observed over time, possibly indicating progressive deposition of insoluble complexes with these two agents.

Figure 5. Levels of Insoluble Gd Forms in the Tissue Homogenates of the 3 Rat Brain Sections (Cerebrum, Cerebellum, and Pons), Measured 3 and 24 Days after the Last Injection of Dotarem (Gd-DOTA), Gadavist (gadobutrol), Magnevist (Gd-DTPA), MultiHance (gadobenate) or Omniscan (gadodiamide)



	saline		Gd-DOTA		gadobutrol		Gd-DTPA		gadobenate		gadodiamide	
	3d	24d	3d	24d	3d	24d	3d	24d	3d	24d	3d	24d
Cerebrum	0.1	0.1	0.5	0.3	0.4	0.4	4.0	4.4	3.3	2.5	2.9	3.6
Cerebellum	0.1	0.1	0.4	0.3	0.4	0.2	2.3	2.2	2.1	1.8	3.2	3.8
Pons	0.1	0.1	0.4	0.2	0.3	0.2	2.0	2.3	1.7	1.2	2.1	2.0

Similar levels of the soluble fraction of either the intact GBCA molecule or Gd or the entire GBCA bound to macromolecules were observed after the administration of Dotarem, Gadavist, Magnevist and Omniscan, while lower levels were measured after the administration of MultiHance (Figure 6 below).

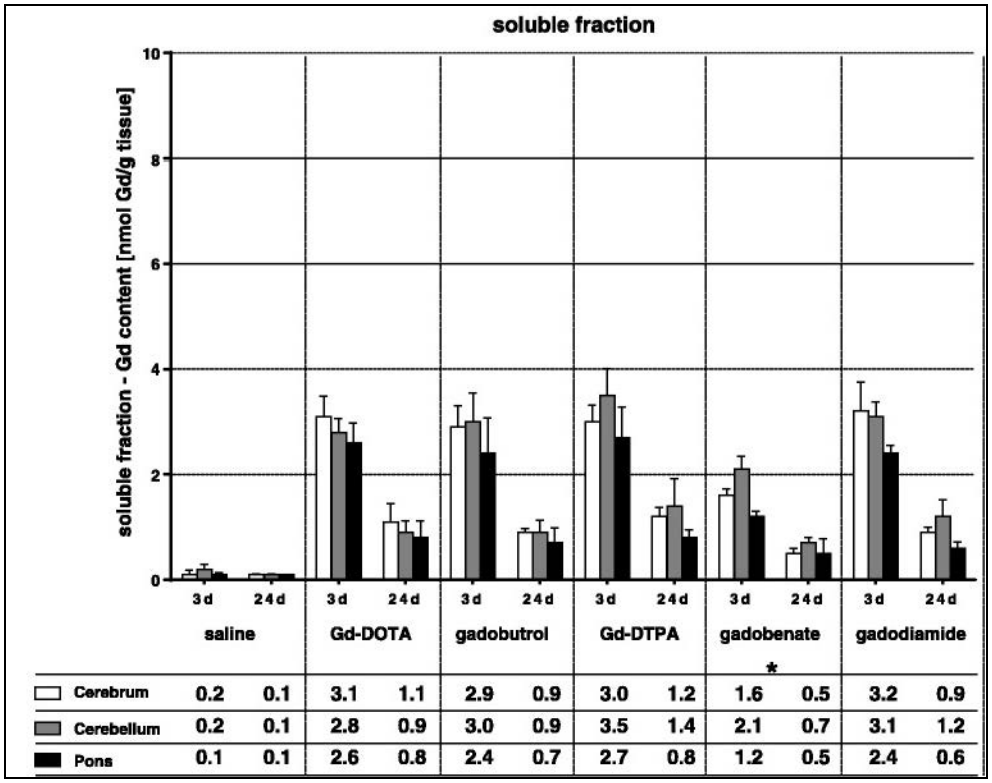
A clear and marked decrease in the concentration of soluble complexes was observed for all the GBCAs, showing rapid clearance of these chemical forms from brain tissues.

Soluble forms were of:

- Low-molecular weight, most likely indicating presence of intact GBCA molecules;
- High-molecular weight, most likely indicating presence of Gd-carrying macromolecules.

The majority of soluble forms were of low molecular weight. In the cerebellum, the high-molecular-weight fraction of soluble complexes was 26.5% for MultiHance, whereas it was 15.5% for Omniscan and 8.9% for Magnevist. This difference may be explained by the fact that the intact molecule of the active ingredient of MultiHance (gadobenic acid) is known to interact with macromolecules in the medium, and such weak intact GBCA-macromolecule interaction, may explain the difference in the concentration of high-molecular-weight soluble Gd complexes after MultiHance.

Figure 6. Levels of soluble Gd forms in the Tissue Homogenates of the 3 Rat Brain Sections (Cerebrum, Cerebellum, and Pons), Measured 3 and 24 days after the Last Injection of Dotarem (Gd-DOTA), Gadavist (gadobutrol), Magnevist (Gd-DTPA), MultiHance (gadobenate) or Omniscan (gadodiamide) (Source: Figure 3 in the original publication by Frenzel et al., 2017)



	saline		Gd-DOTA		gadobutrol		Gd-DTPA		gadobenate		gadodiamide	
	3d	24d	3d	24d	3d	24d	3d	24d	3d	24d	3d	24d
Cerebrum	0.2	0.1	3.1	1.1	2.9	0.9	3.0	1.2	1.6	0.5	3.2	0.9
Cerebellum	0.2	0.1	2.8	0.9	3.0	0.9	3.5	1.4	2.1	0.7	3.1	1.2
Pons	0.1	0.1	2.6	0.8	2.4	0.7	2.7	0.8	1.2	0.5	2.4	0.6

Surprisingly, the known interaction of gadobenic acid with macromolecules was considered unlikely by the Bayer investigators, again quoting an inappropriate reference. Also, it is surprising that the Bayer investigators concluded that no relevant differences between MultiHance, Magnevist and Omniscan were observed, since:

- The Gd concentrations in the brain after administration of MultiHance were lower;
- The clearance of insoluble complexes was faster after MultiHance;

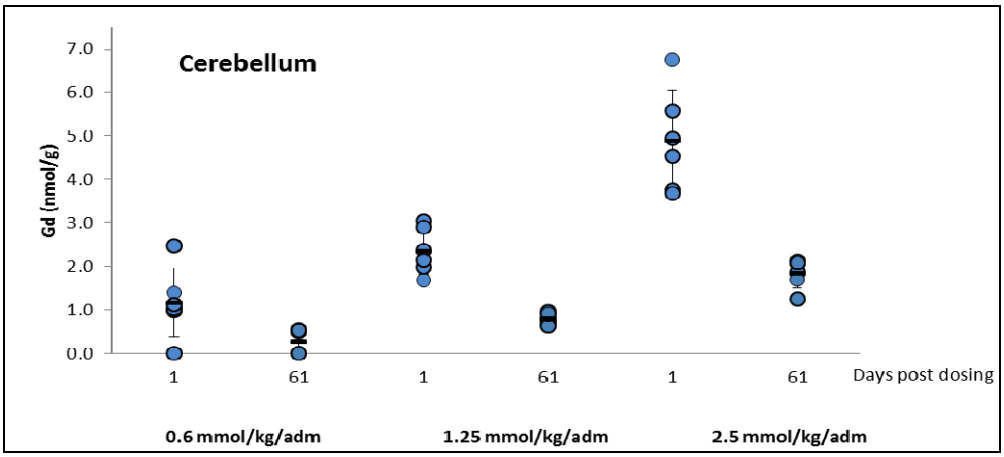
- The higher levels of high-molecular-weight complexes are likely to depend upon interaction of the entire gadobenic acid molecule with macromolecules. As a matter of fact, the Sponsor Bayer cannot determine whether Gd has de-chelated from the parent GBCA ligand. Also, it is important to note that following sonication and homogenisation of brain tissue the GBCAs present would be exposed to a plethora of macromolecules not encountered *in vivo* (in blood or tissues), and this could lead to a wide range of agent specific interactions that may not be relevant to the *in vivo* situation. However, if such work is undertaken, it is of course important that certain processing controls are included to account for any artefactual effects that may occur during the sonication, homogenisation and chromatography process. These were not presented in the main manuscript and are critical to its interpretation and validity of this study.

2.1.1.1.4 Retention of Gd Complexes in Brain Tissues Over Time

Bracco conducted a study (*Study AB21194*) to assess toxicity and Gd retention in juvenile rats (30 males and 30 females) after either a single intravenous administration of MultiHance at 10 days of age (postnatal day [PND] 10) or 6 repeated intravenous MultiHance injections every 4 days from PND 10 to PND 30 at dosages of 0.6, 1.25 or 2.5 mmol/kg per injection (total cumulative doses: 3.6, 7.5 and 15 mmol/kg in 2 days). Levels of elemental Gd were measured using ICP-MS in the brain (cerebral cortex, subcortical brain and cerebellum) and in body organs (liver, skin, femur, and kidneys) on the day after the last injection (Day1) and 60 days later.

Gd content was much lower in brain tissues vs. body tissues, and relatively similar in the cerebral cortex, subcortical brain and cerebellum. Gd levels at Day 61 were also significantly lower, showing clearance of Gd complexes over time.(see Figure 7, from original figure in the Study Report, showing Gd levels in the cerebellum).

Figure 7. Levels of Elemental Gd Forms in the Total Cerebellum of Juvenile Rats Treated with Repeated Doses of 0.6, 1.25 or 2.5 mmol/kg MultiHance(Total Cumulative Dose: 3.6, 7.5 or 15 mmol/kg)
(Source: Study Report AB21194, 2016)



A similar reduction in Gd levels over time was also observed in a study in healthy rats following a total cumulative dose of 12 mmol/kg Omniscan, with 50% reduction from 1 week to 20 weeks after dosing (*Smith et al., 2017*). An extension of data already published was reported to the European Authorities (GE internal study B041015) and included the persistence of very low amounts of Gd in the brain of rats 50 weeks post-dosing with Omniscan, importantly, without histopathological sequelae. Gd levels measured in the brain at 20 and 50 weeks post-dosing (1.38 ± 0.10 and 1.56 ± 0.30 respectively) were a small fraction of the total dose (~1/1,000,000th of the injected dose) and were significantly lower than those measured at 1 week post-dosing (2.49 ± 0.30 nmol/kg) *(Smith et al. 2017; PRAC Re-Examination Rapporteur's Assessment Report 2017, Page 41)*. However, no further significant reduction in Gd levels from week 20 until week 50 after Omniscan dosing was observed (*PRAC Re-Examination Rapporteur's Assessment Report 2017, Page 41, Page 39)*.

2.1.1.1.5 Conclusions on Gd Retention Data from Nonclinical Tissue-Sample Studies

The experimental animal studies of Gd retention in brain tissues showed that:

- Retention of Gd complexes in brain tissues is observed following exposure to all GBCAs;
- Gd levels measured in the brain are only an extremely small fraction of the injected dose (following the administration of Omniscan, the agent that causes the highest levels of Gd retention, less than 0.0002% of the injected dose per gram of tissue in the first days, and less than 0.000001% at 20 weeks post-injection);
- Significantly higher Gd levels in brain tissues are observed following repeated exposure to linear GBCAs;
- Differences were observed among individual agents in the same stability class:
    - Among the macrocyclic GBCAs, brain Gd levels after Dotarem and Gadavist are higher than those following ProHance, and Gd deposits were not observed following administration of ProHance while they were detected following treatment with Gadavist;
    - Among the linear GBCAs, Omniscan was shown to be associated with the highest brain Gd levels, whereas the lowest Gd levels were observed following administration of MultiHance (and differences in the elimination pattern between MultiHance and the other linear agents do not explain the observed differences in Gd brain levels);
- Impairment of renal function did not show an influence on Gd retention and clearance in mice models, while opposite results were found in a rat model. This inconsistency might indicate differences between animal species and, possibly, between animals and humans, and warrants further investigations.
- Limited data are available about speciation of Gd complexes retained in brain tissues and retention of the metal complexes over time, and more data are definitely warranted. Preliminary conclusions from the available data are the following:

- Free Gd3+ cannot survive in biological fluids or tissues because it has a high affinity for abundantly present anions like phosphates, carbonates and citrates, and, to a lesser extent, macromolecules. As a matter of fact, preliminary speciation analysis showed that Gd appears to be retained in insoluble complexes (most likely bound to anions), soluble high-molecular-weight complexes (Gd or gadobenic acid bound to macromolecules), or low-molecular-weight complexes (most likely intact GBCA molecules).
- Following administration of the macrocyclic GBCAs Dotarem and Gadavist, Gd retained was mostly in the form of soluble low-molecular-weight complexes. Insoluble forms were mainly present after administration of linear GBCAs, whereas soluble high-molecular-weight complexes were only present after the administration of the linear GBCAs.
- Soluble complexes seem to be cleared more efficiently than insoluble forms from brain tissues, and this may explain why the clearance of macrocyclic GBCAs seems to be more rapid.
- Insoluble forms seem to be cleared only after the administration of the macrocyclic GBCAs and the linear agent MultiHance. In contrast, no change or even an increase in insoluble forms was observed over time with Magnevist and Omniscan, possibly indicating progressive deposition of insoluble complexes with these two agents.

2.1.1.2 Clinical Tissue-Sample Studies

Five investigations were aimed at measuring levels of elemental Gd in brain autopsy specimens from decedents exposed to GBCAs (cases, N=35) versus specimens obtained from decedents never exposed to GBCAs (controls, N=37). Cases consisted of patients exposed either to Omniscan (N=21), ProHance (N=5), Magnevist (N=3), Gadavist (N=2), MultiHance (N=1), Eovist (N=1), or multiple agents (N=2). Patients in the contrast group were either adults (N=32) or pediatric patients (N=3). Two studies were performed in patients with no intracranial abnormalities who underwent body MRI examinations (cases: N=14; controls: N=19).

One study assessed the form and distribution of Gd deposits in brain and body tissues in a patient with NSF, and one study assessed whether Gd deposits were evident in 30 brain tumor biopsies from 28 patients with normal renal function who were exposed to GBCAs.

2.1.1.2.1 Levels of Elemental Gd in Brain Tissues

Kanda et al. (*Kanda et al., 2015b*) conducted an investigation using brain tissues obtained at autopsy in five subjects who were exposed to multiple administrations of GBCAs (GBCA group) and five subjects with no history of GBCA administration (non-GBCA group). No patient had severe impairment of renal function. The GBCAs involved were the Magnevist, Omniscan, and ProHance. Three patients had received only Magnevist, one patient Magnevist and ProHance, and one patient all three GBCAs.

By using ICP-MS, the investigators determined levels of elemental Gd in the brain tissues. Higher levels were found in the DN (range 0.067-2.1 μg of Gd/g of tissue). The highest Gd

levels were found when the interval between last exposure and autopsy was the shortest (patient 3 died of malignant lymphoma, exposed to three 0.1 mmol/kg doses of Magnevist; brain specimens obtained 15 days after last exposure). No clear association between level of exposure, type of agents administered, or time between last exposure and autopsy could be observed.

In a study by **McDonald et al.** (*McDonald et al, 2015*), levels of elemental Gd were measured in autopsy tissues harvested from the posterior fossa (DN and pons) and basal ganglia (globus pallidus, GP and thalamus) of 13 patients exposed to multiple doses of Omniscan. The effects of intravenous administration of a GBCA on the amount of elemental Gd quantified in neuronal tissues with ICP-MS showed that all patients exposed to Omniscan had elevated levels of elemental Gd in the four neuroanatomic regions of interest, ranging from 0.1 to 58.8 µg Gd/g of tissue. Among all sampled neuroanatomic locations, the DN contained the highest median Gd concentrations (6.6 µg/g vs. 1.7 µg/g in the GP, 0.5 µg/g in the thalamus, and 0.3 µg/g in the pons). For each neuroanatomic location, cumulative GBCA dose showed a very strong correlation with tissue Gd concentration, but no correlation with patient age, sex, baseline renal function, or interval between GBCA exposure and death. Moderate-to-strong dose-dependent correlations were observed between T1-weighted SI changes and tissue concentrations of elemental Gd in all four neuroanatomic regions, ranging from r = 0.49 (P = .08) in the GP to r = 0.89 (P, .0001) in the DN.

Using transmission electron microscopy with energy-dispersive x-ray spectroscopy, McDonald et al. showed that the majority of the observed Gd deposits were in the endothelial walls, with a smaller fraction in the brain interstitium.

Murata et al. (Murata et al., 2016) conducted a study aimed at determining whether Gd is deposited following exposure to either the macrocyclic GBCAs ProHance or Gadavist, or the linear protein-interacting GBCAs MultiHance or Eovist in patients with no known brain disease. Brain tissue was collected at autopsy from decedents with available medical records that documented past history of single-agent exposure. Nine decedents with no prior MRI or only unenhanced MRI served as controls. Tissue samples were collected from white matter, putamen, GP, caudate nucleus, pons and DN, and analyzed for elemental Gd using ICP-MS. Bone tissue from rib was also analyzed as a reference tissue in each case. Results were correlated with type of agent received, cumulative dose, time since dosing and clinical and laboratory data.

From a total of 134 autopsy cases, the investigators identified 9 patients who received 1 or more injections of a single macrocyclic or protein-interacting GBCA during life. These included 5 patients that received ProHance, 2 received Gadavist, and 1 each had received MultiHance or Eovist. The clinical information and specific type of Gd agent exposure are detailed in Figure 8 (original table from Murata et al., 2016a).

Figure 8. Characteristics of Subjects and Exposure (Source: Original Table from Murata et al., 2016a)

TABLE 1. General Information

Case ID	Age	Sex	Major Diagnosis	GBCA	No. of CEMRI	Total Dose, mL	Last-First CEMRI Before Death, d	Renal Function (eGFR)
1	26	F	SLE	Gadobutrol (Gadovist)	1	5	5	>60
2	65	F	Liver cirrhosis	Gadobutrol (Gadovist)	2	20	392–441	Recorded only as >30
3	50	M	AML	Gadoteridol (ProHance)	1	24	15	>60
4	31	F	DLBCL	Gadoteridol (ProHance)	11	126	19–318	>60
5	71	M	Gastric cancer	Gadoteridol (ProHance)	3	57	53–818	>60
6	80	F	Bladder cancer	Gadoteridol (ProHance)	1	18	118	>60
7	57	M	AML	Gadoteridol (ProHance)	1	20	90	>60
8	67	M	HCC	Gadoxetate (Eovist)	10	100	90–819	>60
9	50	M	HCC	Gadobenate (MultiHance)	1	20	83	>60
10	79	F	Interstitial pneumonia	Control				>60
11	67	M	AML	Control				>60
12	28	M	Lung tumor	Control				>60
13	54	F	CAD	Control				>60
14	54	F	Myocarditis	Control				23
15	76	M	Interstitial pneumonia	Control				32
16	77	M	Bladder cancer	Control				28
17	19	F	Heart disease	Control				>60
18	68	M	CAD	Control				>60

GBCA indicates gadolinium-based contrast agent; CEMRI, contrast-enhanced magnetic resonance imaging; SLE, systemic lupus erythematosis; AML, acute myelogenous leukemia; DLBCL, diffuse large B-cell lymphoma; HCC, hepatocellular carcinoma; CAD, coronary artery disease.

Gd was found with all agents in all brain areas sampled with highest levels in the GP and DN (Figure 9, data from Murata et al., 2016a). Also, the highest levels were observed following the macrocyclic agent Gadavist.

One of the two Gadavist cases was a decedent who received Gadavist only 5 days before death when terminally ill with septic shock and possibly with multiple organ failure. The other patient had an interval of 392 days between last Gadavist dose and death; still, the measured Gd levels were higher than in patients exposed to MultiHance, Eovist and, especially, ProHance, despite a shorter interval occurring between last dose of the 3 agents and death.

Bone levels measured 23 times higher (median) than brain levels ($P = 0.008$ for bone vs. GP) and showed a significant correlation with Gd levels in brain tissue ($r = 0.81$, $P = 0.022$). In controls, Gd levels in the brain were at or below limits of measurement and were significantly lower compared with GBCA-exposed patients ($P = 0.005$ for GP).

Figure 9. Levels of Gd Deposition in Brain and Bone (Source: Original table from Murata et al., 2016a)

TABLE 2. Level of Gd Deposition by Tissue Type

		ICP-MS Results (µg/g tissue)							
Case ID	GBCA	PT	GP	CA	WM	Pons	DN	Skin	Bone
1*	Gadobutrol (Gadovist)	0.216	0.625	0.201	0.205	0.107	1.07	NA	NA
2	Gadobutrol (Gadovist)	0.069	0.188	0.040	0.018	0.005	0.111	NA	5.280
3	Gadoteridol (ProHance)	0.039	0.066	0.019	0.020	0.020	0.078	NA	0.754
4	Gadoteridol (ProHance)	0.013	0.039	0.011	0.023	NA	NA	NA	1.620
5	Gadoteridol (ProHance)	0.011	0.023	0.008	0.006	NA	NA	NA	0.428
6	Gadoteridol (ProHance)	0.008	0.008	<0.004	0.003	<0.004	<0.004	0.005	0.098
7	Gadoteridol (ProHance)	<0.004	<0.005	<0.004	<0.004	<0.003	<0.005	0.002	0.094
8	Gadoxetate (Eovist)	0.067	0.148	0.051	0.013	NA	NA	NA	1.300
9	Gadobenate (MultiHance)	0.028	0.052	0.031	0.010	0.009	0.078	0.057	2.380
10	Control	<0.0006	<0.0005	<0.0005	<0.0003	NA	NA	NA	0.0006
11	Control	<0.0006	0.0008	<0.0005	0.0004	NA	NA	NA	0.0018
12	Control	0.0005	0.0008	<0.0006	0.0003	<0.0010	<0.0010	NA	0.0008
13	Control	<0.0005	<0.0007	<0.0006	<0.0004	NA	NA	NA	0.0009
14	Control	0.0025	0.0005	<0.0005	0.0003	NA	NA	NA	0.0031
15	Control	0.0007	<0.0010	<0.0009	0.0006	NA	NA	NA	0.0046
16	Control	<0.0030	<0.0030	<0.0060	<0.0020	NA	NA	NA	0.0020
17	Control	<0.0007	<0.0008	<0.0004	<0.0004	0.0007	0.0004	NA	0.0023
18	Control	<0.0008	<0.0009	<0.0007	<0.0006	<0.0010	0.0008	NA	0.0067

*Gadobutrol administered only 5 days before death.

Gd indicates gadolinium; GBCA, gadolinium-based contrast agent; ICP-MS, inductively coupled plasma mass spectrometry; PT, putamen; GP, globus palidus; CA, caudate nucleus; WM, white matter; DN, dentate nucleus; NA, not available).

McDonald et al. (*McDonald et al., 2017b*) also compared postmortem neuronal tissue samples from 5 patients with normal brain morphologic characteristics at autopsy who had undergone 4 to 18 Omniscan-enhanced MR examinations (contrast group) with samples from 10 Gd-naive patients who had undergone at least one unenhanced MR examination during their lifetime (control group). Neuronal tissues from the DN, pons, GP, and thalamus were harvested and levels of elemental Gd measured using ICP-MS. Transmission electron microscopy with energy-dispersive x-ray spectroscopy was used to detect and localize Gd deposits. The results showed a dose-dependent retention of Gd complexes in the contrast group, with Gd deposits localized to the capillary endothelium and neuronal interstitium and, in two cases, within the nucleus of the cell.

McDonald et al. (*McDonald et al., 2017c*) also assessed levels of elemental Gd in brain tissues of 3 pediatric patients exposed to 4 or more injections of Omniscan (contrast group) and compared them with 3 pediatric patients never exposed to GBCAs (control group). Tissue samples were harvested from formalin-fixed whole-brain specimens at neuroanatomic locations (DN, pons, GP, and thalamus). Elemental levels of Gd were measured using ICP-MS. Localization of Gd deposits was assessed using transmission electron microscopy with energy dispersive spectroscopy. Elemental Gd was detected in all 4 neuroanatomic regions in the contrast group, with the highest concentrations detected in the DN or, differently from adults, in the pons. Similarly to findings in adults, prominent clusters of Gd deposits were observed within the endothelium and scattered smaller foci within the neural interstitium.

2.1.1.2.2 Distribution and Speciation of Gd Deposits

Sanyal et al. (*Sanyal et al., 2011*) performed an analysis of tissues from an autopsy case with verified advanced nephrogenic systemic fibrosis (NSF): using light microscopy and scanning electron microscopy/energy-dispersive X-ray spectroscopy, the investigators found Gd deposits (in the form of insoluble phosphates) in the perivascular glial cells in the cerebellum but not in the pons, thalamus and corpus striatum. Insoluble Gd–phosphate deposits were also detected in the skin, liver, lungs, intestinal wall (ileum), kidney, lymph node, skeletal muscle, and dura mater.

Xia et al. (*Xia et al, 2010*) conducted a retrospective investigation with the aim of detecting Gd deposits in 30 brain tumor biopsies from 28 patients with normal renal to whom either only gadodiamide (Omniscan) or only MultiHance (gadobenate dimeglumine) was administered one or more times (mean: 4.6 contrast-enhanced exams) for contrast-enhanced MRI exams of the brain. Scanning electron microscopy/energy dispersive X-ray spectroscopy was used for detection of Gd-containing deposits, which were primarily found in highly vascularized areas, frequently within the wall of blood vessels, and in association with calcifications. Gd deposits contained calcium, phosphorus, and sodium. A lesser amount of iron and zinc was observed in many deposits. A pattern from the study data was that the Gd deposits were more common in brain tumors from patients who had their scan(s) with Omniscan rather than with MultiHance. Five of the 13 (38%) patients who had their scan(s) with Omniscan showed Gd-containing deposits in the included biopsies while none of the 11 patients who had their scan(s) with MultiHance showed deposits ($p = 0.04$).

2.1.1.2.3 Conclusions on Gd Retention Data from Clinical Tissue-Sample Studies

Since Gd is not an essential element and is not ubiquitous in the environment, there is no or very low background signal to contend with. The elemental analysis technique used in five studies of Gd retention in brain tissues of decedents exposed one or multiple times to GBCAs (ICP-MS) is highly sensitive and allows accurate quantitation of very low levels of the metal in tissues. The same method was used to quantify levels of elemental Gd in the animal studies.

Although the available data are still limited, the following preliminary conclusions can be made. Similarly to nonclinical findings, tissue-sample studies in human patients showed that:

- Gd retention was observed in every patient exposed to either linear or macrocyclic GBCAs;
- Gd levels in GBCA-exposed patients were significantly higher than in controls;
- ProHance was associated with the lowest levels of elemental Gd in brain tissue;
- A majority of elemental Gd deposits were found within the endothelial wall, with a smaller fraction identified in the tissue interstitium (and within the nucleus of a neuronal cell in 2 human patients).

Unlike studies in man, in which the highest levels of elemental Gd were found in the DN, in the animal studies no differences in Gd levels were observed among the various brain areas tested (cerebral cortex, subcortical brain and cerebellum).

Also, differently from findings in animals, and with the exception of ProHance, preliminary data in patients do not show a clear demarcation in brain Gd retention among the other individual GBCAs tested (Gadavist, Magnevist, MultiHance, Omniscan). No data on Dotarem and OptiMARK are available yet, so that no conclusions can be drawn for these agents.

A summary of the experimental data is reported in Table A. The total dose,is given in mL of solution and in mmol Gd, in the interval between the last administration and death; the concentration of Gd per gram of tissue, expressed both as µg/g and as nmol/g and the normalized concentration ($[Gd]_{norm,}$ expressed as µg/g/mmol) of Gd with respect to the injected GBCA total dose were calculated for both the globus pallidus (GP) and the dentate nucleus (DN).

Looking at the two largest patient groups, a correlation between total cumulative dose and Gd levels was observed in the Omniscan-treated patient group (N=21), not in the ProHance-treated group (N=5). On the other hand, the interval between last dose and death seems to play a role in the ProHance-treated group, but not in the Omniscan-treated group.

No major difference can be observed between patients with or without manifest intracranial abnormalities following exposure to multiple doses of Omniscan.

Finally, ICP-MS is a destructive technique that reports only on elemental composition and not on form of the Gd, i.e., whether it is still present as intact GBCA molecules or as other chemical forms. Sanyal et al. used scanning electron microscopy/energy-dispersive X-ray spectroscopy and showed that the Gd deposits observed in the perivascular glial cells in the cerebellum were composed of insoluble gadolinium phosphates. It is also possible, however, that the chemical form of Gd complexes in the DN and other brain areas may vary over time. Finally, the presence and/or concentration of other metals in brain tissues (e.g., manganese, copper or iron) was not determined in any of the published studies.

More, and possibly larger, studies in humans aimed at determining presence and levels of elemental Gd in brain tissues are needed to understand possible differences among the various GBCAs approved and used in the United States. The presence and concentration of other metals and/or factors, e.g., form of the retained Gd, that could contribute to signal intensity increase in the DN and GP on T1 weighted images should also be investigated.

Table A: Summary of Data from Clinical Tissue-Sample Studies: Absolute and Normalized Gd Retention Following Exposure to a Single GBCA

Paper	Case ID	GBCA	Tot Dose [mL]	Total dose [mmolGd]	Last GBCA dose before death, [days]	GP			DN		
						[□g/g tissue]	[nmol/g tissue]	[□g/g/mmol]	[□g/g tissue]	[nmol/g tissue]	[□g/g/ mmol]
McDonald et al. 2015	1	Omniscan	52	26	18	0.0	0.000	0.000	0.100	0.64	0.0038
	2	Omniscan	75	37.5	13	0.6	3.8	0.016	4.4	28.0	0.117
	3	Omniscan	85	42.5	86	0.2	1.3	0.0047	0.3	1.9	0.007
	4	Omniscan	129	64.5	29	0.6	3.8	0.0093	2.1	13.4	0.033
	5	Omniscan	140	70	511	3.6	22.9	0.051	1	6.4	0.014
	6	Omniscan	160	80	197	0.8	5.1	0.010	8.2	52.1	0.103
	7	Omniscan	162	81	44	2.1	13.4	0.026	3.9	24.8	0.048
	8	Omniscan	117	58.5	623	1.3	8.3	0.022	8.5	54.1	0.145
	9	Omniscan	199	99.5	20	1.7	10.8	0.017	6.6	42.0	0.066
	10	Omniscan	241	120.5	17	3.3	21.0	0.027	11.7	74.4	0.097
	11	Omniscan	252	126	53	5.2	33.1	0.041	25.4	161.5	0.202
	12	Omniscan	500	250	62	11.4	72.5	0.046	40	254.4	0.160
	13	Omniscan	420	210	106	17.2	109.4	0.082	58.8	373.9	0.280
Kanda et al., 2015b	1	Magnevist	56	28	450	0.48	3.05	0.017	0.500	3.18	0.018
	3	Magnevist	42	21	15	0.78	4.96	0.037	2.1	13.35	0.100
	4	Magnevist	28	14	120	0.027	0.17	0.0019	0.067	0.43	0.0048
Murata et al., 2016	1	Gadavist	5	5	5	0.625	3.97	0.125	1.070	6.80	0.214
	2	Gadavist	20	20	392	0.188	1.20	0.0094	0.111	0.71	0.0056
	3	ProHance	24	12	15	0.066	0.42	0.0055	0.078	0.50	0.0065
	4	ProHance	126	63	19	0.039	0.25	0.00062	NA	NA	NA
	5	ProHance	57	28.5	53	0.023	0.15	0.00081	NA	NA	NA
	6	ProHance	18	9	118	0.008	0.051	0.00089	<0.004	<0.025	<0.001
	7	ProHance	20	10	90	<0.005	<0.032	<0.001	<0.005	<0.032	<0.001
	8	Eovist	100	25	90	0.148	0.94	0.0059	NA	NA	NA
	9	MultiHance	20	10	83	0.052	0.33	0.0052	0.078	0.496	0.0078
McDonald et al.. 2017b	1	Omniscan	421	210.5	56	19.4	123.37	0.09	8.9	56.60	0.042
	2	Omniscan	244	122	53	9	57.23	0.07	6.7	42.61	0.055
	3	Omniscan	109	54.5	1257	11.1	70.59	0.20	2.6	16.53	0.048
	4	Omniscan	100	50	1	2.1	13.35	0.04	2.2	13.99	0.044
	5	Omniscan	76	38	333	3.2	20.35	0.08	6.1	38.79	0.161
McDonald et al. 2017c	1	Omniscan	42	21	59	0.1	0.64	0.00	3	19.08	0.143
	2	Omniscan	40	20	8	0.2	1.27	0.01	2.2	13.99	0.110
	3	Omniscan	57	28.5	52	1.1	7.00	0.04	0.8	5.09	0.028

2.1.2 Imaging Studies

2.1.2.1 Available Evidence

Some patients exposed to multiple administrations of GBCAs may exhibit progressively increased signal intensity (SI) in the GP, the DN and possibly other deep brain areas on unenhanced T1-weighted MR images of the brain. This imaging finding is putatively correlated with abnormal retention of Gd complexes.

Bracco reviewed data from 38 publications in peer-reviewed journals about imaging studies conducted to detect or exclude abnormal T1 shortening in deep brain areas in adult patients. Bracco is also aware of one additional imaging study conducted in adult patients which has been accepted for publication in Radiology (*PRAC Re-examination Rapporteur's Assessment Report, 2017, Page 36*). Bracco also assessed data from 7 imaging studies conducted in pediatric patients. Summary results of the 46 imaging studies are provided in Table B.

There are a number of limitations that may affect the value of the information deriving from imaging studies:

- *Study design:* all imaging studies available to date have been single-center and retrospective in design. Patients were selected from hospital databases using different selection criteria, and selection and information bias cannot be excluded.
- *Study populations*: There were studies that specifically excluded patients with conditions possibly affecting SI in the DN (e.g., multiple sclerosis, brain irradiation) (*Kanda et al, 2104; McDonald et al, 2015; Ramalho et al, 2015; Radbruch et al, 2015a; Radbruch et al, 2015b; Ramalho et al, 2016a*) and studies that included patients specifically excluded from other studies, e.g., patients who had undergone brain irradiation or had multiple sclerosis (*Errante et al, 2104; Quattrocchi et al, 2015; Adin et al, 2015; Stojanov et al, 2016; Tedeschi et al, 2016; Tanaka et al, 2016; Eisele et al, 2016; Eisele et al, 2017a; Forslin et al, 2017; Eisele et al, 2017b*).

  There were studies that included mostly patients with primary malignant tumors, (*Kanda et al, 2014; Kanda et al, 2015a; McDonald et al, 2015; Adin et al, 2015; Radbruch et al, 2015a; Radbruch et al, 2015b; Radbruch et al, 2016; Cao et al, 2016b; Zhang et al, 2017; Hinoda et al, 2017; Radbruch et al, 2017b*) and 2 studies that focused primarily on patients with metastases (*Weberling et al, 2015; Bae et al, 2017*), one including almost exclusively patients with metastases from melanoma (*Weberling et al, 2015*). These differences in patient populations may be important when considering that Adin et al. showed that patients with primary malignant tumors are less prone to develop hyperintensity in the DN compared with patients with other types of brain diseases (*Adin et al, 2015*). For example, MultiHance was shown to produce a significant increase in SI in the DN in patients with metastases from melanoma (*Weberling et al, 2015*), while the same effect was not seen in patients with other types of brain diseases (*Ramalho et al, 2015; Ramalho et al, 2016b)* or with no known intracranial abnormalities *(Schneider et al., 2017*).
- *Potential for carryover effects*: some studies allowed prior scans with other GBCAs before patients' first scan with the GBCA under investigation (*Weberling et al, 2015; Radbruch et*

al, 2015a; Radbruch et al, 2015b). However, one study showed a potential for a significant carryover effect and the need for careful assessment of history of patient exposure when considering effects of a specific GBCA (*Ramalho et al, 2016b*).

- *Imaging technique*: Different field strengths (1.5T and 3.0 T) and different acquisition sequences (T1-weighted Spin Echo, Fast Spin Echo and MPRAGE) have been used to acquire brain MR images, in some cases within the same study or individual patient studies. Moreover, the acquisition parameters used by different authors are not identical. As a consequence, experimental data are largely variable, both within and between patient studies.
- *Quantitative assessments:* All investigators, with the exception of one study (*Tedeschi et al, 2016*), used the MRI SI ratio between regions of interest drawn on target area (DN or GP) and a reference area of the brain (normally the pons, the thalamus and/or the cerebrospinal fluid) as primary quantitative endpoint. The value of this SI ratio, and in turn its sensitivity, depends on the applied magnetic field and on the acquisition sequence and parameters including TE, TR, and surface coils.
- ROI drawing and positioning has been rarely standardized leading to inconsistent results even with the same tested GBCA.

  More importantly, there is no scientifically rigorous means for quantifying the concentration of Gd complexes in a target tissue using MRI. This excludes all of the methods used in the listed papers, including the relaxometric approach, as long as:
  - The concentration of other metals known to affect SI or relaxation rates in the area of interest is not known, and
  - The longitudinal and transverse relaxivities of the Gd moiety responsible for the observed effect on SI and relaxation rates are not known, as the chemical forms in brain tissues are not known.

  Surprisingly, while the effects of field strength and sequences on SI, SI enhancement after contrast, and diagnostic quality of MR images are well known in neuroradiology, only two studies attempted to address the issue of variability related to the MR sequence used, sometimes interchangeably, in the studies, and the issue of the accuracy of the quantification method: Tedeschi et al, 2016, used MR relaxometry instead of T1-weighted SI analysis in order to avoid sources of potential inaccuracy, such as variability in receiver coil sensitivity profile and in transverse relaxation rate and proton density. One study showed that two sequences, T1-weighted Spin Echo and MRPAGE, should not be used interchangeably when assessing effects of GBCAs on SI in the DN as the methods can result in statistically significant differences in signal output (*Ramalho et al, 2016a*).
- *Methodology of image evaluation*: Most studies used two readers affiliated with the study centers, so that blinding to clinical information and to the GBCA used, in case of comparative studies, could have been affected. Three of the studies used only one reader, and always the same reader for the three studies (*Weberling et al, 2015; Radbruch et al, 2015a; Radbruch et al, 2015b*). This reader also selected patients and images from the hospital database. Of note, two of these studies were non-comparative, so that the reader

was fully aware of the GBCA under investigation (*Weberling et al, 2015; Radbruch et al, 2015b*).

When two readers were used, reader discordance was managed by consensus, so that the readers were never completely independent, and inter-reader variability could not be properly assessed.

Only one study used 5 independent readers, unaffiliated with the enrolment center and fully blinded to clinical information (*Schneider et al, 2017*). However, this was not a comparative study, and the readers were aware of the GBCA under investigation.

Bearing in mind the limitations of the imaging studies conducted to date, and the limited value of MRI to quantify levels of Gd retained in the brain, the following summary can be provided:

- Independent of the tested GBCA, abnormal T1 shortening has been observed mainly in the DN, in agreement with the human Gd deposition studies to date;
- An increase in the total number of contrast-enhanced exams and thus total amount of Gd administration has been reported to significantly increase the risk for developing hyperintensity in the DN. It has been reported that, after ≥4 contrast-enhanced MRI scans and a total dose ≥77 mL of GBCA, there is a significant increase in the likelihood of abnormal T1 shortening (*Adin et al, 2015*). However, an absolute threshold for the number of contrast-enhanced exams resulting in this finding has not been identified;
- Abnormal T1 shortening has never been reported after a single dose of any GBCA;
- Impaired renal function seems to increase the probability of T1 hyperintensity in the DN, but not in the GP (*Cao et al, 2016b*).

Table B provides a summary of the results of the studies of the individual GBCAs.

- All the imaging studies of Magnevist and Omniscan showed significant changes in SI or relaxation rates in deep brain areas, and were more marked when compared with Dotarem, Gadavist, ProHance and MultiHance.
- No significant effect on SI was observed after multiple exposures to ProHance, either in adult or pediatric patients.
- The majority of imaging studies of Dotarem and Gadavist did not show an effect of these two GBCAs on SI in the DN.
- Inconsistent results were observed with the GBCAs Eovist and MultiHance.
- No imaging studies have been conducted to assess the effect of OptiMARK on SI or relaxations rates in deep brain areas.

Table B: Summary Results of Imaging Studies

GBCA	Significant effect on SI or relaxation rate a. Adult patients b. Pediatric patients	No significant effect on SI or relaxation rate a. Adult patients b. Pediatric patients	Notes
Dotarem	a. Tedeschi et al, 2016 b. Rossi-Espagnet et al, 2017	a. Radbruch et al, 2015b; Eisele et al, 2016; Bae et al, 2017; Radbruch et al, 2017b; Eisele et al, 2017b b. Tibussek et al 2017	The study by Tedeschi et al. used relaxometry to assess presence of retained Gd
Eovist	a. Kahn et al, 2017 b. No data available	a. Ichikawa et al 2017; Conte et al 2017 b. No data available	
Gadavist	a. Stojanov et al, 2016; Tedeschi et al., 2016; Bjornerud et al , in press b. No data available	a. Radbruch et al, 2015a; Cao et al, 2016a; Radbruch et al, 2016; Schlemm et al, 2017; Kromrey et al, 2017; Bae et al, 2017; Radbruch et al, 2017b; Langner et al, 2017; Müller et al, 2017; Yoo et al, 2017 b. No data available	The study by Tedeschi et al. used relaxometry to assess presence of retained Gd
Magnevist	a. Kanda et al, 2014; Kanda et al, 2015; Adin et al, 2015; Radbruch et al, 2015a; Radbruch et al, 2015b; Tedeschi et al, 2016; Cao et al, 2016a; Tanaka et al, 2016; Radbruch et al, 2016; Cao et al, 2016b; Zhang et al, 2017; Schlemm et al, 2017; Kuno et al, 2017; Bae et al, 2017; Radbruch et al, 2017b; Forslin et al, 2017 b. Hu et al, 2016; Roberts et al, 2016a; Flood et al, 2017		Significantly more marked effect vs. Dotarem, Gadavist and ProHance (Kanda et al.2015; Radbruch et al, 2015a; Tedeschi et al. 2016; Schlemm et al, 2017)
MultiHance	a. Weberling et al. 2015	a. Ramalho et al. 2015; Ramalho et al 2016b b. Schneider et al. 2017	Significantly less effect vs.Omniscan (Ramalho et al. 2015) Significant effect of MultiHance on relative change in SI ratio in the DN (Ramalho et al. 2015, endpoint not used in other studies). Such significant change was observed only if exposure to MultiHance followed exposure to Omniscan (Ramalho et al 2016b) The study by Scheider et al. used 5 blinded readers unaffiliated with enrolment center

Table B: Summary Results of Imaging Studies

GBCA	Significant effect on SI or relaxation rate a. Adult patients b. Pediatric patients	No significant effect on SI or relaxation rate a. Adult patients b. Pediatric patients	Notes
Omniscan	a. Kanda et al, 2014; Errante et al, 2104; Quattrocchi et al, 2015; McDonald et al, 2015; Ramalho et al, 2015; Ramalho et al, 2016a; Tanaka et al, 2016; Ramalho et al, 2016b; Cao et al, 2016b; Zhang et al, 2017; Bae et al, 2017; Ichikawa et al, 2017; Radbruch et al, 2017b; Forslin et al, 2017		More marked effect vs. MultiHance (Ramalho et al. 2015)
OptiMARK	No data available	No data available	
ProHance		a. Kanda et al., 2015 b. Tibussek et al, 2017	

2.1.2.2 Conclusions on Data from Imaging Studies

All the imaging studies aimed at assessing abnormal T1 shortening are affected by potential sources of bias, some clustering in the same investigation, which could be less relevant if a systematic trend of a certain variable is observed regardless of variability in the experimental design and setup. Unfortunately, this was not always the case in the retrospective studies performed to date. While a consistent trend was observed for the GBCAs Magnevist and Omniscan, this was not the case with other agents. For instance, MultiHance was shown to produce a significant effect in one study (*Weberling et al, 2015*) and not in others (*Ramalho et al, 2015; Ramalho et al, 2016b*, *Schneider et al., 2017*), and Gadavist was shown to produce a significant effect on DN SI values or R1 values in three studies (*Stojanov et al, 2016; Tedeschi et al, 2016; Bjornerud et al, in press*) but not in others (*Radbruch et al, 2015a; Cao et al, 2016a; Schlemm et al, 2017; Kromrey et al, 2017; Radbruch et al, 2017b; Langner et al, 2017; Müller et al, 2017; Yoo et al, 2017*).

Further studies, possibly prospective and involving larger and homogeneous patient populations, using meaningful endpoints (possibly incidence of hyperintensity in deep brain areas rather than mean SI changes from baseline) and sound and standardized methodology for image acquisition and evaluation, eliminating any source of bias, and using rigorous statistical methods, should confirm the preliminary findings available to date. Important, prospective studies should also evaluate whether or not T1 hyperintensity in deep brain areas may be associated with development of clinical deficits.

2.1.3 Overall Conclusions on Gd Retention in Brain Tissues

Since retention of Gd compounds is the key potential safety issue under assessment, direct demonstration of the presence of the metal in the human brain following exposure to GBCAs, i.e., data from tissue-sample studies, should be considered the highest level of evidence. The

elemental analysis technique used in tissue-sample studies of Gd retention, i.e., ICP-MS is highly sensitive and allows accurate quantitation of very low levels of the metal in tissues.

Both experimental animal studies and post-mortem, tissue-sample studies in man showed that the intravenous administration of GBCAs is associated with retention of Gd complexes in brain tissues. Gd retention was observed in every patient exposed to either linear or macrocyclic GBCAs. One animal study and post-mortem, tissue-sample data following multiple administrations of Omniscan showed a correlation between the cumulative GBCA dose and tissue Gd concentration. However, it is important to point out that the Gd levels measured in the brain were only an extremely small fraction of the injected dose, and were markedly lower than those measured in body tissues.

The available evidence shows clear differences among the individual agents, with the macrocyclic GBCA ProHance determining the lowest levels of Gd concentrations in brain tissues both in animals and man, often at the limit of quantitation by ICP-MS, and lower than those measured after administration of the other macrocyclic GBCAs Dotarem and Gadavist. Animal studies showed that all the macrocyclic GBCAs, Dotarem and Gadavist included, caused significantly lower levels of Gd retention compared with the linear GBCAs. However, first and limited data from post-mortem, tissue-sample studies in man do not show a clear demarcation between Gd levels retained after exposure to Gadavist and those following the administration of linear GBCAs. Among the linear GBCAs, Omniscan was shown to be associated with the highest brain Gd levels, whereas the lowest Gd levels were observed following administration of MultiHance.

Clinical data are too limited to evaluate whether patient characteristics, including underlying diseases, renal function, or type of brain disease may affect Gd retention in brain tissues. Impairment of renal function did not show to influence Gd retention and clearance in mice models, while opposite results were found in a rat model. One imaging study showed higher frequency of T1 hyperintensity in the DN of patients on dialysis compared with patients with normal or near-normal renal function.

Free Gd3+ cannot survive in biological fluids or tissues because it has a high affinity for abundantly present anions like phosphates, carbonates and citrates, and, to a lesser extent, macromolecules. Evidence from testing in animals showed that Gd3+ may be retained in the intact GBCA molecule, in insoluble complexes, most likely bound to anions, or soluble high-molecular-weight complexes, most likely bound to macromolecules. For protein-interacting GBCAs like MultiHance and Eovist, high-molecular-weight complexes could also consist of the intact GBCA molecule bound to macromolecules. Significantly higher levels of insoluble complexes are observed following repeated doses of linear GBCAs, especially after the administration of Magnevist and Omniscan.

Soluble complexes, like the intact GBCA molecule, Gd3+ bound to macromolecules, or the GBCA bound to macromolecules seem to be cleared more efficiently from brain tissues. Clearance of insoluble complexes has been observed following repeated doses of macrocyclic GBCAs and MultiHance, but not after the administration of Magnevist and Omniscan, possibly because of progressive deposition of insoluble complexes with these two agents. Preliminary

data suggest that clearance of Gd complexes from brain tissues seems to be more efficient following repeated doses of macrocyclic GBCAs.

Both in animals and in man, a majority of elemental Gd deposits were found within the endothelial wall, with a smaller fraction identified in the tissue interstitium. In animals, Gd deposits were observed after repeated doses of Gadavist, MultiHance and Omniscan but not after repeated doses of ProHance. Presence and localization of Gd deposits in brain tissues have been investigated in man only after multiple exposure to Omniscan. Of note, Gd deposits were detected within the nucleus of a neuronal cell in 2 patients who received multiple doses of this GBCA.

More, and possibly larger studies in humans aimed at determining levels of elemental Gd in brain tissues are needed to understand possible differences among the various GBCAs approved and used in the United States. Also, additional clinical studies are needed to determine the chemical form of the Gd complexes retained in human tissues, as well as retention over time and presence and localization of Gd deposits in the brain. The presence and concentration of other metals that could contribute to SI increase in the DN and GP on T1-weighted images should also be investigated.

All the imaging studies available to date are affected by potential sources of bias. All were small-scale, single-center, retrospective investigations. Patients were selected from hospital databases using different selection criteria, and selection and information bias cannot be excluded. Differences in patient populations and image acquisition protocols, the inappropriate methodology used for image evaluation, as well as different level, frequency and time of exposure to GBCAs, and potential carryover effects deriving from previous exposures to a GBCA different from the one under study limit the precision in the determination of changes in SI or R1 values associated with a certain number of administrations of a specific GBCA. The results of imaging are affected by unknown variables which could not be addressed in the design. Gd may be retained in forms (mainly insoluble forms) that do not affect SI on the MR image. In addition, paramagnetic materials and other coexisting metals such as manganese or iron, may also affect the SI of brain MRI, so that, when analyzing Gd retention using MRI, caution should be exercised when interpreting the validity and reliability of the obtained data. Further, imaging data, from either T1-weighted SI analysis or relaxometry, cannot be used to quantify Gd deposition in brain tissues since the longitudinal and transverse relaxivities of the form of the Gd compounds responsible for the observed changes in SI or R1 values are not known.

The value of the imaging studies available to date is that they clearly showed that T1 hyperintensity in deep brain areas may be observed in deep brain areas following repeated doses of GBCAs, especially in the DN and GP. Healthcare professionals should be aware of such potential source of abnormal T1 shortening in deep brain areas, and of the need to be provided with appropriate clinical information on the patient for proper interpretation of such finding.

2.2 Gd Retention in Body Tissues

2.2.1 Nonclinical Studies

Bracco is aware of 12 published studies in adult healthy rats, 10 studies in renally impaired rats or mice, 1 study in ovariectomized rats, and 6 studies only aimed at histopathological examination of body tissue samples. Bracco has also conducted one study in juvenile rats (*Study AB21194*) and other study in adult healthy rats that is not published (*Study TOX-05-07)* dealing with histopathological examination of main organs only.

The cumulative dose injected in adult healthy rats ranged between 6 and 90 mmol/kg (considering that a dose of 0.6 mmol/kg corresponds to 0.1 mmol/kg in adult humans of 60 kg body weight (*Food and Drug Administration, Guidance for Industry*), those doses in rats correspond to a total cumulative GBCA dose of 1 to 15 mmol/kg, i.e., to 10 to 150 times 0.1 mmol/kg doses in a human being of 60 kg body weight, usually administered in a short period of time). The off dose period (for adult healthy rats) ranged between 1 day and 93 weeks.

ICP-MS was used to determine Gd levels in tissues. The most significant results in skin, femur, liver, kidneys and spleen are summarized and discussed in the following sections.

2.2.1.1 Levels of Elemental Gd in Skin Following Exposure to Different GBCAs in Healthy Rats

A comparison of results of levels of elemental Gd can be directly done only between two studies carried out by the Sponsor Bayer with similar methodology (*Sieber et al. 2008a; Lohrke et al. 2017)*. In these studies, healthy rats received 20 intravenous injections (4 injections per week for 5 consecutive weeks) of 2.5 mmol Gd/kg (cumulative dose: 50 mmol/kg) of different GBCAs. An additional control group receiving matched volumes of saline was included. The off-dose period before animal sacrifice was 5 days and 8 weeks, respectively, in the study by Sieber et al. and in the study by Lohrke et al.

Energy dispersive x-ray spectroscopy was applied to analyze the elemental composition, including the presence of Gd in the skin on serial sections. The presence of Gd-containing domains in the connective tissue of the subcutis was only observed for Magnevist and Omniscan, with Omniscan having approximately 3- to 4-fold more Gd domains in the skin than Magnevist. Such deposits were not observed in animals treated either with ProHance or Gadavist (*Lohrke et al, 2017*).

The lowest amount of elemental Gd (close to the limit of quantification) was observed following repeated administrations of ProHance or Gadavist. Levels of elemental Gd following treatment with Magnevist were at least 47 times higher than those measured for the macrocyclic GBCAs and levels of Gd deposition following Omniscan were 8 and 18 times higher than Magnevist after 8 weeks or 5 days off dose periods, respectively, suggesting a slower elimination of Omniscan from skin. In fact, between 5 days and 8 weeks after the end of dosing Gd contents decreased by about 60% in animals treated with Magnevist and only by about 13% in animals treated with Omniscan (Table C).

Table C: Gd Concentration in Skin Tissues after Repeated Administrations of GBCAs: Data from Sieber et al. 2008 and Lohrke et al. 2017

Skin nmol/g fresh tissue	ProHance	Gadavist	Magnevist		Omniscan	
	b	b	a	b	a	b
5 days off dose Mean (SD)	nd	nd	210 (100)	nd	1700 (200)	nd
8 weeks off dose Mean (SD)	1.7 (0.8)	1.1 (0.5)	nd	80.8 (6.2)	nd	1472 (115)
a Sieber et al. 2008a **b** Lohrke et al. 2017 nd = not determined						

2.2.1.2 Retention of Gd in Skin Tissues Over Time

In a study by the Sponsor Bayer, 7 different marketed GBCAs (Omniscan, OptiMARK, Magnevist, MultiHance, Gadavist, ProHance, and Dotarem) were intravenously injected for 5 consecutive days at a dose of 2.5 mmol/kg to healthy rats (cumulative dose: 12.5 mmol/kg) and the determination of levels of elemental Gd was performed using ICP-MS in skin biopsies taken at various time-points up to a year after the last injection (*Pietsch et al., 2009a*). On day 35 post-injection, the highest amount of Gd was observed after treatment with Omniscan (132 ± 23 nmol Gd/g) and OptiMARK (47 ± 5.0 nmol Gd/g). Lower Gd contents in the skin were measured in animals treated with Magnevist (36 ± 6.0 nmol Gd/g) and MultiHance (7 ± 1 nmol Gd/g). Even lower Gd contents were observed in biopsies taken from animals injected with the macrocyclic GBCAs, ProHance (1 ± 1 nmol Gd/g skin), Dotarem (2 ± 1 nmol Gd/g) or Gadavist (2 ± 1 nmol Gd/g). On day 364 after injection, the highest amount of Gd was, again, observed after treatment with Omniscan (72.2 ± 11.9 nmol Gd/g) and OptiMARK (18.2 ± 4.8 nmol Gd/g). Lower Gd values in the skin were again measured in animals treated with Magnevist (8.5 ± 1.5 nmol Gd/g) and MultiHance (1.4 ± 0.4 nmol Gd/g). For biopsies taken from animals treated with ProHance (0.08 ± 0.02 nmol Gd/g), Dotarem (0.22 ± 0.17 nmol Gd/g) or Gadavist (0.06 ± 0.03 nmol Gd/g), the Gd content was close to the detection limit. Results are summarized in Table D.

Table D: Gd Levels in Skin of Rats Following a Cumulative Dose of 12.5 mmol/kg (Data from Pietsch et al., 2009a)

GBCA	35 days off dose	364 days off dose
	Healthy animals nmol Gd/g [Mean±SD]	
Omniscan	132 ± 23	72.2 ± 11.9
OptiMARK	47 ± 5.0	18.2 ± 4.8
Magnevist	36 ± 6.0	8.5 ± 1.5
MultiHance	7 ± 1	1.4 ± 0.4
Gadavist	2 ± 1	0.06 ± 0.03
ProHance	1 ± 1	0.08 ± 0.02
Dotarem	2 ± 1	0.22 ± 0.17

Most of the administered Gd was eliminated from the skin within a time-period of about 2 months. However, the repeated administration of linear GBCAs resulted in long-term retention of a small portion of the administered Gd, with substantially higher values observed in animals

treated with Omniscan, OptiMARK, or Magnevist than in those that received MultiHance or, especially, Gadavist, ProHance or Dotarem. Among linear and macrocyclic GBCAs, the lowest Gd contents were observed with MultiHance and ProHance, respectively.

Results of the above mentioned study were in agreement with those of Bussi et al. (*Bussi et al, 2017*), in which, after a cumulative dose of 12 mmol/kg and an off dose period of 4 weeks, Gd content in skin was below the limit of quantitation for the three tested macrocyclic GBCAs (ProHance, Dotarem and Gadavist).

In a juvenile toxicity study of Dotarem performed by the Sponsor Guerbet (*Giorgi et al., 2015*), Gd concentration in tissues was measured either after a single intravenous administration at 10 days of age (Postnatal Day [PND] 10) or 6 repeated intravenous injections every 4 days from PND 10 to PND 30 at dosages of 0.6, 1.25 or 2.5 mmol/kg per injection (total cumulative doses: 3.6, 7.5 and 15 mmol/kg). Levels of elemental Gd were measured using ICP-MS the day after the last injection (Day 1) and 60 days later. As shown in Table E below, Gd levels increased with increasing dose in all animals, being significantly lower at Day 60, showing clearance of Gd complexes over time.

Table E: Gd Concentration in Skin in a Juvenile Toxicity Study with Dotarem: Data from Giorgi et al., 2015

Dose mmol Gd/kg	Single administration 1 day off dose	Single administration 60 days off dose	Repeated administrations 1 day off dose	Repeated administrations 60 day off dose
0.6	34.3 ± 36.7	BLQ	9.8 ± 1.8	BLQ
1.25	95.79 ± 101	BLQ	21.2 ± 3.2	BLQ
2.5	109 ± 43.2	BLQ	48.3 ± 9.1	BLQ
BLQ, below the limit of quantitation				

A study sponsored by Bracco (*Bracco Study AB21194, 2016*) used the same exact experimental model in juvenile animals exposed to MultiHance. The Gd levels measured in skin were markedly lower than those of Dotarem following a single administration of MultiHance, as shown in Table F, while similar levels were observed following repeated doses.

Table F: Gd Concentration in Skin in a Juvenile Toxicity Study with MultiHance: Data from Study AB21194

Dose (mmol Gd/kg)	Single administration 1 day off dose		Single administration 60 days off dose		Repeated administrations 1 day off dose		Repeated administrations 60 day off dose	
	Males	Females	Males	Females	Males	Females	Males	Females
0.6	3.53±3.33	3.73±2.45	BLQ	BLQ	8.27±3.55	7.09±1.56	BLQ	BLQ
1.25	10.1±4.4	10.5±4.99	BLQ	BLQ	22.1±8.15	21.5±4.87	BLQ	0.18±0.27
2.5	29.9±6.78	24.6±14.0	BLQ	BLQ	48.3±5.71	39.9±12.6	0.67±0.11	1.22±0.36
BLQ, below the limit of quantitation								

Also for MultiHance, Gd levels increased with increasing dose in all animals, and became significantly lower at Day 60, showing clearance of Gd complexes over time.

2.2.1.3 Levels of Elemental Gd in Skin Following Exposure to Different GBCAs in Animals with Impairment of Renal Function

Omniscan, OptiMARK, Magnevist and Gadavist were studied by the Sponsor Bayer (*Pietsch et al., 2009b*) in a model of 5/6 nephrectomized rats using the same methodology described for testing in healthy animals. (*Pietsch et al., 2009a*). Differences in the skin Gd concentrations were observed among the 4 investigated GBCAs. For Omniscan and OptiMARK, high Gd concentrations were maintained in the skin over the observation period of up to 168 days post-injection. For Magnevist, comparatively lower Gd retention in the skin was observed over time. For the macrocyclic compound, Gadavist, the Gd values in the skin were even lower, and significantly lower than Gd values in the skin in Omniscan- and OptiMARK-treated animals.

Compared to healthy rats (*Pietsch et al., 2009b),* renally impaired rats showed Gd contents 2 to 4 times higher (*Pietsch et al., 2009b*) 5-7 weeks after the end of dosing.

Sato et al. (*Sato et al., 2013*) studied Gd deposition in skin after 12 repeated administrations of 2.5 mmol Gd/kg of Omniscan or ProHance (cumulative dose: 30 mmol/kg), and at a dose of 0.625 mmol/kg of Primovist (cumulative dose: 7.5 mmol/kg) in 5/6 nephrectomized rats. The off dose period was 7 days. Gd content in the skin was 2506±728 nmol Gd/g in the Omniscan group, while it was below the limit of quantification both for ProHance and Primovist.

Omniscan and Dotarem were studied in a mouse model of renal failure, performed by electrocoagulation of the entire kidney excluding the hilum (*Kartamihardja et al., 2016b*). Mice were intravenously administered 5 mmol/kg (7 administrations/week for 4 consecutive weeks, cumulative dose: 140 mmol/kg). Gd content in skin was assessed 3 and 45 days after the last injection and compared to data obtained in healthy animals. A significantly higher Gd retention was found with Omniscan both under conditions of normal and impaired renal functions. Differently from the study in rats by Pietsch et al. (*Pietsch et al., 2009b*), long-term Gd retention for the GBCAs was almost unaffected by renal function.

Haylor et al. assessed the distribution of Gd deposits in skin by using an animal model of NSF and comparing Omniscan with Dotarem administered at 2.5 mmol/kg for 5 days (*Haylor et al, 2012*). After an off dose period of 28 days, skin was analyzed by means of morphometric and immunohistochemical techniques and transmission electron microscopy (TEM). Skin Gd deposits were located by means of energy-filtered transmission electron microscopy (EFTEM). Following administration of Omniscan, Gd deposits were detected in the skin of rats with renal insufficiency associated with collagen fibrils, both extracellularly within collagen bundles and intracellularly within the fibroblast associated with collagen fibril fragments.
Presence of Gd around the extracellular collagen fibril may enhance its susceptibility to degradation and endocytosis by fibroblasts. Skin Gd deposits were not detected following administration of Dotarem.

2.2.1.4 Levels of Elemental Gd in Bone Tissues (Femur) Following Exposure to Different GBCAs in Healthy Rats

In a study by the Sponsor Bayer (*Sieber et al. 2008a*)*,* healthy rats received 20 intravenous injections (4 injections per week for 5 consecutive weeks) of 2.5 mmol Gd/kg (cumulative dose:

50 mmol/kg) of Omniscan or Magnevist. Gd content in bone tissue (femur) was analysed using ICP-MS after a 5 days off dose period. The concentration of Gd detected in the femur was 600 ± 100 nmol/g and 200 ± 100 nmol/g, for Omniscan and Magnevist, respectively.

In a study by Bracco (*Bussi et al., 2017*) following a cumulative dose of 12 mmol/kg of Dotarem, Gadavist and ProHance, and an off dose period of 4 weeks, Gd levels in bone tissue samples from femur measured by ICP-MS were 5.69±1.75 nmol/g, 7.48±1.38 nmol/g, and 8.60±2.04 nmol/g, respectively.

In the juvenile toxicity study of Dotarem performed by the Sponsor Guerbet (*Giorgi et al., 2015*), Gd concentration in bone tissues (femur) measured using ICP-MS the day after the last injection (Day 1) and 60 days later. As shown in Table G below, Gd levels increased with increasing dose in all animals, being significantly lower at Day 60 and close to or below the limit of quantitation, showing clearance of Gd complexes over time.

Table G: Gd Concentration in Bone Tissue (Femur) in a Juvenile Toxicity Study with Dotarem: Data from Giorgi et al., 2015

Dose mmol Gd/kg	Single administration 1 day off dose	Single administration 60 days off dose	Repeated administrations 1 day off dose	Repeated administrations 60 day off dose
0.6	6.1± 4.1	BLQ	5.9 ± 1.2	BLQ
1.25	20.9 ± 9.6	BLQ	13.3 ± 1.8	BLQ
2.5	35.7 ± 1.4	BLQ	30.2 ± 4.7	0.4±0.6
BLQ, below the limit of quantitation				

In a similar study performed by Bracco (Study AB21194, 2016), bone Gd levels measured one day after a single dose of MultiHance were similar to those measured after a single dose of Dotarem. However, higher levels were observed following repeated doses and at Day 60 (Table H), even if Gd concentrations were significantly lower at the same time point, showing clearance of Gd complexes over time also after exposure to MultiHance.

Table H: Gd Concentration in Bone Tissue (Femur) in a Juvenile Toxicity Study with MultiHance: Data from Study AB21194

Dose mmol Gd/kg	Single administration 1 day off dose		Single administration 60 days off dose		Repeated administrations 1 day off dose		Repeated administrations 60 day off dose	
	Males	Females	Males	Females	Males	Females	Males	Females
0.6	7.34±6.30	8.12±5.80	0.26±0.64	0.72±1.33	21.7±6.18	22.0±4.64	9.74±3.17	10.3±4.96
1.25	18.0±6.40	24.5±12.0	2.00±1.92	2.22±1.51	39.8±16.3	40.6±12.8	17.7±4.83	21.3±4.31
2.5	47.2±10.7	39.0±24.2	2.17±1.73	2.51±1.94	78.0±13.1	67.0±25.7	28.6±6.90	19.3±7.83

2.2.1.5 Levels of Elemental Gd in Bone Tissues (Femur) Following Exposure to Different GBCAs in Animals with Impairment of Renal Function

Omniscan and Dotarem were studied in a mouse model of renal failure, performed by electrocoagulation of the entire kidney excluding the hilum (*Kartamihardja et al., 2016b*). Mice were intravenously administered at 5 mmol/kg (7 administrations/week for 4 consecutive weeks,

cumulative dose: 140 mmol/kg). Gd content in femur was assessed 3 and 45 days after the last injection and compared to data obtained in healthy animals. Higher Gd levels were observed in renally impaired mice at Day 3. Omniscan showed higher Gd levels at Day 5 compared to Dotarem; however, while Gd clearance and lower levels over time were observed after Omniscan, Gd levels following Dotarem did not show a decrease over time.

2.2.1.6 Levels of Elemental Gd in Liver Following Exposure to Different GBCAs in Healthy Rats

Determination of levels of elemental Gd was performed using ICP-MS in liver of animals in a study by the Sponsor Bayer (*Sieber et al., 2008a)*. In this study, healthy rats received 20 intravenous injections (4 injections per week for 5 consecutive weeks) of 2.5 mmol Gd/kg (cumulative dose: 50 mmol/kg) of Omniscan and Magnevist. An additional control group receiving matched volumes of saline was included. The off-dose period was 5 days. Gd contents were 400±100 nmol/g and 200±20 nmol/g after Omniscan and Magnevist, respectively.

Results of a study by Bracco (*Bussi et al., 2017*) showed that after a cumulative dose of 12 mmol/kg and an off dose period of 4 weeks, Gd content in liver tissue samples was below the limit of quantitation by ICP-MS for the 3 tested macrocyclic GBCAs (ProHance, Dotarem and Gadavist), thus showing clearance of Gd complexes over time.

In the juvenile toxicity study of Dotarem performed by the Sponsor Guerbet (*Giorgi et al., 2015*), Gd concentration in liver was measured using ICP-MS the day after the last injection of the agent (Day 1) and 60 days later. Gd levels increased with increasing dose in all animals, and were below the limit of quantitation at Day 60, showing clearance of Gd complexes over time (Table I).

Table I: Gd Concentration in Liver Tissue in a Juvenile Toxicity Study with Dotarem: Data from Giorgi et al., 2015

Dose mmol Gd/kg	Single administration 1 day off dose	Single administration 60 days off dose	Repeated administrations 1 day off dose	Repeated administrations 60 day off dose
0.6	16.7± 11.7	BLQ	15.7 ± 3.1	BLQ
1.25	72.4 ± 33.8	BLQ	35.0 ± 6.1	BLQ
2.5	114 ± 76.3	BLQ	78.3 ± 10.4	0.2±0.5
BLQ, below the limit of quantitation				

The level of elemental Gd measured following single and repeated doses of Dotarem in the study by Giorgi et al. were very similar to those observed following administration of MultiHance in a similar study performed by Bracco (*Study AB21194, 2016*), both at Day 1 and Day 60 after injection of the two agents (Table J).

Table J: Gd Concentration in Liver Tissue in a Juvenile Toxicity Study with MultiHance: Data from Study AB21194

Dose mmol Gd/kg	Single administration 1 day off dose		Single administration 60 days off dose		Repeated administrations 1 day off dose		Repeated administrations 60 day off dose	
	Males	Females	Males	Females	Males	Females	Males	Females
0.6	13.8±13.5	14.4±8.80	BLQ	BLQ	19.1±6.39	16.2±3.86	BLQ	BLQ
1.25	43.1±19.7	45.9±23.5	BLQ	BLQ	48.4±13.1	43.5±4.46	BLQ	0.09±0.21
2.5	113±24.8	83.3±48.5	BLQ	BLQ	80.9±15.2	72.1±19.9	BLQ	0.84±0.27
BLQ, below the limit of quantitation								

2.2.1.7 Levels of Elemental Gd in Liver Following Exposure to Different GBCAs in Animals with Impairment of Renal Function

Sato et al. (*Sato et al., 2013*) studied Gd deposition in liver tissue samples following 12 repeated doses of 2.5 mmol Gd/kg Omniscan or ProHance (cumulative dose: 30 mmol/kg) or 12 doses of 0.625 mmol/kg Eovist (cumulative dose:7.5 mmol/kg) in 5/6 nephrectomized rats. The off dose period was 7 days. Gd content in the liver was 794±162 nmol Gd/g in the Omniscan group, while it was 69±24 nmol Gd/g for ProHance and 16.5±6.4 nmol Gd/g Eovist.

Omniscan and Dotarem were studied in a mouse model of renal failure, performed by electrocoagulation of the entire kidney excluding the hilum (*Kartamihardja et al., 2016b*). Mice were intravenously administered at 5 mmol/kg (7 administrations/week for 4 consecutive weeks, cumulative dose: 140 mmol/kg). Gd content in liver was assessed 3 and 45 days after the last injection and compared to data obtained in healthy animals. Gd levels in liver were higher in renally impaired mice, but only after Omniscan and at Day 3. No difference between healthy and renally impaired animals was observed at Day 3 for Dotarem, and at Day 45 for both agents. Gd levels after Omniscan were higher compared to those measured after Dotarem at Day 3, both in healthy and renally impaired mice; however, while a decrease in Gd levels was observed at Day 45 following Omniscan, an increase was observed for Dotarem, as shown in Table K.

Table K: Gd Levels in Mouse Liver Following Administration of Omniscan and Dotarem: Data from Kartamihardja et al., 2016b

	3 days off dose		45 days off dose	
	Healthy animals	Renally impaired animals	Healthy animals	Renally impaired animals
GBCA	nmol Gd/g [Mean±SD]			
Omniscan	1506±307	3341±1442	1590±345	1140±320
Dotarem	997±163	812±151	1140±320	1102±638

2.2.1.8 Levels of Elemental Gd in Kidney Following Exposure to Different GBCAs in Animals with Impairment of Renal Function

McDonald et al. determined Gd contents in kidneys of healthy animals treated with Omniscan, MultiHance, Gadavist and ProHance after a cumulative dose of 50 mmol/kg and an off dose

period of 7 days (*McDonald et al., 2017a*). Gd contents were respectively 11187±3912 nmol/g, 7645±608 nmol/g, 3307±1051 nmol/g and 1055±458 nmol/g following Omniscan, MultiHance, Gadavist and ProHance, respectively.

Results of a study conducted by Bracco (*Bussi et al., 2017*) showed that after a cumulative dose of 12 mmol/kg and an off dose period of 4 weeks, Gd content in kidneys was significantly lower with ProHance (25±13 nmol/g) than with Dotarem (139±88 nmol/g) and Gadavist (204±109 nmol/g).

In the juvenile toxicity study performed with Dotarem (*Giorgi et al., 2015*), Gd concentration in kidneys was measured using ICP-MS the day after the last injection (Day 1) and 60 days later. As shown in the table below, Gd content in kidneys was detectable both after single and repeated dosing after an off dose period of 60 days, decreasing by about 99% from 1 to 60 days. Gd levels increased with increasing dose in all animals, and were significantly lower at Day 60, showing clearance of Gd complexes over time (Table L).

Table L: Gd Concentration in Kidneys in a Juvenile Toxicity study with Dotarem: Data from Giorgi et al., 2015

Dose mmol Gd/kg	Single administration 1 day off dose	Single administration 60 days off dose	Repeated administrations 1 day off dose	Repeated administrations 60 day off dose
0.6	278±225	0.3±0.5	448±71	1.4±1.6
1.25	1014±458	0.4±0.6	915±138	2.7±1.8
2.5	1595±1013	1.0±0.6	1805±300	9.8±6.2

In a study conducted by Bracco (*Bracco Study AB21194, 2016*), using the same experimental design, Gd levels in kidney following MultiHance were consistently lower than those observed for Dotarem (Table M).

Table M: Gd Concentration in Kidneys in a Juvenile Toxicity Study with MultiHance: Data from Study AB21194

Dose mmol Gd/kg	Single administration 1 day off dose		Single administration 60 days off dose		Repeated administrations 1 day off dose		Repeated administrations 60 day off dose	
	Males	Females	Males	Females	Males	Females	Males	Females
0.6	105±103	100±63.5	BLQ	BLQ	197±85.2	158±41.0	0.85±0.11	1.33±0.21
1.25	307±139	341±182	0.44±0.58	0.80±0.53	530±134	420±78.3	1.62±0.35	2.05±0.31
2.5	836±191	668±377	0.69±0.13	1.00±0.62	859±141	785±204	2.25±0.36	3.98±1.02
BLQ, below the limit of quantitation								

2.2.1.9 Levels of Elemental Gd in Kidney Following Exposure to Different GBCAs in Animals with Impairment of Renal Function

In a study of renally impaired mice (*Kartamihardja et al., 2016b*), mice were intravenously administered 5 mmol/kg of Omniscan or Dotarem (5 administrations/week for 4 consecutive weeks, cumulative dose: 100 mmol/kg). Gd content in kidneys was assessed 3 and 45 days after the last injection and compared to data obtained in healthy animals. Omniscan levels were higher

in renally-impaired mice compared with healthy animals, but not in animals treated with Dotarem (Table N). Similar to what was observed in juvenile healthy rats treated with Dotarem and MultiHance (*Giorgi et al, 2015; Bracco Study AB21194, 2016),* Gd levels in healthy mice were higher after repeated doses of the macrocyclic Dotarem compared with the linear Omniscan.

Table N: Gd Levels in Mouse Kidney Following Administration of Omniscan and Dotarem. Data from Kartamihardja et al., 2016b

	3 days off dose		45 days off dose	
	Healthy animals	Renally impaired animals	Healthy animals	Renally impaired animals
GBCA	nmol Gd/g [Mean±SD]			
Omniscan	2293±378	4153±1532	258±113	1003±1011
Dotarem	5994±970	3379±676	1189±250	212±141

Sato et al. (*Sato et al., 2013*) studied Gd deposition in kidneys after 12 repeated administrations at 2.5 mmol Gd/kg of Omniscan and ProHance (cumulative dose: 30 mmol/kg) and at 0.625 mmol/kg of Eovist (cumulative dose: 7.5 mmol/kg) in 5/6 nephrectomized rats. The off dose period was 7 days. Gd content in the kidneys was 8305±3852 nmol Gd/g in the Omniscan group, while it was 818±567 nmol Gd/g for ProHance and 88±21 nmol Gd/g after Eovist.

2.2.1.10 Conclusions on Gd Retention Data in Body Tissues from Nonclinical Tissue-Sample Studies

The experimental animal studies of Gd retention in body tissues showed that:

- Retention of Gd complexes in skin, bone, liver, and kidneys is observed following exposure to all GBCAs and is dose-dependent;
- Clearance of retained Gd complexes over time has been observed for all GBCAs, both in adult and juvenile animals;
- Impairment of renal function increases Gd retention in rats but not in mice. In general, Gd clearance following Omniscan was more effective in renal failure mice, all organs. This is difficult to explain as the opposite would be expected, and it is not clear how Gd could be effectively eliminated by the renally impaired animals after exposure to Omniscan. Of note, liver Gd levels also decreased over time in mice exposed to Omniscan, while long-term Gd accumulation in the liver and bone was observed following Dotarem (active ingredient: gadoterate meglumine, or Gd-DOTA). This finding is consistent with a previous study by **Wadas et al**. (*Wadas et al., 2010*), which demonstrated that the biodistribution of 153/NatGd-DOTA at 7 days after injection was higher in the liver of renally-impaired mice than normal mice, together with 3-fold ($p<0.037$) more activity in their bone tissue.

There are differences in Gd retention among the various individual GBCAs that depend on animal species, the individual organs, and the experimental models used. In general, it seems that higher Gd levels are observed following administration of Magnevist, and especially, Omniscan. When comparing similar studies in juvenile animals, higher Gd levels and less effective

clearance were observed after MultiHance in bone tissue (femur), but similar levels and clearance were observed after single and repeated doses Dotarem and MultiHance in skin and liver tissues, and higher levels were observed in kidneys following Dotarem compared with MultiHance.

2.2.2 Clinical Studies

This section will review tissue-sample studies conducted to detect or exclude Gd retention in extra-cerebral (body) organs and tissues either in patient with normal renal function or with renal impairment from a pool of 19 peer-reviewed publications (*Gibby et al., 2004; White et al., 2006; Boyd et al., 2007; Lim YL et al., 2007; Singh M et al., 2008; Khurana A et al., 2008; Schroeder JA et al., 2008; Darrah TH et al., 2009; Thakral and Abraham, 2009; Boyd et al., 2010; High WA et al., 2010; Aggarwal A et a., 2011; Baker-Griffith A et al., 2011; Christensen KN et al., 2011; Sanyal et al., 2011; Birka M et al., 2015; Murata et al., 2016; Maximova N et al., 2016; Roberts DR et al., 2016b*).

2.2.2.1 Retention in Bone

Four studies have assessed whether Gd deposits were evident in bone from patients with normal renal function who were exposed to GBCAs (*Gibby et al., 2004; White et al., 2006; Darrah et al., 2009; Murata et al., 2016a*) .

Gibby et al. conducted an investigation using femoral heads removed from 18 patients undergoing a total hip arthroplasty (*Gibby et al., 2004*). The GBCAs involved were Omniscan and ProHance. Both GBCAs were injected intravenously at the dose of 0.1 mmol/kg not less than 3 days and not more than 8 days before surgery. Ten patients had received Omniscan and eight patients ProHance. An age-matched control population (N=7) of patients undergoing hip replacement with no history of GBCA administration was also obtained. By using inductively coupled plasma atomic emission spectroscopy (ICS-AES), the investigators determined the presence of and Gd concentration in the bone tissue. Tissue retention was 1.18 ± 0.787 µg Gd/g bone (n=10) for Omniscan and 0.466 ± 0.387 µg Gd/g bone (n=8) for ProHance, whereas it was -0.117 ± 0.227 µg Gd/g bone in the control group (n=7).

A study from **White et al**. (*White et al., 2006*) was conducted to confirm the above reported findings of Gibby et al. The investigators used different samples from the original bone specimens used in the study by Gibby et al. (Omniscan N=9; ProHance N=10, controls N=8). A more sensitive analytical method, ICP-MS, was used instead of ICS-AES. Again, Gd levels in bone tissues were markedly higher following administration of Omniscan: 1.77 ± 0.704 µg Gd/g bone (n=9) for Omniscan and 0.477 ± 0.271 µg Gd/g bone (n=10) for ProHance.

Darrah et al. investigated the relation between Gd concentrations in trabecular and cortical bone tissues and exposure to GBCAs (*Darrah et al., 2009*). Thirty-five femoral heads removed during hip replacement surgery were analyzed for Gd retention by using ICP-MS. The GBCA group included 13 patients with medical records documenting exposure to GBCAs, with maximum time elapsed since exposure of 8 years prior to surgery. For 11 patients, the contrast agent type was known: Omniscan in 6 patients and ProHance in 5. The control group included 18 osteoarthritis patients with no exposure to GBCAs. There were also bone samples from

4 additional patients with incomplete associated medical records with no history of GBCA exposure who were not included in comparisons of control and GBCA group.

Gd concentrations in both cortical and trabecular tissues were significantly higher ($p<0.001$) in patients of the GBCA group (n=13) compared to controls (N=18). Differently from the studies by Gibby et al. and White et al., which both assessed Gd retention 7 days after exposure, no significant difference in bone Gd levels was observed between patients who received Omniscan or ProHance 8 years after last exposure.

Furthermore, the effect of bone pathology on Gd incorporation and retention was investigated by comparing Gd concentration in the bone of patients with osteoporotic fractures to those of patients with osteoarthritis. Osteoporotic bone (samples from 7 patients) had significantly ($p<0.001$) lower Gd concentrations in both cortical and trabecular tissues than areas of osteoarthritis (samples from 9 patients), with no overlap in 95% limits of the confidence interval.

Murata et al. conducted a conducted a study with the aim of determining whether Gd is retained in the brain as well as in the bone, following exposure to the GBCAs Eovist, Gadavist, MultiHance, or ProHance. (*Murata N et al., 2016a* – see Section 2.1.1.2.1 of this document for details on brain retention). Brain and bone tissue from rib were collected at autopsy from decedents with available medical records that documented past history of single-agent exposure. Nine decedents with no prior MRI or only unenhanced MRI served as controls. From a total of 134 autopsy cases, the investigators identified 9 patients who received 1 or more injections of a single macrocyclic or linear GBCA during life. This included 5 patients who received ProHance, 2 who received Gadavist, and 1 each who had received MultiHance or Eovist.

Gd levels in bone were >20 times higher compared to brain levels, and levels in GBCA-exposed patients were significantly higher than in controls. Similarly to brain, normalized Gd retention ratios (Gd retained in 1 g of tissue per millimole of GBCA administered) showed highest levels following administration of Gadavist, followed by MultiHance and Eovist. The lowest levels were observed after administration of ProHance. Correlation between normalized Gd levels in brain and bone was good (correlation for GP versus bone: $r = 0.81$ ($P = 0.022$, $n = 8$).

2.2.2.2 Retention in Skin

Despite that the skin is primarily involved in the development of NSF, data on Gd retention in cutaneous tissues are limited to case reports or small scale studies.

Birka et al. conducted a combination of elemental bioimaging and speciation analysis on skin samples obtained with tissue biopsy from a patient with suspected NSF who was exposed to Magnevist and ProHance 11 and 8 years before the study (*Birka et al., 2015*). Quantitative analysis of elemental Gd in skin biopsy specimens was conducted using ICP-MS. After determination of the total Gd concentration, distribution maps of calcium, phosphorous and Gd were generated by means of laser ablation with inductively coupled plasma mass spectrometry (LA-ICP-MS). Speciation analysis of water-soluble Gd species was achieved by hydrophilic interaction liquid chromatography with ICP-MS (HILIC-ICP-MS). The elemental Gd concentration in skin samples was found to be between 3.02 and 4.58 mg/kg in the three skin samples. LA-ICP-MS revealed a distinctly inhomogeneous distribution of Gd. The correlation

between the distributions of phosphorus and Gd suggested the presence of Gd phosphates in the skin. Speciation analysis by means of HILIC-ICP-MS showed the presence of the intact ProHance molecule retained as such 8 years after the last administration of this GBCA to the patient.

Thakral and Abraham studied 29 patients with known NSF who underwent skin biopsies 2 weeks to 3 years after GBCA exposure (*Thakral and Abraham, 2007*). Gd deposits were present in 53 of 57 skin biopsies. The concentration of elemental Gd ranged from 1 to 2270 cps/mm2, and was detected predominantly in the deep dermis and subcutaneous fibrous septa. Gd was found associated with calcium, phosphorus and sometimes iron or zinc. Patients with sequential biopsies showed persistence or increase of Gd concentration in tissues (6 of 11). Transmission electron microscopy (TEM) identified the intracellular deposits of insoluble Gd complexes in fibrocytes and macrophages.

Sanyal et al. performed an analysis of tissues from an autopsy case with verified advanced nephrogenic systemic fibrosis (NSF) (*Sanyal et al., 2011*). Using light microscopy and scanning electron microscopy/energy-dispersive X-ray spectroscopy, the investigators found Gd deposits (in the form of insoluble Gd-phosphate deposits) in the perivascular glial cells in the cerebellum, and in the skin, liver, lungs, intestinal wall (ileum), kidney, lymph node, skeletal muscle, and dura mater, primarily in vascular walls. Some, but not all, Gd deposits were seen in fibrotic areas.

High et al. quantified Gd retention in skin and soft tissue of two patients with NSF using ICP-MS and mapped Gd deposition using synchrotron x-ray fluorescence spectroscopy (*High et al, 2010*). A gradient of Gd deposition in tissue correlated with fibrosis and cellularity. Gd deposited in peri-adnexal locations within the skin, including hair and eccrine ducts, where it co-localized to areas of high calcium and zinc content.

Christensen et al. conducted a study using fresh skin tissue samples from 13 patients with NSF obtained from skin areas with clinically manifest skin lesions and non-lesional areas, and skin samples from 13 controls (*Christensen et al, 2011*). Determination of Gd levels was performed using ICP-MS. In patients with NSF, the mean ratio of paired Gd concentrations of affected skin to unaffected skin was 23.1, ranging from 1.2 to 88.9 ng/mL. Therefore, there were significant differences in the amounts of Gd in involved versus nonlesional skin of patients with NSF.

Boyd et al described a hemodialysis-dependent liver transplant recipient, still with no clinical signs of NSF, who received a GBCA and demonstrated insoluble Gd deposition in a fibrotic dermis and subcutaneous septum using scanning electron microscopy/energy dispersive x-ray spectroscopy, higher in the deep fibrous area (287 Gd cps/mm2 compared with the rest of the tissue (128 Gd cps/mm2), and in association with phosphorous, calcium, sodium, and iron (*Boyd et al, 2010*).

Roberts et al. investigated Gd deposition in the skin of a patient with normal renal function, based on estimated glomerular filtration rate (eGFR) value greater than 59 mL/min/1.73 m^2, after exposure to large cumulative doses of GBCAs (*Roberts et al., 2016b*). The patient, with the history of glioblastoma resection at the age of 19 years, underwent 61 contrast-enhanced MRI scans over the next 11 years. Multiple GBCAs were administered, including MultiHance,

Magnevist, Omniscan and ProHance. Following an episode of cholecystitis and subsequent cholecystectomy the patient developed severe progressive generalized contractures not explained by his brain tumor or prior treatments. Although no external skin changes of NSF were present (no complaints of rashes, pruritus, sclerosis of the skin, no superficial plaque, woody induration, peau d'orange, hyperpigmentation, or violaceous coloration involving any skin surfaces), he underwent skin biopsy from the forearm and lower extremity. The skin biopsy samples were formalin fixed and paraffin embedded. They included epidermis, dermis, and subcutaneous adipose tissue. Three 3-μm-thick formalin fixed and paraffin embedded sections from each site were stained with hematoxylin and eosin and antibodies against CD34. Eight micron-thick unstained sections were sent for gadolinium analysis and were analyzed with ICP-MS, LA- ICP-MS, and HILIC-ICP-MS. The ICP-MS demonstrated high levels of Gd retention (14.5 ± 0.4 μg/g). Review of histologic sections revealed normal skin and subcutis. There was no evidence of increased dermal cellularity, fibrosis, abnormal collagen bundles, osseous metaplasia, or abnormal numbers of fibroblast-like cells or macrophages.

While no typical clinical or histopathological signs of NSF were present, there was increased CD34 immunoreactivity in the connective tissue septations of the subcutaneous adipose tissue, indicating inflammation and/or tissue injury. Similar to previous findings in patients with NSF, LA-ICP-MS demonstrated deposition of Gd within the deep layers of the subcutis closely associated with the connective tissue septations of the subcutaneous adipose tissue. Unlike the skin samples from the patient with NSF, there was no significant co-localization of phosphorous with the observed high levels of Gd retention. Of note, speciation analysis showed presence of intact molecules of gadopentetic acid (Magnevist) and gadobenic acid (MultiHance) months after their last administration.

2.2.2.3 Retention in Liver

A study of Gd retention in liver tissue following exposure to Dotarem has been recently conducted by **Maximova et al.** who included 21 pediatric patients who were recipients of allogeneic hematopoietic stem cell transplants and underwent multiple Dotarem-enhanced MRI examinations for suspected infection or relapse followed by liver biopsy (*Maximova N et al., 2016*). Mean age at first liver biopsy was 11.05 years (age range, 2–17 years). The mean number of Dotarem–enhanced MR examinations per patient was 1.8 (range, 1–6). The mean interval between the first Dotarem–enhanced MR examination and the first liver biopsy was 10.1 months (range, 1–42 months), and the mean interval between the first Dotarem administration and the last liver biopsy was 16.6 months (range, 1–49 months). All patients had normal liver function and normal or near-normal renal function. Eight patients had siderosis and underwent chelation therapy. The study group was compared with 4 control patients who were never exposed to any GBCA.

The number of Dotarem-enhanced MR examinations and cumulative Gd dose for each patient was analyzed by comparing liver histologic analysis and iron and Gd liver concentration using ICP-MS. Iron overload was observed in 19 of 21 patients (90.5%). Three of 21 patients (14.3%) had mild iron overload, six of 21 patients (28.6%) had moderate iron overload, six of 21 patients (28.6%) had severe iron overload, and four of 21 patients (19.0%) had very severe iron overload. Evaluation of liver biopsy samples from the study and control groups revealed 4 of 21 (19.0%)

patients with no ductopenia. There were findings in 17 patients that were consistent with graft-versus-host disease: 11 of 21 (52.4%) with severe ductopenia and 6 of 21 (28.6%) with total ductopenia.

Mean level of elemental Gd in liver tissue was 0.29 μg/g (range, 0.03–0.96 μg/g). There was a positive association between the number of Dotarem–enhanced MR examinations per patient and liver Gd levels ($r = 0.4486$; $p<0.05$). Moreover, there was an association between the degree of Gd accumulation and the degree of iron overload. The concentration of Gd in liver tissues was correlated with levels of iron ($r = 0.5493$; $p < 0.05$), while no correlation was found with the presence or severity of ductopenia.

To reduce levels of iron in tissues, 8 patients were treated with chelation therapy or phlebotomy: 5 were treated with deferoxamine, one was treated with deferasirox, and two were treated by monthly phlebotomy. The mean duration of deferoxamine treatment was 9.6 weeks (range, 7–14 weeks), the duration of deferasirox treatment was 2 years, and the duration of phlebotomy was one year for one patient and two years for the other patient. In these patients, there was a reduction of Gd levels in the liver, which decreased on average by 70.72% (range, 52.1%-99.8%) in all patients who had received deferoxamine treatment, which suggests that chelation therapy was effective in reducing Gd in liver and dependent on the duration of the treatment. While chelation therapy with deferoxamine was associated with significant reduction of Gd levels in the liver (from 0.64 to 0.20 μg/g, $p < 0.05$), there was no correlation with reduction of iron overload. This suggests that deferoxamine also exerted a chelating action relevant to Gd.

2.2.2.4 Conclusions on Clinical Body Tissue-Sample Studies

Despite data on Gd retention in human body tissues is limited, the following general preliminary conclusions may be made:

- The available evidence shows that Gd complexes are retained not only in brain tissues, but also, and at higher levels, in extra-cranial (body) tissues, such as bone, skin and liver, and for a long time;
- There may be a significant correlation between Gd levels in bone and brain;
- Speciation analysis showed that Gd is retained in insoluble complexes (mostly phosphates), but the intact GBCA molecule may be retained for a long time. This was shown for both a macrocyclic GBCA (ProHance) and two linear GBCAs (Magnevist and MultiHance) in the skin of subjects, independently of renal function;
- In the skin of patients with NSF, Gd deposits seem to be associated with presence of other metals, such as calcium, zinc, sodium, and iron. Co-localization with phosphorous was not found in a patient with normal renal function without clinical or pathology signs of NSF;
- In the bone and skin, the distribution of Gd deposits is inhomogeneous and seems to be higher in areas of inflammation, increased cellularity, or lesional areas in patients with NSF. In the bone, Gd levels were significantly higher in areas of osteoarthritis compared with areas of osteoporosis. In the skin of patients with NSF, TEM identified the intracellular deposits of insoluble Gd complexes in fibrocytes and macrophages.

- In a patient with normal renal function and no signs of NSF, LA-ICP-MS demonstrated deposition of Gd within the deep layers of the subcutis closely associated with the connective tissue septations of the subcutaneous adipose tissue and increased CD34 immunoreactivity;
- Only few studies report the type and the number of doses of GBCAs (*Murata et al., 2016; Gibby WA et al., 2004; White GW et al., 2006; Darrah TH et al., 2009; Birka M et al., 2015; Roberts DR et al., 2016; Maximova N et al., 2016*), so that it is difficult to draw conclusions about possible differences among the various GBCAs. In the bone, while levels of retained Gd are 3-4 times higher following a single dose of Omniscan compared with ProHance, no difference in Gd retention between these two agents was observed at a longer time point.
- An association between Gd levels in liver tissues and dose was suggested following multiple exposures to Dotarem in pediatric patients who were recipients of allogeneic hematopoietic stem cell transplants. In patients with siderosis, the presence of severe iron overload that may create an abnormal iron availability, and gadoterate meglumine (active ingredient of Dotarem) may not be dissociated, but could be associated in some form with the bulk or specific forms of the iron. Alternatively, gadoterate meglumine could be dissociated because of a combination of competing conditions, including acidity of a specific (i.e., hydrogen ion) or general (e.g., ferric ion) nature, to undergo transmetallation and then can be effectively chelated and cleared by deferoxamine.

2.2.3 Overall Conclusions on Gd Retention in Body Tissues

Both experimental animal studies and clinical tissue-sample studies show that:

- Retention of Gd complexes in body tissues is observed following exposure to all GBCAs and may be dose-dependent;
- Gd levels in body tissues seem to be markedly higher than those observed in brain tissues, even if there is a correlation between lvels in brain and levels in body tissues;
- Clearance of retained Gd complexes over time has been observed for all GBCAs both in adult and juvenile animals. However, Gd complexes (insoluble Gd phosphates or even intact GBA molecules) have been observed in human patients months, or even years after exposure to either linear or macrocyclic GBCAs;
- The effect of impairment of renal function on Gd levels in body tissues is not thoroughly clear. Data obtained in mice and rat models do not show consistent results;
- In the skin of patients with NSF, Gd deposits seem to be associated with presence of other metals, such as calcium, zinc, sodium, and iron. Co-localization with phosphorous has been also observed in NSF patients, but was not found in a patient with normal renal function without clinical or pathology signs of NSF;
- The distribution of Gd deposits in man is inhomogeneous and seems to be higher in areas of inflammation, increased cellularity, or lesional areas in patients with NSF. In a patient

with normal renal function, deposition of Gd was associated with the connective tissue septations of the subcutaneous adipose tissue and increased CD34 immunoreactivity;

- In animal studies, Omniscan and Magnevist were associated with the highest levels of Gd retained in body tissues. Data obtained in humans do not allow to draw firm conclusions about possible differences between the various GBCAs.

3 Assessment of Potential Toxicity of Gd Retained in Brain and Body Tissues

This section of the document will report and assess data from:

- Acute neurotoxicity studies of GBCAs;
- Structural histopathology and electron microscopic analysis of brain tissues and clinical assessments performed during non-clinical studies aimed at assessing levels of retention of Gd complexes in brain tissues;
- Assessment of neurotoxic potential from data reported in clinical, post-mortem tissue-sample studies, imaging studies, and epidemiologic studies.

3.1.1 Acute Neurotoxicity Studies of GBCAs

Four studies have been conducted to assess the acute neurotoxicity of GBCAs after a single intrathecal administration. Overall, they allowed to determine the NOEL (No Observed Effect Level) and the LOEL (Lowest Observable Effect Level) of Magnevist, Omniscan and MultiHance, with respect to the induction of acute or sub-acute behavioural changes and/or histopathological lesions after intrathecal injections of the GBCAs, as summarized in Table O.

Table O: Neurotoxicity of Intrathecal Doses of Magnevist, Omniscan and MultiHance

Source	GBCA	Evaluated Endpoint	NOEL (µmol/g brain)	LOEL (µmol/g brain) Inducing Acute or Sub-acute Symptoms and/or Histopathological Lesions
Ray et al., 1996	gadopentetate dimeglumine (Magnevist)	behavioural assessment + histopathology	3.3	5.0
Ray et al., 1998	gadodiamide (Omniscan)	behavioural assessment + histopathology	0.5	1.25
Toney et al., 2001	gadopentetate dimeglumine (Magnevist)	behavioural assessment + histopathology	2.5	Single intrathecal dose - no information on the toxicity of higher doses
Bracco Study RF994-1, 1993	gadobenate dimeglumine (MultiHance)	behavioural assessment + histopathology	3.0	6.0
NOEL, no observed effect level; LOEL, lowest observable effect level				

The acute neurotoxicity of macrocyclic and linear contrast agents after intrathecal injection was compared in a paper by Vogler et al (*Vogler et al., 1995*) who determined the Median Effective

Dose (ED_{50}) for presence of signs of neurotoxicity, and the Median Lethal Dose (LD_{50}). Data could not be inserted in Table O above as no NOELs or LOELs were provided. Gadavist (gadobutrol), ProHance, Dotarem, Omniscan and Magnevist were injected into the cisterna magna of rats after mild anesthesia with ether. The results are reported in Figure 10 below. Consistent with the results of the acute neurotoxicity studies reported in Table O above, the neurotoxicity of Omniscan was higher than that of Magnevist. The macrocyclic GBCAs were significantly more neurotoxic than Omniscan and, especially, Magnevist, when considering both the ED_{50} and LD_{50} values.

Figure 10. Acute Neurotoxicity of Linear and Macrocyclic GBCAs

Table 6
LD_{50} and ED_{50} of different contrast media after intracisternal application in rats

	LD_{50}		ED_{50}	
	Median value (μmol per kg)	Confidence interval ($P < 0.05$)	Median value (μmol per kg)	Confidence interval ($P < 0.05$)
Gadobutrol	86	64–115	18	8–33
ProHance®	46	34–62	20	8–29
Dotarem®	58	43–78	31	19–44
Omniscan®	208	149–308	47	22–86
Magnevist®	740	480–1250	73	33–165

In order to evaluate whether all these studies are relevant in relation to long-term retention effects and possible chronic toxicity, one should first understand what fraction of the intravenously-injected GBCA in a typical MR procedure is expected to access the CSF.

Two preclinical studies (*Morris et al.,1991; Mamourian et al, 2000,*) addressed directly this point by measuring using ICP-MS or atomic emission spectroscopy (AES) the Gd content of CSF samples collected after intravenous administration of Magnevist to healthy dogs (0.1 and 0.3 mmol/kg) and in healthy rabbits (0.2 mmol/kg). The measured Gd concentration ranged between 7 and 100 μM, with the latter value reached only in 2 of 5 examined rabbits and at approx. 15 min after injection. This Gd concentration in CSF (100 μM) is by far the highest reported value after IV administration of a GBCA. Taking into account the NOEL of Omniscan (the lowest of the linear agents, i.e., 0.5 μM/g brain, corresponding to 1 μM/rat assuming a mean brain weight of 2 g) and considering a volume for the rat CSF of 250 μL (*Hamann et al., 2003*, reporting the highest value found in literature), one can estimate that Gd concentration, immediately after the intrathecal injection at the NOEL, reached a maximum value of 4 mM, approximately 40-fold the highest concentration (100 μM) of Gd dosed in the CSF of rabbits after IV administration of 0.2 mmol/kg of Magnevist. For this calculation, the intrathecal NOEL of Omniscan was used because it is the lowest available for a linear GBCA. Indeed, the same calculation would lead to much higher safety margin (>240-fold). Even if no NOEL is available for the macrocyclic GBCAs, a significant safety margin could be implied for these agents as well.

3.1.2 Assessment of Neurotoxicity of Retained Gd Complexes in Animal Studies

In studies of Gd deposition after repeated intravenous administrations in mice and rats, dosing was set to simulate exposure of humans to repeated contrast-enhanced MRI procedures, although high cumulative doses were administered to the study animals in a short period of time in order for studies to be completed in a reasonable timeframe.

Several of these studies also included structural histopathology assessment of brain tissues as well as clinical and behavioural examination on a daily basis during the treatment and wash-out periods (from 1 to 50 weeks after the end of dosing) to detect signs of histopathological damage or abnormal changes suggestive of a putative neurological toxicity. (*Robert et al., 2015; Robert et al., 2016; Kartamihardja et al., 2016a; Kartamihardja et al., 2016b; Bussi et al., 2017; Frenzel et al., 2017; Lohrke et al, 2017; McDonald et al., 2017a; Smith et al., 2017; Bracco Study FRCG-03-15, 2016*.

In addition to observation of the study animals and structural histopathology, four studies conducted additional assessments.

The Sponsor Bayer conducted a study to determine the potential correlation of histopathological changes with Gd distribution in rodent brain after a very high cumulative intravenous dose (50 mmol/kg in 4 weeks, corresponding to 83 standard 0.1 mmol/kg doses in a human being of 60 kg body weight) of the GBCAs Gadavist, Magnevist, Omniscan and MultiHance (*Lohrke et al, 2017).* In addition to structural histopathology assessment of olfactory bulb, caudate/putamen, cerebral cortex, corpus callosum, GP, subfornical organ, hippocampus, thalamus, hypothalamus, choroid plexus, pyramids, cerebellum, trigeminal nuclei and tracts, lateral (dentate) cerebellar nuclei (lateral DN), and medulla oblongata, serial sections were prepared from selected brain sections (brain II and VI where the GP, subfornical organ, and lateral DN are located) for special staining, immunohistochemistry, and ultrastructural analysis using scanning electron microscopy coupled to energy dispersive x-ray spectroscopy (SEM-EDX) and transmission electron microscopy (TEM-EDX). Cresyl violet stains for Nissl bodies, which in normal neurons are composed of rough endoplasmatic reticulum intermingled with myriad polyribosomes and widely disseminated in the cytoplasm, were used to facilitate detection. In injured neurons, the Nissl bodies undergo partial to complete dissolution, thus releasing ribosomes needed to manufacture new proteins required to repair the damaged cell infrastructure. A polyclonal rabbit anti-glial fibrillary acidic protein (GFAP) antibody was used to assess astrocyte number and morphology. Resident astrocytes respond regarding a retention of metabolic byproducts/fluid by expansion of cytoplasm and/or membrane-bound organelles and by proliferation to repair tissue defects. The astrocyte end-feet projections interact with endothelial cells by encircling the abluminal side making up an important part of the blood-brain barrier (BBB). A polyclonal goat anti-ionized calcium-binding adapter molecule 1 (Iba1) antibody was used to identify early activation of microglia (the resident phagocytic cells of the CNS), because microglial response to neuronal damage ends up in phagocytosis of the degenerating neurons. The histopathological evaluation was conducted according to the best practice guidelines of the Society of Toxicologic Pathology (*Bolon et al, 2013*).

One study also assessed ultrastructural changes using TEM-EDX in addition to structural pathology assessments (*McDonald et al., 2017a.* This study performed the pathology

assessments 7 days after a total cumulative dose of 50 mmol/kg of Omniscan, MultiHance, ProHance and Gadavist.

Bracco (*Bracco Study AB21194*) conducted a study in juvenile rats (30 males and 30 females) exposed to intravenous MultiHance doses up to 15 mmol/kg from 10 days of age (Post-Natal Day, PND) 10 to PND 30. Besides assessment of systemic Gd retention in target organs (liver, skin, femur, kidneys, cerebral cortex, subcortical brain and cerebellum, including the DN), the study was conducted to detect any possible sign of toxicity deriving from exposure to the agent during a 60-day treatment-free period, and to monitor development up to sexual maturity. Particular attention was given to behavioural changes and histopathological examination of different brain areas. Because neonatal/juvenile rats do not have a fully formed BBB, the results of this study are particularly relevant. The following behavioural and neurological tests were performed:

- water maze test on PND 64 and PND 71
- open field test on PND 78 (approximately 11 weeks of age)
- FOB (Functional Observation Battery) on PND 36, at approximately 4 weeks of age and on PND 85, at approximately 12 weeks of age evaluating: grip strength measurement (forelimbs only), gait (forelimb and hind limb assessment), pupillary reflex, auditory reflex.

Brain was sampled according to the to the best practice guidelines of the Society of Toxicologic Pathology (*Bolon et al, 2013*). The brains were sectioned and examined at 6 levels, including cerebral cortex, subcortical nuclei and cerebellum (with a focus on the DN).

No abnormality was ever detected in any of the above experimental animal studies. In particular:

- The clinical evaluation of the experimental animals conducted inside and outside of their cages did not show any neurological disorders, behavioral abnormalities, physiological dysfunctions, and any other signs of nervous system toxicity. During the study in juvenile rats, no changes in spatial learning (water maze test), general locomotor activity (open field test) or grip strength or gait were observed up to 60 days after the end of dosing. No signs of effect on organ maturation or development up to sexual maturity were observed.
- No gross histologic changes were ever observed in hematoxylin-eosin–stained brain tissue samples;
- Electron microscopic examination always revealed a regular tissue architecture with the neurons, glial cells, and focally typical cerebellar structure (granular and Purkinje cells) in animals administered high GBCA doses, and no ultrastructural abnormalities;

Immunohistochemistry with astrocyte (GFAP) and microglia markers (Iba1) and cresyl violet staining of the Nissl substance on consecutive sections, did not reveal any adversity compared with control animals that were administered saline.

3.1.3 Assessment of Neurotoxic Potential from Clinical Data

3.1.3.1 Post-mortem Tissue-Sample Studies

Four post-mortem, tissue-sample studies assessed potential signs of neurotoxicity using structural histopathology assessments (*McDonald et al., 2015; Murata et al., 2016b; McDonald et al., 2017b; McDonald et al., 2017c*). Three studies also assessed ultrastructural changes using TEM-EDX in addition to structural pathology assessments.

The results of these studies can be summarized as follows:

- Histologic examination of brain tissues did not show gross histologic changes between contrast and control groups in hematoxylin-eosin–stained tissue samples examined with visual light microscopy. In one study of pediatric patients, dentate tissue from two patients, one with pontine glioma and one with neuroblastoma who received high cumulative doses of Omniscan had mildly to severely gliotic regions with prominent axonal spheroids. It is unclear whether these pathologic changes are associated with prior external beam radiation therapy or with Omniscan exposure (*McDonald, 2017c*).
- Electron microscopic examination did not show ultrastructural abnormalities. One study showed Gd deposits identified within the nucleus of a neuronal cell in two patient samples (McDonald et al, 2017b).

3.1.3.2 Imaging and Epidemiologic Studies

Most of the imaging studies aimed at assessing presence of abnormal T1 shortening were not designed to determine the correlation between increase in SI in deep brain areas and potential neurotoxicity. The only exceptions were a study by Cao et al. and a study by Forslin et al.

Cao et al. (*Cao et al., 2016b*) conducted a retrospective study aimed at comparing brain T1 signal changes in patients exposed to GBCAs while on chronic hemodialysis to matched control patients with near-normal renal function (eGFR >60 mL/min per 1.73 m2). Because dialysis patients are regularly evaluated by nurses and physicians, typically 3 times per week, there was also an opportunity to search for patterns of clinical events that might be occurring after each GBCA exposure in these patients. An electronic medical records search of 2 large medical centers identified 25 patients who received linear GBCAs while on hemodialysis and had unenhanced T1-weighted images of the brain before and after. T1-weighted SI analysis was performed to detect abnormal T1 shortening in the dentate nucleus (DN) and globus pallidus (GP) in the patients on hemodialysis compared with 25 age/sex/GBCA exposure–matched control patients with normal or near-normal renal function (estimated glomerular filtration rate >60 mL/min per 1.73m^2). Two additional control groups included 13 patients on hemodialysis without GBCA exposure and 13 age/sex-matched patients with estimated glomerular filtration rate greater than 60 mL/min per 1.73 m^2.

The 25 dialysis patients received 44 GBCA administrations including 6 MultiHance, 25 Omniscan, and 13 Magnevist. A greater T1 SI change in the DN (but not in the GP) was observed in dialysis patients receiving GBCAs compared to matched patients with normal or near-normal renal function. These patients were monitored at regular dialysis visits occurring

3 times per week. Despite evidence of abnormal T1 shortening, particularly in the DN, there was no increase or unique pattern of clinical events occurring within 30 days after any of the 44 GBCA exposures in this cohort. In particular, 7 neurological issues described during the 30 days after GBCA administration, including migraine headache, loss of consciousness, memory loss (2 patients), falling, lightheadedness, and ataxia, were also present before GBCA exposure.

Forslin et al. (*Forslin et al., 2017*) conducted a retrospective longitudinal study aimed at investigating the relationship of multiple GBCA administrations with SI increase in the DN and GP, and any association with cognitive function in patients with multiple sclerosis. GBCAs used were Dotarem, Magnevist and Omniscan. A total of 23 patients with multiple sclerosis and 18-year follow-up, and 23 healthy, age-/gender-matched single time point controls underwent one unenhanced MRI scan. T1-weighted SI analysis was performed by placing ROIs in the DN and GP, using pons and thalamus as references, respectively. Patients underwent neurological and neuropsychological evaluations at 3 time points during the study, at baseline, 9-year follow-up, and 18-year follow-up. Physical disability was assessed using the Expanded Disability Status Scale (EDSS). Neuropsychological testing included the following: the Symbol Digit Modalities Test to assess information-processing speed; the F-A-S Test for evaluating phonologic verbal fluency; the Rey–Osterrieth Complex Figure Test-Copy for evaluating visiospatial ability; and the Rey Auditory Verbal Learning Test with encoding and delayed recall at 30 minutes for episodic auditory-verbal memory. The raw test scores were converted to normalized *z*-scores on the basis of age, sex, and educational level.

The results of this study showed that increase in SI in the DN was higher and was observed only among patients with multiple sclerosis compared to controls (1.05 vs. 0.99, $p<0.001$). Multivariate linear regression with corrections for MRI sequence and scanner showed a remaining association with higher SI increase in the DN in patients with multiple sclerosis ($\beta = 0.05$, $p<0.001$). Increase in SI in the DN was also associated with number of exposures to GBCA injections ($\beta = 0.008$, $p<0.001$). No significant association between SI increase and GP was seen. Duration of disease was not associated with changes in SI in either the DN or GP. An increased SI in the DN among patients with multiple sclerosis was associated with lower verbal fluency scores, which remained significant after correction for several aspects of disease severity ($\beta = -0.40$; $P = .013$).

However, a decrease in verbal fluency is common in multiple sclerosis, occurring at all stages of the disease (*Henry and Beatty, 2006*), and its isolated decline retrospectively observed by Forslin et al. in a small cohort of patients could be related more to progression of the disease than to possible Gd retention in the DN.

As a matter of fact, a much larger study did not see any association between multiple doses of Omniscan, i.e., of the agent causing the highest level of Gd retention in brain tissues, and a decline in verbal fluency, or in cognitive function in general.

McDonald et al. (*PRAC Re-Examination Rapporteur's Assessment Report, 2017, Pages 46-52*) recently provided the European Authorities with preliminary results from a post-hoc analysis of data obtained from the clinical database of the Mayo Clinic Study of Aging (MCSA), a large study of the incidence and natural history of normal and abnormal (mild cognitive impairment

and dementia) cognitive decline over time (*Roberts et al, 2008*). While this analysis was not initially conceived to identify the neurologic effects of GBCA exposure, it remained an ideal dataset for such an investigation as it is a prospectively enrolled, population-based cohort with unique linkage to demographic, clinical and vital records data provided from the Rochester Epidemiology Project (REP), a large population-based study of human health (*Melton et al., 1996*).

The MCSA has continuously enrolled patients in a prospective manner since study initiation on October 1, 2004. After enrolment, all patients underwent extensive longitudinal clinical (neurologic evaluation, neuropsychological testing) and imaging (unenhanced MRI and PET/CT) assessment at baseline and 15-month follow-up intervals. In the assessment of possible association between GBCA exposure and cognitive function, MCSA participants were limited to those enrolled between 2004 and 2012 who were cognitively normal at initial screening to: a) maximize available data on outcomes and mitigate confounding bias from exposure to multiple GBCA classes (Omniscan was the only used during that study period), and b) the effects from premature cognitive impairment. After searching the electronic medical record and REP data, patients were segregated into those with no history of prior GBCA exposure (control group) and those who underwent prior Gd-enhanced MRI (Omniscan-exposed group). GBCA exposure in this cohort was independent of MCSA participation and, as a result of MCSA inclusion and exclusion criteria, was not related to an underlying neuropathologic process that could confound these results. All the participants (patients and controls) underwent the clinical, laboratory and imaging tests in Table P.

Table P: Data Elements Included in Mayo Clinic Study on Aging (Source: McDonald et al., 2017d)

Clinical Metrics	Laboratory Tests	Imaging Studies
Psychometric Summary	Apo E genotyping	Unenhanced Brain MRI
Financial Capacity Instrument - Short Form MCI	CSF	PIB-PET Imaging
The Brief Smell Identification Test		Tau-PET Imaging
Blessed Memory Test		
MCSA Interview/Risk Factor Assessment (Baseline & F/U)		
BDI II (Beck Depression Inventory)		
BAI (Beck Anxiety Inventory)		
Clinical Dementia Rating - Interview		
Clinical Dementia Rating Scoring		
Neuropsychiatric Inventory Questionnaire		
Everyday Cognition Index (ECog)		
Mayo Sleep Questionnaire		
Physical and Cognitive Activities Questionnaire		
Telephone Instrument for Cognitive Status - Modified		
Neurologic Exam and Modified UPDRS Q 1-23		
Neurologic Exam and Modified UPDRS Q 24 + Hachinski		
Short Test of Mental Status		
GAITRite		
Computerized Testing		
Food Frequency Questionnaire		
Montreal Cognitive Assessment		

Neurologic and neurocognitive scores from the MCSA from Table P were compared between study participants with no history of prior Omniscan exposure (control group) and those who underwent prior Omniscan-enhanced MRI (Omniscan-exposed group), using standard multivariate regression models. In these models, neurologic and neurocognitive performance scores were expressed as changes in performance over the observation period (most recent evaluation minus the initial clinical evaluation) and were controlled by accounting for baseline neurologic and neurocognitive performance scores. Progression from normal cognitive status to mild cognitive impairment or dementia were assessed using Cox-Proportional Hazard and multi-state Markov model analysis. In both the Cox and Markov hazard-based models, age was used as the time variable and all models were adjusted for sex, years of education, and other variables (e.g., gender, race, body mass index, smoking history, alcohol use, history of diabetes, head trauma, stroke, cardiac arrhythmia, hypertension, etc.). The Schoenfeld method was used to estimate statistical power to detect differences between groups.

Among 4261 cognitively normal study participants aged 50-89, 1092 patients (25.6%) received one or more Omniscan doses (mean (SD): 3 doses (2.1); median (IQR): 2 doses (1-4); range: 1-28 doses) unrelated to their participation in the MCSA. The Omniscan exposed group was followed prospectively for a total of 7,104 person years and had a total of 83,119 person-years of retrospective clinical data available from the REP. Similarly, the control group was followed prospectively for a total of 16,078 person years with a total of 236,384 person years of

retrospective clinical data available from the REP. For all study participants, the mean age at the time of enrolment was 71.9 yrs (SD = 10.7). Mean study participation duration for the entire cohort was 3.7 yrs (SD = 2.6). In the Omniscan exposed groups, the mean time since first Omniscan exposure was 6.1 years (SD = 3.7) with a median time of 5.6 years (IQR=2.2-9.3 years). At the time of initial study recruitment, Omniscan-exposed patients had slightly higher comorbid burden and had slightly lower normalized *z*-scores in their performance on language and visual tasks.

After adjusting for over 20 demographic and clinical variables including age, sex, education level, baseline neurocognitive performance, Charlson comorbidity index, and ApoE4 status, Omniscan exposure was not a significant predictor of excess cognitive decline when compared to control populations with respect to changes in mental status exam score (p=.16), normalized neuropsychological memory, language, attention, and visual task test performance (p=.58 - .96), or motor performance (Unified Parkinson's Disease Rating Scale (p=.22); Table Q. No dose-related effects were observed among these metrics (p=.20-.89).

Table Q: Multivariate Analysis of the Effects of Omniscan Exposure on Neurologic Outcomes

Neurologic Outcomes	Odds Ratio (95% CI)	P-value
Mini-mental status exam	0.95 (0.89-1.04)	.16
Memory Z score	1.04 (0.97-1.12)	.58
Language Z score:	1.01 (0.98-1.05)	.96
Attention Z score	0.97 (0.92-1.02)	.79
Visual Z score:	1.02 (0.98–1.05)	.80
Dementia (UPDRS) score:	1.01 (0.96-1.07)	.22

A total of 670 (16%) of the 4261 participants who were initially cognitively normal at the time of enrolment progressed to mild cognitive impairment during the study timeframe. The incidence of progression to cognitive decline in the Omniscan-exposed group, standardized by age, sex, and education level to the Olmsted County population, was 29.1 per 1,000 person-years (95% CI: 25.7-31.2) while the control group was 27.6 per 1,000 person-years (95% CI: 24.9-30.4). After adjusting for age, sex, education level, and other covariates, neither Omniscan exposure (HR = 1.02 (95% CI: 0.95-1.20), p = .77) nor cumulative lifetime Omniscan dose (HR = 0.99 (95% CI: 0.95-1.08), p = .85) was associated with significant excess risk of conversion to cognitive impairment in the study population. With the sample size and event rates described above assuming alpha = .05, hazard ratios of 1.09 or higher could be detected with 80% power, and we had at least 99.9% power to detect moderate and larger effect sizes (hazard ratios greater than 1.5).

Welk et al. (*Welk et al., 2016*) conducted a population-based study to assess the association between Gd exposure and parkinsonism using multiple and linked administrative databases from Ontario, Canada. Using fee codes submitted by radiologists, all patients who underwent an initial

magnetic resonance imaging (MRI) exam between April 2003 and March 2013 were identified. Patients who were exposed to GBCA-enhanced MRIs, modeled as a time-varying, cumulative count variable, were compared with patients who received unenhanced MRIs and were never exposed to GBCAs.

The primary outcome, assessed from the initial MRI until death, emigration, or March 2015, was a new diagnosis of parkinsonism based on a validated definition (sensitivity, 81.7%; specificity, 99.7%; positive predictive value, 78.0%; negative predictive value, 99.8%; accuracy, 99.5%; and disease prevalence, 1.4%) using diagnosis codes from hospital admissions and physician visits or a dispensed Parkinson disease–specific medication (*Butt et al., 2014*). Since medication coverage is provided in Ontario, Canada for those older than 65 years, only patients older than 66 years were included in the study. At the same time, to avoid confounding factors, patients who underwent MRI of the central nervous system (CNS), neurosurgery or had pre-existing Parkinson's disease or parkinsonism were excluded.

Comparability of the two study groups was tested using 105 covariates. A subset of 38 covariates that were particularly relevant to parkinsonism (based on associations reported in the literature) or were significantly different at baseline (standardized difference >10%) were included in a multivariable time-dependent extended Cox regression model using SAS (SAS Institute), version 9.4. The hazard ratio (HR) was interpreted as the hazard of parkinsonism per additional exposure to GBCAs. Sensitivity analyses adjusting for covariates with standardized differences of 9% or 10% and defining parkinsonism without medications were performed. A 2-sided P value less than 0.05 was considered significant.

Of the 246,557 patients (median age, 73 years [interquartile range, 69-78]; women, 54.9%) undergoing at least 1 non-CNS MRI during the study period, 146,818 (59,5%) underwent 1 or more unenhanced MRI and 99,739 (40.5%) received at least 1 GBCA dose. Among patients who underwent contrast-enhanced MRIs, 81,827 (81.5%) were exposed to a single GBCA injection, 11,278 (15.8%) to 2-3 injections, and 6,634 to 4 or more injections.

Incident parkinsonism developed in 1.16% of unexposed patients and in 1.17% of those exposed to GBCAs. In adjusted analysis, there was no significantly increased hazard of parkinsonism among patients with cumulative exposure to GBCAs compared with those never exposed to the agents (HR, 1.04 [95% CI, 0.98-1.09], $P = .18$). No significantly increased HR was found in either sensitivity analysis. Of note, the incidence rate of parkinsonism was lower (0.70%) in the cohort of patients exposed 4 or more times to GBCAs. More detailed results are reported in Figure 11 below (reporting Table 2 from the original publication).

Figure 11. Results of Study by Welk et al.

Table 2. New Diagnoses of Parkinsonism After MRIs (Not of the Brain or Spine) With or Without Gadolinium Exposure

Primary Analysis	Entire Cohort (N = 246 557)	Exposed to Only Non-Gadolinium-Enhanced MRIs (n = 146 818)	Exposed to Gadolinium-Enhanced MRIs		HR (95% CI)	*P* Value
			≥1 MRI (n = 99 739)	≥4 MRIs[a] (n = 2446)		
Total follow-up, person-years	991 937	625 185	366 752	6634		
Primary outcome, No. (%)	2861 (1.16)	1697 (1.16)	1164 (1.17)	17 (0.70)		
Rate (95% CI)[b]	2.88 (2.78-2.99)	2.71 (2.59-2.84)	3.17 (2.99-3.36)	2.56 (1.54-4.02)		
Unadjusted analysis[c]		Reference			1.08 (1.04-1.13)	<.001
Adjusted analysis[d]		Reference			1.04 (0.98-1.09)	.18
Sensitivity analysis						
Post hoc analysis 1[e]		Reference			0.99 (0.94-1.03)	.58
Post hoc analysis 2[f]		Reference			1.03 (0.98-1.09)	.29

Abbreviations: HR, hazard ratio; MRI, magnetic resonance imaging; SSRIs, selective serotonin reuptake inhibitors.

[a] Patients exposed to 4 or more MRIs with gadolinium are a subset of those who are exposed to 1 or more MRIs with gadolinium.

[b] Per 1000 person-years of observation.

[c] HR per additional gadolinium exposure.

[d] Adjusted analysis used the same statistical model and included 38 covariates selected from the 105 measured covariates that were either potential confounders or unbalanced at baseline (standardized difference, >10%). Specific covariates included demographics (age, sex, year of cohort entry, MRI study body part), comorbid conditions (dementia, stroke, solid organ cancer [bowel, lung, breast, prostate, rectal], melanoma, seizure, comorbidity score, congestive heart failure, coronary artery disease, hypertension, chronic liver disease, chronic kidney disease), medications (antipsychotics, atypical antipsychotics, antidepressants, cholinesterase inhibitors, SSRIs, anticholinergics, androgen deprivation therapy, antiplatelets, β-blockers, calcium-channel blockers, angiotensin-converting enzyme inhibitors, corticosteroids, total number of unique drug names), and health care utilization (number of hospitalizations, number of neurology visits, computed tomography of the head, echocardiogram, Holter monitor, carotid ultrasound).

[e] Post hoc analysis 1 had further adjustment for 12 additional covariates with standardized differences of 9% or 10%: atrial fibrillation or flutter, peripheral vascular disease, antineoplastic agents, narcotics, non–potassium-sparing diuretics, number of family physician visits, bone scan, cardiac catheterization, cardiac stress test, prior spine MRI, prior urine culture, prior chest x-ray.

[f] Post hoc analysis 2 was an adjusted analysis using an outcome definition independent of parkinsonism medications.[4]

Source: Original data from Table 2 in Welk et al

3.1.4 Conclusions on Potential Effects of Gd Retention in Brain Tissues

The review of acute neurotoxicity data leads to the conclusion that experimental animal studies specifically designed to assess (or exclude) acute neurotoxicity (clinical signs and histopathological lesions) after single, very high intrathecal doses are of little relevance in relation to long-term retention effects and possible chronic toxicity that may occur in humans following possible long-term retention of very small levels of Gd complexes in brain tissue following single or multiple intravenous doses of GBCAs. Data from these studies may be relevant to predict neurotoxic effects of intrathecal injection of GBCAs when used off-label for MR myelography or cisternography for presurgical localization of cerebrospinal fluid leaks. Following intrathecal doses of 0.5 to 1.0 mL, usually minor adverse reactions are reported (headache most commonly), and more severe/serious adverse events, e.g., transient hemiparesis or gait disturbance, are infrequent. However, inadvertent injection of significantly higher doses may lead to diffuse brain function impairment usually identified as “gadolinium encephalopathy”. The neurological disorders of gadolinium encephalopathy develop in hours following the intrathecal GBCA overdose, and resemble neurological abnormalities observed in preclinical studies (*Artl et al., 2007; Kapoor et al., 2010; Park et al., 2010*).

The experimental animal studies aimed at determining the potential correlation between chronic Gd retention in brain tissues and neurological changes did not detect any sign of neurotoxicity,

neurological disorders, behavioral abnormalities, physiological dysfunctions, and any other signs of nervous system toxicity.

Histologic examination of human brain tissues did not show gross histologic changes between contrast and control groups in hematoxylin-eosin–stained tissue samples examined with visual light microscopy nor statistical difference in number of glial cells and neurons between exposed patients and controls that would suggest reactive changes. Severely gliotic regions with prominent axonal spheroids observed in two pediatric patients could be related to prior external beam radiation therapy. Electron microscopic examination did not show ultrastructural abnormalities.

The population study by Welk et al. (*Welk et al., 2016*) seems to exclude an association between exposure to GBCAs and development of parkinsonism. This is of particular relevance because studies aimed at detecting and measuring elemental Gd in brain tissues following exposure to GBCAs showed that the highest levels of the metal were in the DN and in the GP (*Kanda et al., 2015; McDonald et al., 2015; Murata et al, 2016; McDonald et al., 2017b; McDonald et al, 2017c*), and GP lesions are typically associated with motor deficits, including parkinsonism, tremor, and dystonia (*Bhatia et al., 1994; Pal et al., 1999; Münchau et al., 2000*). Therefore, the results of this study do not support the hypothesis that Gd deposits in the GP may lead to neuronal damage manifesting as parkinsonism.

Dysfunction of the DN has been shown to result in deficits in coordination (planning and initiation) of limb movement while dysfunction of the basal ganglia has been shown to result in deficits in learning and memory, coordination, balance, and has been shown to be involved in anxiety and mood disorders (*Krack et al., 2010*). Such neurologic deficits, if present, could be easily detected using standard neurologic and neuropsychological testing performed in the MCSA. However, based upon the preliminary data from the analysis by McDonald et al. (*PRAC Re-Examination Rapporteur's Assessment Report, 2017, Pages 46-52*), single or repeated administrations of Omniscan administration did not appear to be associated with worse overall neurologic or neurocognitive performance, nor did GBCA exposure significantly affect the natural progression of cognitive decline in this large population-based cohort.

In conclusion, the available evidence seems to exclude that brain retention of Gd complexes is associated with harmful effects and potential interaction with neurological disease processes.

3.2 Assessment of Potential Effects of Gd Retention in Body Tissues

3.2.1 Nonclinical Evidence

While most nonclinical publications are focused on Gd retention in tissues, a few reports describe the results of histologic assessment of several tissues, with emphasis on the skin. The available evidence can be summarized as follows:

- In healthy rats histologic evidence of skin lesions have been observed only in animals administered Omniscan or OptiMARK, but not with other GBCAs in a series of studies conducted by the Sponsor Bayer (*Sieber et al., 2008a; Sieber et al., 2008b; Sieber et al., 2008c; Pietsch et al., 2009b; Pietsch et al., 2009c; Steger-Hartmann et al., 2009; Lohrke et*

al., 2017; Pietsch et al., 2011). The observed skin changes were characterized by dermal fibrosis and infiltration of different cells, including mononuclear cells and CD34-positive cells. The results of the studies conducted by Bayer were confirmed by a study by Sato et al. (*Sato et al. 2013*), in which macroscopic changes of the skin, such as reddening, scab formation and ulceration, were observed in animals treated with Omniscan but in those treated with ProHance or a lower dose of Eovist.

- The severity of the observed skin lesions caused by solutions of gadodiamide and gadoversetamide (active ingredients of Omniscan and OptiMARK) were dependent on the amount of excess ligand in solution (*Sieber et al., 2008c*).
- Shorter injection intervals resulted in more severe skin reactions in animals treated with a formulation of gadodiamide without excess ligand (*Pietsch et al. 2011*). In contrast, the injection interval did not influence the long-term presence and level of Gd concentration in skin.
- Fretellier et al. (*Fretellier et al., 2012*) demonstrated that hyperphosphatemia induced in partially nephrectomized rats by a high phosphate diet increased the frequency and severity of Omniscan-induced skin lesions.
- The skin lesions in rats were not completely consistent with histopathology of NSF in humans. Some of the difference between the cutaneous changes described in the Omniscan-treated healthy rats and human NSF are possibly due to anatomical differences, such as the presence of the panniculus carnosus in rats, a structure that separates the dermis from the subcutis and explains the fact that the lesions are restricted to the dermis in this animal species (*Sieber et al., 2009*).
- One study (*Steger-Hartmann et al., 2009*) showed elevation of several proinflammatory cytokines in the serum of Omniscan-treated rats, suggesting the involvement of inflammatory pathways in the pathogenesis of skin damage.
- In one study (*Grant et al., 2009*), naïve and partially nephrectomized rats administered a solution of gadodiamide (active ingredient of Omniscan) without excess ligand and the commercially available formulation of Omniscan showed marked skin lesions, characterized by epidermal hyperplasia and hyperkeratosis, crusting and ulceration, and dermal infiltration by acute and chronic inflammatory cells, mast cells and mineralization. No fibrosis was demonstrated in these animals. There were no cutaneous lesions in animals treated with Magnevist or different Gd salts. In addition, no difference was observed between naïve and nephrectomized rats. The authors concluded that the superficial skin changes were likely caused by trauma (scratching) due to severe pruritus. On the other hand, other studies (*Haylor et al., 2010; Sato et al., 2013)* confirmed dermal fibrosis in partially nephrectomized rats.
- One study confirmed the fibrotic process using special stains for CD34 expression, collagen deposition, and prolyl-4-hydroxylase (*Haylor et al., 2010*). Dermal fibrosis was not observed following equivalent doses of Dotarem. The injection of gadodiamide was followed by a rapid and transient induction of signaling molecules in the serum (MCP1,

MCP3, IL1, IP-10, Osteopontine SCF and Timp1), as well as in the skin (MCP1 and TGF-beta).

- In the study by Sato et al. (*Sato et al., 2013*), using Masson's trichrome staining of skin samples, increased cellularity was observed in animals exposed to Omniscan, with CD34-positive expression in mesenchymal spindle-shaped cells (most likely fibroblasts), whereas no significant changes were found in the Eovist or ProHance groups compared with controls. No increase in collagen fibers was observed in any animal group in this study.

3.2.2 Clinical Evidence

Retention of Gd complexes in body tissues has been associated with several clinical conditions, namely nephrogenic systemic fibrosis and, more recently, non-NSF delayed adverse events.

3.2.2.1 Nephrogenic Systemic Fibrosis (NSF)

NSF has been reported as a delayed adverse reaction to GBCAs, and is the most significant clinical syndrome associated with retention of Gd complexes in body tissues. NSF cases have been observed weeks, months or even years after the administration of certain GBCAs. It has been observed only in patients with acute or chronic severe renal insufficiency (estimated glomerular filtration rate, eGFR <30 mL/min/1.73 m^2), end-stage renal disease, or with acute renal insufficiency of any severity due to hepatorenal syndrome or in the perioperative liver transplantation period (*ACR, 2017c*).

It has been suggested that cumulative lifetime GBCA exposure increases the risk of NSF (*Othersen et al., 2007*). However, GBCA exposure and renal insufficiency cannot be the sole effectors of NSF; the vast majority of patients with end-stage renal disease on chronic dialysis do not acquire the disease, even following multiple exposure to the GBCAs that have been associated with the highest number of NSF cases. Also, a few cases of NSF have been reported in patients with no history of GBCA exposure (*Wahba et al., 2007; Anavekar et al., 2008; Lemy et al., 2010*).

No cases of NSF could be identified before 1997 (*Galan et al, 2006*). This truly new disease entity should have then resulted from exposure of patients with advanced renal failure to one or more new exogenous agents, e.g., a new medication, toxin or infectious agent, or new ways of using previously existing medications (*Shellock and Spinazzi, 2008*). The first suspects were exposure to high-dose erythropoietin and lack of angiotensin converting enzyme inhibitor therapy, in the presence of co-factors such as hypercoaguable states, various forms of vascular injury, vascular surgical procedures, and liver failure, in particular hepatorenal syndrome and liver transplantation (*Shellock and Spinazzi, 2008*).

Since January 2006, when the study by Grobner was published (*Grobner T., 2006*), GBCAs became the prime suspect, even if GBCAs had been available for clinical use since the late 1980's, i.e., at least 10 years before the first cases of NSF were identified. The working hypothesis is that Gd complexes may stay for weeks, months or even years within the skin and other tissues. In the skin of patients with advanced renal failure, the Gd complexes would attract and/or activate circulating fibrocytes, bone marrow-derived cells that participate in normal

wound healing and fibrosis and are believed to underlie aberrant fibrosis in NSF (*Wagner et al., 2016*). These cells are distinct from other fibrocytes in that they have a specific immunophenotype, i.e., the CD34⁄ procollagen I dual positive profile (*Wagner et al., 2016*). As a matter of fact, histology of the skin of patients with NSF shows an increase in cellularity, with CD34- and procollagen I-expressing spindle cells, occasional histiocytes, and factor XIIIa^{+} dendritic cells, and the higher levels of retained Gd have been observed in areas of increased cellularity (*Wagner et al., 2016*).

NSF cases occurring after the sole administration of one GBCA are defined as "single-agent" or "unconfounded." If a case of NSF follows the administration of two or more agents, it is more difficult to determine which agent is associated with the development of the disorder, and the case is reported as "multiple-agent" or "confounded". (*Shellock and Spinazzi, 2008*)

Monitoring of NSF based on spontaneous reporting of adverse drug reactions and literature case reports may lead to underreporting of NSF cases. Missing or inconclusive information in spontaneously reported cases is also a limitation of the monitoring of NSF, as for some reports it may not be possible to confirm or exclude a diagnosis of NSF using the clinicopathological criteria developed by Girardi et al (*Spinazzi et al., 2009; Girardi et al., 2011*). However, clinical data deriving from studies of individual GBCAs in high-risk patients, with prolonged and extensive follow-up and systematic collection of all the clinical and dermopathological information that is needed to make a diagnosis of NSF, overcome the limitations of spontaneous reporting of NSF cases and provide better information on NSF risk deriving from exposure to individual GBCAs, providing the patient population is large enough to draw meaningful conclusions.

This section will review the number of single-agent NSF cases (Table R) from the literature case reports, clinical investigations of the rate of single-agent, unconfounded NSF cases from clinical studies from a pool of 16 peer-reviewed publications *(Todd DJ, 2007; Reilly et al., 2008; Rydahl et al., 2008; Abujudeh et al., 2009; Altun et al., 2009; Bryant et al., 2009; Martin et al., 2010; Elmholdt et al., 2011; Wang Y et al., 2011; Amet et al., 2013; Lauenstein et al., 2015; Nandwana et al., 2015; Soulez et al., 2015; Bruce et al., 2016; Martin et al., 2017; Michaely et al., 2017).*

Table R reports the number of NSF cases from clinical studies of patients at high risk for the development of NSF, i.e., patients with end-stage renal disease or eGFR <30 mL/min/1.73 m^2, and/or during the perioperative liver transplantation period.

Despite the extensive testing in clinical studies, with prolonged follow-up of 8,486 patients at high risk, no cases of NSF have been observed following exposure to MultiHance. The number of high-risk patients in the MultiHance clinical studies was more than twice the number of high-risk patients in all the studies of the other GBCAs, and more than nine times higher than the number of high-risk patients in all the studies of the macrocyclic GBCAs taken together. No NSF cases were observed following single-agent exposure to Dotarem, Eovist, Gadavist, or ProHance, while the rate was 2.4% for Magnevist and 4.7% for Omniscan. The upper bound of 95% confidence interval (CI) was computed using Clopper-Pearson exact method because of the observed zero count of NSF for some GBCAs (*Clopper-Pearson et al., 1934*) and an improved normal approximation Wilson Score Interval method (*Wilson et al., 1927*). The results from both

methods were consistent and very close to each other because of sufficiently large sample size (per central limit theorem, n≥30 required) for each of the GBCAs.

Comparisons of upper bounds across GBCAs provides insight regarding the likelihood of NSF. With a relatively large number of patients exposed, the upper bound of 95% CI for Magnevist and Omniscan were 4.1713% and 5.8506%, respectively, indicating higher risk of NSF compared to other GBCAs. For MultiHance, no cases of NSF were observed among 8,486 patients, resulting in a very small upper bound (0.0435%), MultiHance is the least likely to be associated with single-agent, unconfounded cases of NSF among all the other GBCAs.

Of note, while the observed counts were zero for all GBCAs except Magnevist and Omniscan, upper bound of CI was dependent on the size of the denominator. Even with zero count for NSF, upper bound of confidence interval will be larger for those GBCAs (Eovist 0/85, ProHance 0/153, Gadavist 0/284, Dotarem 0/502) with sample size smaller than MultiHance (0/8,486).

Table R: Proportion of Single-Agent NSF Cases from Clinical Investigations in High-Risk Patients

GBCA	Incidence of NSF (N of NSF cases / N of high-risk patients [a])	Upper Bound of 95% Confidence Interval (Clopper-Pearson Exact Method)	Upper Bound of 95% Confidence Interval from Wilson Score Interval Approximate Method
MultiHance	0/8486 [b]	0.0435%	0.0452%
Dotarem	0/502 [c]	0.7321%	0.7594%
Gadavist	0/284 [f]	1.2905%	1.3346%
ProHance	0/153 [e]	2.3822%	2.4493%
Eovist	0/85 [d]	4.2470%	4.3239%
Optimark	NA	NA	NA
Magnevist	12/498 [g] (2.4%)	4.1713%	4.1642%
Omniscan	79/1673 [h] (4.7%)	5.8506%	5.8463%

NA, not available
[a] Patients with end-stage renal disease or eGFR <30 mL/min/1.73 m^2, and/or during the perioperative liver transplantation period
[b] Data from: Bryant et al., 2009; Abujudeh et al., 2009; Altun et al, 2009; Martin et al., 2010; Wang et al., 2011; Soulez et al., 2015; Nandwana et al., 2015; Bruce et al., 2016; Martin et al., 2017.
[c] Data from: Elmholdt et al., 2011; Amet et al., 2013.
[d] Data from: Lauenstein et al., 2015.
[e] Data from: Soulez et al., 2015; Reilly et al.. 2008.
[f] Data from: Michaely et al., 2017.
[g] Data from: Todd et al., 2007; Wang et al., 2011.
[h] Data from: Bruce et al., 2016; Rydahl et al., 2008; Altun et al., 2009

3.2.2.2 Non-NSF Delayed Adverse Events

With the term "non-NSF delayed adverse events", the Sponsor refers to any adverse event that does not occur within the first hour of the GBCA administration, and reported signs and

symptoms are suggested to be associated with Gd retention in tissues, but the condition does not meet the clinical and pathological criteria used to establish a diagnosis of NSF developed by a group of experts (*Giradi et al, 2011*).

Included in the definition of non-NSF adverse events are several conditions that have been described in the last 5 years, such as "gadolinium-associated plaques", "gadolinium-deposition disease" and a case report of late-onset joint contractures with no signs of NSF in a patient with normal renal function exposed to a high cumulative GBCA dose.

3.2.2.2.1 Gadolinium-associated Plaques

"Gadolinium-associated plaques" is the proposed name of an entity first described in 2013, for a total of 3 case reports between the first report and 2015 (*Bhawan et al., 2013; Gathings et al., 2015*). Eosinophilic, collagenous, round, or ovoid sclerotic bodies in various stages of calcification are the pathognomonic histopathologic feature of the condition. The sclerotic bodies were observed in the dermis of patients exposed to GBCAs and with no signs of NSF. Patients presented with 0.5-2.5 cm plaques that resembled dermal infiltrative processes such as granuloma annulare or cutaneous sarcoidosis in two cases (*Gathings et al., 2015*); in one case, it was an accidental finding in excisional specimens obtained during surgical removal of squamous cell carcinoma (*Bhawan et al., 2013*).

Renal function was impaired in two patients and normal in the remaining one. Two cases have been reported after administration of Omniscan (total cumulative volume: 90 mL in one case, 100 mL in the other), and one following exposure to an unknown dose of an unknown GBCA. The interval between last exposure and onset of the skin lesions ranged between 3.5 and 5 years. Plaques were asymptomatic in two cases, and pruritic and burning in one.

The association between the observed skin lesions and exposure to GBCAs was suggested because sclerotic bodies and osseous metaplasia have been described as a late finding in the course of NSF, and the observed plaques were seen in patients exposed to a GBCA (Omniscan) known to be associated with NSF. The diagnosis of NSF was ruled out, however, as no other clinical or pathological sign of the disease was observed.

Of note, these skin lesions were never associated with the administration of MultiHance or ProHance.

3.2.2.2.2 Gadolinium-Deposition Disease

Semelka et al. *(Semelka et al., 2016a)* proposed to use the term "gadolinium storage condition" for the demonstrated retention of Gd complexes in the brain or body tissues in patients with impaired or normal renal function, when no concomitant clinical syndromes are present and while clinical implications are not understood. The authors also proposed the term "gadolinium deposition disease" for a condition observed in subjects with normal or near-normal renal function who develop persistent symptoms that arise hours to 2 months after the administration of GBCAs. Typical clinical symptoms of this "gadolinium deposition disease" reportedly include persistent headache and bone and joint pain. Patients often complain of clouded mentation that many describe as a "brain fog." More distinctive features are comparable with those observed in NSF but of lesser severity; patients may experience subcutaneous soft-tissue thickening that

clinically appears somewhat spongy or rubbery without the hardness and redness observed in NSF. Tendons and ligaments in a comparable distribution may also be painful and have a thickened appearance. Patients may complain of tightness of the hands and feet that resembles the feeling of being fitted with extremely tight gloves or socks. Patients may experience excruciating pain, typically in a distal distribution, of the arms and legs but that may also be in the torso or generalized in location. This pain is often described as feeling like sharp pins and needles, cutting, or burning.

Symptoms of the "gadolinium deposition disease" would occur only in some subjects exposed to GBCAs. The authors imply that: a) the "gadolinium storage condition" may evolve into "gadolinium deposition disease"; b) "gadolinium deposition disease" may occur in the presence of host factors such as genetic susceptibility and/or adaptive immune response; c) the contribution of an immune response may make this condition observed with either macrocyclic or linear GBCAs, i.e., independent of the level of Gd complexes retained in tissues. However, possible predisposing conditions or risk factors have not been identified.

Semelka et al. also reported four cases of patients who experienced adverse events after exposure to GBCAs (*Semelka et al., 2016b*). These four patients developed symptoms with an onset time from between hours to 4 weeks after exposure. Two patients presented months after receiving GBCAs (Patient 2 months and Patient 3 months) and two presented years after first exposure (Patient 7 years and Patient 8 years).

- Patient 2 months received one dose of Magnevist, and experienced symptoms 24 hours after administration. The symptoms were 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. Gadolinium was present in serum at 0.7 ng/mL (normal range: <0.5 ng/mL) 31 days after the exposure, and in a 24-hour urine sample at 18 μg/24 hours (normal range: 0–0.4 μg/24 hours) 33 days after the exposure.
- Patient 3 months with a history of Guillain-Barre' syndrome received Optimark, Eovist (Eovist in Europe), and 2 doses of Gadavist. The day after the final administration of Gadavist, the patient reported nonpitting oedema in the injection site and ipsilateral hand which progressed over several weeks to involve the face, both arms and hands, feet, legs, and torso, preceded by tingling neurological pain. The patient also experienced diminished memory, fatigue and subjective muscle weakness. The patient showed subcutaneous lesions, skin tightness, and shiny appearance of skin over fingers 3.5 months after the last MRI. Total gadolinium in urine per 24 hours was measured. At 28 days after the last exposure, this was 82 μg/24 hours, this declined to 3.3 μg/24 hours at 103 after the last exposure (reference values: 0.0-0.4 μg/24 hours).
- Patient 7 years received one dose of MultiHance. Within 24 hours of MultiHance administration, the patient complained of intense pain in the torso, peripheral arm and legs that she describes as the feeling of being "on fire." Pain described as excruciating persisted for 4 months with slight relief afterward. Other signs and symptoms included rash, nausea, burning bladder, headache, and spikes of high blood pressure. Clouded mentation and disorientation as well as a metallic taste that persisted for 3 months after the examination were also reported. Quantitative analysis 4 years after GBCA exposure detected

gadolinium in hair at a concentration of 0.0007 µg/g (normal ≤0.0005) and in urine at 0.0644 µg/g (normal≤0.019). Seven years after GBCA administration, the most striking features were mild skin discoloration of the hands with tightness sensation in webs between fingers.

- Patient 8 years received 5 doses of Omniscan and 1 dose of 0.05 mmol/kg MultiHance. Two to three weeks after the last GBCA administration she experienced intense pain involving a distribution from the forearms to the hands, feet, and toes with a sensation that felt like a sharp knife in her skin. Within 2 to 3 months of the initial pain sensation, she could no longer wear her wedding ring because of expansion of her hand subcutaneous tissue, and redness and swelling appeared in the distal arms and legs. Eight years after the last MRI, the skin and subcutaneous tissues of her hands and feet were thickened and red and had doughy consistency. Gadolinium was detected in a section of vein from a saphenous vein resection 8 years after the last GBCA exposure, at a concentration of 4.4 pg/g of tissue.

The authors noted some consistency in the symptoms that are reported, noting that "glove-and-sock" pain occurred in all cases, torso pain in three, skin thickening in two, and cognitive symptoms in two. Pain in the extremities, torso pain, and skin thickening can all occur in NSF. However, a diagnosis of NSF was ruled out in all four cases by a physician experienced in NSF.

3.2.2.2.3 Case Report of Joint Contractures in a Patient with Normal Renal Function

Roberts et al. (*Roberts et al., 2016)* reported a case of a patient with a diagnosis of glioblastoma, who underwent surgery, chemotherapy and radiation treatment, and a total of 61 contrast-enhanced MRI examinations with use of different GBCAs in 11 years (Magnevist, Omniscan, ProHance, and MultiHance). This case was previously mentioned in Section 2.2.2.2 of this document. During the 11-year period, 167 renal function tests were performed, and the eGFR was reported as >59 mL/min/1.73 m^2 at all times.

Following an episode of cholecystitis and subsequent cholecystectomy, the patient developed severe progressive generalized contractures not explainable by his brain tumor or prior treatments. Although no external skin changes of NSF were present (no complaints of rashes, pruritus, sclerosis of the skin, no superficial plaque, woody induration, peau d'orange, hyperpigmentation, or violaceous coloration involving any skin surfaces), he underwent skin biopsy from the forearm and lower extremity. The skin biopsy samples were formalin fixed and paraffin embedded. They included epidermis, dermis, and subcutaneous adipose tissue. Three 3-µm-thick formalin-fixed and paraffin-embedded sections from each site were stained with hematoxylin and eosin and antibodies against CD34. Eight-micron-thick unstained sections were sent for gadolinium analysis and were analyzed with ICP-MS, LA- ICP-MS, and HILIC-ICP-MS. The ICP-MS demonstrated high levels of Gd retention (14.5 ± 0.4 µg/g). Review of histologic sections revealed normal skin and subcutis. There was no evidence of increased dermal cellularity, fibrosis, abnormal collagen bundles, osseous metaplasia, or abnormal numbers of fibroblast-like cells or macrophages.

While no typical clinical or histopathological signs of NSF were present, there was increased CD34 immunoreactivity in the connective tissue septations of the subcutaneous adipose tissue,

suggesting inflammation and/or tissue injury. Similar to previous findings in patients with NSF, LA-ICP-MS demonstrated deposition of Gd within the deep layers of the subcutis closely associated with the connective tissue septations of the subcutaneous adipose tissue.

3.2.2.3 Conclusions on Potential Effects of Gd Retention in Body Tissues

NSF

To date, NSF is the most significant clinical syndrome associated with retention of Gd complexes in body tissues, even if the exact pathogenesis of NSF is still not known, nor how GBCAs can have a role in the development of the disease.

Data from clinical studies in high-risk patients are not consistent with the dissociation kinetics of the various GBCAs. However, clinical data seem more consistent with Gd retention data from animal studies:

- The highest rate of NSF cases has been observed following exposure to Magnevist and, especially, Omniscan; both agents were associated with significantly higher levels of Gd retained in skin tissues. Omniscan and OptiMARK were also shown to be associated with proinflammatory and profibrotic effects as well as skin lesions partially resembling NSF in animal studies. Similar skin lesions were not observed following exposure of the other GBCAs;
- MultiHance was associated with markedly lower levels of Gd complexes retained in skin compared with Omniscan and Magnevist. Comparisons with the macrocyclic agents Dotarem, Gadavist and ProHance showed slightly higher levels following MultiHance in adult rats, and similar levels in juvenile animals. The available clinical evidence shows no cases of NSF in high-risk patients exposed to MultiHance, and that this agent is the least likely to be associated with single-agent, unconfounded cases of NSF among all the other GBCAs.
- No cases of NSF were observed in any of the clinical studies of Dotarem, Eovist, Gadavist, and ProHance, even if in a more limited number of high-risk patients.

It is clear that GBCA exposure and renal insufficiency cannot be the sole effectors of NSF, as the vast majority of patients with end-stage renal disease on chronic dialysis do not acquire the disease, even following multiple exposure to Omniscan. It is important to note that important and effective risk minimization measures were introduced in 2010 (*FDA, 2010*), and, to our knowledge, no new cases of NSF have been reported since.

Non-NSF Delayed Adverse Events

Non-NSF delayed adverse events include case reports of skin changes and/or symptoms that have been associated with Gd retention in tissues.

The three cases of gadolinium-associated plaques were characterized by skin lesions (presence of sclerotic bodies and osseous metaplasia) known to be a late histopathological change (7-8 years after exposure to GBCAs) observed in patients with NSF. Strength of these cases is that the cutaneous lesions have been detected and assessed by physicians experienced with

histopathologic signs of NSF, and that all patients had been exposed to GBCAs 3.5 to 5 years before development of the condition. However, only 3 cases have been reported to date, and all cases were non-serious: in two cases, the observed skin changes were asymptomatic, in one case they were pruritic. It is difficult to draw conclusions about the potential association of sclerotic bodies and exposure to GBCAs; of note, similar lesions were never observed in animal experiments. Finally, the Sponsor is not aware of any cases of gadolinium-associated plaques following administration of MultiHance or ProHance.

Four cases of "gadolinium-deposition disease" were medically confirmed and showed retention of Gd complexes in tissues (skin, vein, hair) and urine. There are some limitations of these cases for assessing causality in relation to GBCA exposure. The patients were self-selected for further investigation of their symptoms. The reported cognitive symptoms are more subjective than the skin changes, and reporting of cognitive symptoms could have perhaps been influenced by publically available information on Gd retention in the brain. The level of exposure varies in the cases, including two patients who received only one dose. The occurrence of symptoms hours or days after even a single exposure to a GBCA seems to separate this condition from possible effects of retention of Gd complexes in brain and body tissues. The physicians reporting these cases indicated that there might be the possibility that this presumed disease process might initially arise as an acute adverse event type process to the GBCAs; therefore it may not be related to Gd deposition but rather involve the immune system. Along this line of explanation, Wermuth et al. found that both linear and macrocyclic agents stimulate release of numerous chemokines, cytokines, and growth factors, which are mediators of acute reactions (*Wermuth et al., 2013*).

At this time, there is insufficient evidence to allow a conclusion concerning any additional risk resulting from these delayed, non-NSF adverse events

The strength of the case reported by **Roberts et al** (*Roberts et al., 2016)* is that high levels of Gd complexes were detected within the skin in a patient with normal renal function, and distribution of Gd retention in the connective tissue septations of the subcutaneous adipose tissue where increased CD34 immunoreactivity was observed. However, the patient reportedly suffered from joint contractures of unknown etiology. In NSF, joint contractures are mainly dependent upon fibrotic skin changes, but no skin lesions were observed in this case. Also, no joint biopsy was performed, so that Gd retention in the affected joints could not be demonstrated.

At this time, besides NSF, there is insufficient evidence of any additional risk resulting from Gd retention in body tissues. The occurrence and frequency of delayed, non-NSF adverse events, as well as possible risk factors need to be further investigated.

4 Benefit-Risk Balance of MultiHance

MultiHance is currently indicated for use in:

- MRI of the central nervous system (CNS) in adults and children over 2 years of age to visualize lesions with abnormal blood brain barrier or abnormal vascularity of the brain, spine, and associated tissues;

- Magnetic resonance angiography (MRA) to evaluate adults with known or suspected renal or aorto-ilio-femoral occlusive vascular disease.

Following the approval granted by the US FDA, several randomized controlled clinical trial have confirmed the benefit provided by MultiHance in the approved indications at the approved dose of 0.1 mmol/kg body weight.

Well characterized risk related to the intravenous administration of GBCAs derives from immediate-type, hypersensitivity reactions and possible occurrence of nephrogenic systemic fibrosis (NSF), a rare clinical syndrome that has been associated with retention of Gd complexes in body tissues. Recently, prolonged Gd retention in brain and body tissues following exposure to GBCAs has raised concern about possible harm to patients.

4.1 Assessment of Clinical Benefit Provided by MultiHance

As indicated in Section 1.2 of this document, all available GBCAs approved for use in MRI of the CNS or MRA show similar r1 relaxivity values in water (*Laurent et al, 2006*). The presence of macromolecules like human serum albumin has no significant effect on the relaxivity of the other GBCAs approved for use in MRI of the CNS or MRA, but markedly increases the relaxivity of MultiHance because of a non-covalent interaction between the active ingredient of the agent with the macromolecules, resulting in slower molecular tumbling rates, longer rotational MR correlation times and faster relaxation rate of surrounding water protons. (*Laurent et al., 2006*). *In vivo*, the resulting increase in the relaxivity of MultiHance depends on the relative amounts of its active ingredient, gadobenic acid not interacting with macromolecules and gadobenic acid bound to macromolecules. Therefore, the resulting relaxivity also depends on the concentration of macromolecules in the medium: the higher the concentration, the higher the relaxivity (*Giesel et al, 2006*). Relaxivity is what provides the signal in MRI: the higher the relaxation rate, the higher the SI on T1-weighted images and the higher the contrast-enhancing efficacy of the GBCA. (*Bleicher et al, 2008*).

To understand whether the higher relaxivity of MultiHance provides any diagnostic advantage in MRI of the CNS and MRA over lower relaxivity GBCAs, several prospective, multicenter, randomized, double-blind clinical trials have been conducted following approval of the agent in the United States, which occurred in 2004.

All these studies were designed as crossover, within-patient comparisons of MultiHance with a lower-relaxivity agent, with study patients undergoing contrast-enhanced MRI or MRA with both agents in a randomized sequence order, and with an interval between scans of more than 2 days to avoid carryover effects, and no more than 14 days to minimize chance of disease progression. All patients underwent two imaging exams using the same, commercially available imaging scanner and the same exact imaging protocol. All study images were evaluated by three independent, experienced radiologists per study, unaffiliated with the enrolment centers and blinded to the contrast agent used, patient clinical and radiologic information, and interpretations by on-site investigators. All the studies had a sample size prospectively defined based on the study objectives, endpoints, and statistical considerations, to enroll the adequate number of patients for 85% to 90% of power.

4.1.1 MRI of the CNS

The primary reason for the use of GBCAs in MRI of the CNS is to detect/exclude presence of brain or spine lesions, determine their location and size, characterise them through assessment of their internal features, delineate their borders and distinguish them from surrounding edema or normal tissues, and/or to define their extent and relationship with adjacent structures in patients with suspected primary or secondary tumors, focal neurologic deficits, endocrinology disorders, or other conditions (*Kanal et al, 2014*).

In the randomized, double-blind clinical comparisons of MultiHance with lower-relaxivity GBCAs (Magnevist, Omniscan, Gadavist and Dotarem), the study endpoints were chosen to provide relevant information on the clinical benefit of contrast enhancement in MRI of the CNS, i.e., the degree of contrast enhancement between lesional areas and surrounding tissues, the delineation of the CNS lesion borders, the definition of the extent of the CNS lesions, and the quality of visualization of the lesion internal morphology. The degree of contrast enhancement was also assessed quantitatively by measuring signal intensity values determined on a pixel-by-pixel basis in CNS lesions and normal tissue, and using them to calculate the percentage enhancement of lesions and the post-contrast-pre-contrast lesion-to-background ratio.

In all these studies, highly significant (all readers, all endpoints, $p<0.001$) better contrast enhancement and better morphologic and morphometric assessment of brain and spine lesions were demonstrated with 0.1 mmol/kg MultiHance vs. 0.1 mmol/kg Magnevist, Omniscan, Gadavist and Dotarem, both at 1.5T and 3.0T (*Maravilla et al., 2006; Kuhn et al., 2007; Rowley et al., 2008; Rumboldt et al., 2009; Seidl et al. 2012; Vaneckova et al., 2015*).

In all these studies, no difference in type, rate or severity of adverse events was observed between MultiHance and the comparators.

4.1.2 MR Angiography (MRA)

Dynamic bolus contrast-enhanced MR angiography is a rapid imaging technique in which images are acquired during the first pass of a GBCA through the vessels of interest and increase in arterial SI because of the shortening of T1 relaxation in blood. Similarly to MRI of the CNS, the higher the relaxivity of the GBCA, the greater the increase in SI within the vessels of interest.

To understand whether the higher relaxivity of MultiHance translates into superior sensitivity and specificity for the detection of clinically significant arterial stenoses when used at the same dose of lower-relaxivity agents, a multicenter, randomized, double-blind, crossover comparison of 0.1 mmol/kg MultiHance with 0.1 mmol/kg Magnevist was conducted in MRA of the peripheral arteries (note: Magnevist is currently not specifically indicated for use in MRA in the United States). The diagnostic performance for the detection of clinically relevant disease (stenosis ≥51%) was significantly improved with MultiHance ($p \leq 0.0028$), with positive predictive values between 90% and 91% and negative predictive values between 89% and 92%, according to the different blinded readers.

4.1.3 Conclusion on the Clinical Benefit of MultiHance in the Approved Indications

All the randomized clinical studies conducted after approval on MultiHance in the United States confirmed the effectiveness of the agent in the approved indications and at the approved dose of 0.1 mmol/kg.

4.2 Assessment of Clinical Benefit Provided by ProHance

The clinical benefit that ProHance provides in MRI of the CNS and of the head and neck is similar to that of other GBCAs with similar relaxivity (4 to 5 $L \cdot mmol^{-1}s^{-1}$) and pharmacokinetics (rapid distribution to the extracellular fluid space and renal elimination).

Following approval in the United States in 1992, large-scale, well-designed clinical comparisons in 599 patients with known or suspected CNS disease showed that ProHance provides similar diagnostic effectiveness when compared to Gadavist in MRI of the CNS.

Gutierrez et al. (*Gutierrez et al., 2015*) published the results of a Phase III, prospective, multicentre, randomized, crossover study that included 390 patients who underwent unenhanced MRI followed by enhanced imaging with 0.1 mmol/kg Gadavist (gadobutrol) or 0.1 mmol/kg ProHance (gadoteridol). Three blinded readers assessed the magnetic resonance images. The study endpoints were those validated by the US FDA as indicative of the clinical benefit of improved morphologic and morphometric assessment of CNS lesions with MRI, i.e., lesion contrast enhancement, delineation of lesion borders, and depiction of lesion internal morphology. Another primary endpoint was the count of the total number of lesions detected on the MR images, even if there is no standard of truth available for CNS lesion detection. The full analysis efficacy set included 336 patients. The analysis was made on average readers' scores ("average reader") for combined unenhanced and enhanced imaging ("combined gadobutrol" and "combined gadoteridol"). For the average reader, combined gadobutrol was non-inferior to combined gadoteridol for contrast-enhancement, lesion border delineation, and depiction of lesion internal morphology. The Authors also report data on the diagnostic performance of gadobutrol- and gadoteridol-enhanced MRI, indicating better sensitivity for lesion characterization for gadobutrol. However, the final diagnosis of lesions was determined by a truth committee on the basis of other imaging findings, not of a validated standard of truth (histology).

Maravilla et al. (*Maravilla et al., 2015*) conducted a prospective, multicentre, randomized, crossover comparison of Prohance and Gadavist that involved 209 patients who underwent unenhanced MRI followed by enhanced imaging with 0.1 mmol/kg Gadavist or 0.1 mmol/kg ProHance. Three blinded readers assessed the magnetic resonance images. The study endpoints were again lesion contrast enhancement, delineation of lesion borders, and depiction of lesion internal morphology. The readers also recorded lesion signal-intensity measurements relative to normal brain parenchyma for up to 3 lesions in each patient. Comparisons of diagnostic performance (lesion detection rate, accuracy for tumor characterization—that is, the distinction between benign and malignant tumors based on World Health Organization brain tumor classification) and confidence for lesion characterization were performed for 139 patients with 308 histologically confirmed brain tumors after biopsy or surgical resection. For these

evaluations, patients with only follow-up diagnostic data from alternative imaging procedures were excluded (while such data were the basis for the assessment of diagnostic performance in the study by Gutierrez et al, 2015). For each lesion, the blinded readers assigned either a single diagnosis or could choose to assign differential diagnoses (i.e., 2, 3, or more than 3 diagnoses). With this approach, a confidence score for correct lesion diagnosis was determined by using a 5-point scale as follows: 5 (single and correct diagnosis); 4 (2 differential diagnoses, one of which was correct); 3 (3 differential diagnoses, one of which was correct); 2 (>3 differential diagnoses, one of which was correct) or 1 (incorrect diagnosis or lesion not detected at MRI).

The morphologic assessment of CNS lesions was considered almost superimposable with the two agents by the 3 readers (p values = 0.69 –1.00). Two readers found no difference in the detection of patients with lesions (p values = 0.32 –0.56), while 1 reader found minimal differences in favor of ProHance (p value = 0.046). Readers 1 and 2 noted no significant differences between ProHance and Gadavist for characterization of detected tumors either at the patient level or at the lesion level. Conversely, improved lesion characterization with ProHance was noted by reader 3. Patient level analysis showed slightly higher mean confidence scores assigned in the ProHance group. Differences were more marked at the lesion level analysis, with significantly higher confidence scores for all 3 readers (p values = 0.033–0.001).

In addition to the two studies described above, a previous crossover comparison of 0.1 mmol/kg gadoteridol (ProHance) with 0.1 mmol/kg gadopentetic acid (Magnevist) in MRI of the CNS did not show any significant difference in terms of degree of contrast enhancement, number of lesions visualized, or level of additional information gained (*Greco et al., 2001*).

Doses higher than 0.1 mmol/kg, up to a dose of 0.3 mmol/kg, have been shown to provide further increase in lesion-to-brain contrast and to improve detection of CNS lesions and tumor infiltration, size and extent of gliomas. (*Yuh et al., 1994; Yuh et al., 1995; Wolansky et al., 1998; Suzuki et al., 2004; Ono et al., 2013*).

4.3 Risk Assessment

4.3.1 Immediate-type Hypersensitivity Reactions

Hypersensitivity reactions are a known risk for all GBCAs, MultiHance and ProHance included. The vast majority of these reactions are non-serious. Serious reactions such as anaphylactoid reactions can occur; they are rare (0.001%-0.01%) but include the possibility of fatal outcomes (*ACR Manual, 2017b*).

Patients at risk for acute hypersensitivity reactions are those with history of hypersensitivity reactions or a history of asthma or other allergic disorders (*ACR Manual, 2017b*).

For serious hypersensitivity reactions, it is the clinical management of the adverse event that is a significant determinant of the eventual outcome, and with timely and correct management, the vast majority patients will make a full recovery. It is reasonable to expect that with appropriate treatment, the rate of fatal outcomes is extremely low. The prescribing information of all the available GBCAs already includes warnings and risk minimisation measures in connection with this risk.

4.3.2 Nephrogenic Systemic Fibrosis (NSF)

NSF is a well characterized risk associated with exposure to GBCAs, limited to patients with acute or chronic severe renal insufficiency (estimated glomerular filtration rate, eGFR <30 mL/min/1.73 m^2), end-stage renal disease, or with acute renal insufficiency of any severity due to hepatorenal syndrome or in the perioperative liver transplantation period.(*ACR Manual, 2017c*).

NSF cases occurring after the sole administration of one GBCA are defined as "single-agent" or "unconfounded." If a case of NSF follows the administration of two or more agents, it is more difficult to determine which agent is associated with the development of the disorder, and the case is reported as "multiple-agent" or "confounded" (*Shellock and Spinazzi, 2008*).

Bracco is not aware of any medically-confirmed, single-agent, unconfounded case of NSF following exposure to MultiHance from any source, including large clinical studies of the individual agents in high-risk patients, with prolonged and extensive follow-up and systematic collection of all the clinical and dermopathological information that is needed to make a diagnosis of NSF (see Section 3.2.2.1).

Bracco is aware of only one spontaneously reported, single-agent, unconfounded case of NSF following exposure to ProHance. No cases of NSF were detected following administration of ProHance involving 154 high-risk patients.

It is important here to note that important and effective risk minimization measures were introduced in 2010 (*FDA, 2010*) and, to Bracco's knowledge, no new cases of NSF have been reported since.

4.3.3 Gadolinium Retention in Brain and Body Tissues

Risk deriving from retention of Gd complexes in brain and body tissues might derive from:

- T1 hyperintensity in deep brain areas that may negatively affect the interpretation of brain MR images;
- Possible toxic effects causing adverse clinical events besides NSF.

4.3.3.1 T1 Hyperintensity in Deep Brain Areas

Independent of exposure to GBCAs, there are several conditions known to be associated with hyperintensity on unenhanced T1-weighted (Spin-Echo, Turbo-Spin-Echo, Gradient-Eco, IR) images observed in the GP and/or DN (*Hedge et al, 2011; Zaitout et al., 2014*), such as: accumulation of manganese due to hepatic failure (*Park et al.; 2003; Klos et al., 2005; Klos et al., 2006*), long term, unbalanced total parenteral nutrition (*Fitzgerald et al., 1999*), occupational exposure (*Kim et al., 2004; Josephs et al., 2005*), iron deficiency (*Herrero et al., 2002*), non-ketotic hyperglycemia (*Lee et al., 2002*), carbon monoxide poisoning (*O'Donnel et al, 2000*), neurofibromatosis type 1 (*Bognanno et al., 1988*), calcification (*Zaitout et al., 2014*), brain irradiation (*Zaitout et al., 2014*), and secondary progressive subtype of multiple sclerosis, characterized by a higher score on the Expanded Disability Status Scale, a higher brain lesion load, and tissue loss (*Roccatagliata et al., 2009*).

Distinction among the different causes of T1-hyperintensity, abnormal T1 shortening induced by GBCAs included, is based on an integrated evaluation of all the other available MR imaging sequences acquired (T2-weighted, T2*-weighted, diffusion-weighted imaging, DWI) as well as findings from CT when available (e.g., for the visualization of calcifications). Most importantly, in order to properly interpret images showing T1 shortening in the DN and/or GP, radiologists should always be provided with appropriate clinical information on the patient. For instance, T1 shortening following multiple exposures to GBCAs should not have any impact on the interpretation of images in typical scenarios, signs, or symptoms that lead to the neuroimaging examination, such as neurofibromatosis type 1, a known suicide attempt, hyperglycemic chorea-ballismus in a diabetic patient, signs of parkinsonism and psychiatric symptoms in a patient with liver cirrhosis and end-stage liver failure, chronic occupational exposure to manganese, or in a patient on total parenteral nutrition.

Clinical evidence shows that such visible abnormal T1 shortening may occur more frequently with linear GBCAs, especially with Omniscan and Magnevist. However, an increase in SI on unenhanced images is possible with macrocyclic agents as well. More importantly, patients that undergo a new contrast-enhanced MR procedure might have undergone multiple procedures with the use of different GBCAs.

Bracco is not aware of any report of visible hyperintensity in deep brain areas negatively affecting the interpretation of brain MR images following the administration of MultiHance, ProHance or other GBCAs. However, a warning in the prescribing information of the agent would be important to increase awareness and avoid any possible, even if unlikely, effect of T1 shortening on the interpretation of brain MR images.

If properly warned, radiologists observing T1 hyperintensity on unenhanced images would be reminded of the importance of gathering information on the patients that would make them understand the possible cause of the abnormal T1 shortening seen on the images.

4.3.3.2 Potential Toxic Effects

There is no evidence of neurotoxicity in adult or juvenile animals exposed to high intravenous doses of any of the available GBCAs (see Section 3.1.2). Similarly, the available clinical evidence seems to exclude that brain retention of Gd complexes is associated with harmful effects and potential interaction with neurological disease processes (see Section 3.1.3).

As for retention of Gd complexes in body tissues, evidence of skin lesions has been observed only in animals administered Omniscan or OptiMARK, but not following exposure to other GBCAs, with involvement of inflammatory pathways in the pathogenesis of skin damage. The administration of Omniscan has been also shown to be associated with a profibrotic effect both in healthy and renally-impaired rats (see Section 3.2.1).

Gd retention in body tissues has been associated with development of NSF (see Sections 3.2.2.1 and 4.3.2) and non-NSF delayed adverse events (see Section 3.2.2.2).

With the term “non-NSF delayed adverse events”, the Sponsor refers to any adverse event that does not occur within the first hour of the GBCA administration, reported signs and symptoms are suggested to be associated with Gd retention in tissues, but the condition does not meet the

clinical and pathological criteria used to establish a diagnosis of NSF developed by a group of experts (*Girardi et al, 2011*).

Included in the definition of non-NSF adverse events are several conditions that have been described in the last 5 years, such as "gadolinium-associated plaques", "gadolinium-deposition disease" and a case report of late-onset joint contractures, with no signs of NSF in a patient with normal renal function exposed to a high cumulative GBCA dose. At this time, besides NSF, there is insufficient evidence of any additional risk resulting from Gd retention in body tissues (see Section 3.2.2.3). The occurrence and frequency of delayed, non-NSF adverse events, as well as possible risk factors need to be further investigated to address current knowledge gaps.

Even if there is currently no evidence that, besides NSF, Gd retention in tissues is associated with harm to patients, to minimize the risk of Gd retention, the prescribing information for all GBCAs, MultiHance and ProHance included, should contain clear, unambiguous wording to make the health care professionals utilizing the products aware and to reduce exposure.

4.4 Conclusions on the Benefit-Risk Balance of MultiHance

In view of:

- The benefit provided by the use of MultiHance and ProHance in the approved indications and at the approved dose;
- The absence of significant differences in the rates of hypersensitivity reactions between MultiHance, ProHance and other GBCAs that could influence benefit-risk considerations, and the presence of specific warnings in connection with this risk in the approved prescribing information of the product;
- The absence of medically-confirmed, single-agent, unconfounded cases of NSF reported in association with the administration of MultiHance, either from spontaneous reporting, literature monitoring, or extensive clinical testing, only one NSF case associated with the administration of ProHance, and the presence of the effectiveness of warnings and risk minimisation measures in connection with NSF;
- The absence of evidence of any additional risk resulting from Gd retention in brain and body tissues,

The Sponsor Bracco concludes that the benefit-risk balance of both MultiHance and ProHance is positive and unchanged in its approved use.

5 Conclusions

The aim of this document was to provide evidence to address four fundamental questions related to possible retention of Gd complexes in brain and body tissues.

The first question is whether exposure to GBCAs is associated with retention of Gd complexes in brain and extra-cerebral (body) tissues, and, if so, whether there are differences among individual

GBCAs. Data from experimental animal studies and clinical investigations were reported in Section 2 of this document. Results from these studies showed that:

- Exposure to GBCAs is associated with retention of Gd complexes in brain and body tissues. Gd retention was observed in every patient exposed to either linear or macrocyclic GBCAs, and may be dependent on the total cumulative dose given to a patient.
- Gd levels measured in the brain are only an extremely small fraction of the injected dose, and are markedly lower than those measured in body tissues, even if there seems to be a correlation between levels in brain and levels in body tissues.
- Clearance of retained Gd complexes over time has been observed for all GBCAs both in adult and juvenile animals, and from both brain and body tissues. However, Gd complexes have been observed in human patients months or even years after exposure to either linear or macrocyclic GBCAs.
- Clinical data are too limited to evaluate whether patient characteristics, including underlying diseases and renal function, may affect Gd retention in brain and/or body tissues. Impairment of renal function did not seem to influence Gd retention and clearance in mice models, while opposite results were found in rat models;
- As far as brain Gd retention is concerned, the available evidence shows clear differences among the individual agents:
    - The intravenous administration of the macrocyclic GBCA ProHance is associated with the lowest levels of Gd concentrations in brain tissues both in animals and man, often at the limit of quantitation by ICP-MS, and lower than those measured after administration of the other macrocyclic GBCAs Dotarem and Gadavist;
    - Animal studies showed that all the macrocyclic GBCAs, Dotarem and Gadavist included, caused significantly lower levels of Gd retention compared with the linear GBCAs. However, first and limited data from post-mortem, tissue-sample studies in man do not show a clear demarcation between Gd levels retained after exposure to the macrocyclic agent Gadavist and those following the administration of linear GBCAs;
    - Among the linear GBCAs, Omniscan was shown to be associated with the highest brain Gd levels, whereas the lowest Gd levels were observed following administration of MultiHance;
    - Post-mortem, tissue-samples studies showed the highest brain concentration of elemental Gd in the DN and GP, with a majority of elemental Gd deposits found within the endothelial wall, and a smaller fraction identified in the tissue interstitium. Of note, following repeated doses of the GBCA Omniscan, Gd deposits were detected within the nucleus of a neuronal cell in 2 patients;
    - Soluble Gd complexes seem to be cleared more efficiently than insoluble complexes. However, Gd complexes have been observed in the brain of human patients months, or even years after exposure to either linear or macrocyclic GBCAs.
- As for retention in body tissues:

  - Animal studies showed that administration of Omniscan or Magnevist was associated with the highest Gd levels retained in skin, bone, liver and kidney. Data obtained in humans do not allow the drawing of firm conclusions about possible differences among the various GBCAs.
  - The distribution of Gd deposits in body tissues in man is inhomogeneous and seems to be higher in areas of inflammation, increased cellularity, or lesional areas in patients with NSF. In a patient with normal renal function, deposition of Gd was associated with the connective tissue septations of the subcutaneous adipose tissue and increased CD34 immunoreactivity.

- More, and possibly larger studies in humans aimed at determining levels of elemental Gd in brain and body tissues are needed to understand possible differences among the various GBCAs approved and used in the United States. Also, additional clinical studies are needed to determine the chemical form of the Gd complexes retained in human tissues, as well as retention over time.

The second fundamental question is whether long-term Gd retention in tissues is associated with damage of the involved tissues. The available evidence, presented in Section 3 of this document, shows that:

- No sign of neurotoxicity was ever detected in any of the experimental animal studies or post-mortem, tissue-sample studies in man, following exposure to MultiHance, ProHance or any other GBCA.
- Omniscan and OptiMARK were shown to be associated with proinflammatory and profibrotic effects, as well as with skin lesions partially resembling NSF in healthy and renally-impaired animals. Similar skin lesions or proinflammatory and profibrotic effects were not observed following exposure to the other GBCAs;
- In human patients, NSF, a disease that resembles scleroderma and eosinophilic fasciitis clinically and scleromyxedema histopathologically, has been associated with exposure to GBCAs in patients with end-stage renal disease or eGFR <30 mL/min/1.73 m^2, and/or during the perioperative liver transplantation period. Besides NSF, eosinophilic, collagenous, round, or ovoid sclerotic bodies, in various stages of calcification, were observed in patients 3.5 to 5 years after exposure to GBCAs (“gadolinium-associated plaques”).

The third question is whether there is evidence of clinically relevant harm to patients. The evidence presented in Section 3 of this document indicate that, besides NSF, there is currently no evidence suggesting that brain or body retention of Gd complexes is associated with harmful effects and potential interaction with disease processes, because:

- The experimental animal studies aimed at determining the potential correlation between Gd retention in brain tissues and neurological changes did not detect any neurological disorders, behavioral abnormalities, physiological dysfunctions, and any other signs of nervous system toxicity, both in adult and juvenile animals.

- Two population studies seem to exclude an association between exposure to GBCAs and an effect on overall neurologic or neurocognitive performance.
- Case reports of non-NSF, delayed adverse events do not provide sufficient evidence of any additional risk resulting from Gd retention in body tissues.

The final question is whether the available evidence on Gd retention in tissues changes the benefit-risk balance of MultiHance and ProHance (see Section 4). The answer is no: the benefit-risk balance of both products is unchanged, because:

- The effectiveness and benefit provided by the two agents has been properly demonstrated before and after their approval by the US FDA.
- There is no evidence of new, additional risk resulting from Gd retention in brain and body tissues. To date, NSF is the most significant clinical syndrome associated with retention of Gd complexes in body tissues, and there are no medically-confirmed, single-agent, unconfounded cases of NSF reported in association with the administration of MultiHance, and only one case following the administration of ProHance. No cases of NSF were observed with either MultiHance or ProHance in clinical investigations carried out in high-risk patients exposed to the agents. Of note, for NSF, important and effective risk minimization measures were introduced in 2010 (*FDA, 2010*), and, to Bracco's knowledge, no new cases of NSF have been reported since.

Even if there is currently no evidence that, besides NSF, Gd retention in tissues is associated with harm to patients, in order to minimize the risk of Gd retention, the prescribing information for all GBCAs (including MultiHance and ProHance) should contain clear, unambiguous wording to make the health care professionals utilizing the products aware and to reduce exposure.

Also, even if Bracco is not aware of any report of visible hyperintensity in deep brain areas negatively affecting the interpretation of brain MR images following the administration of MultiHance, ProHance or other GBCAs, further precautions in the prescribing information of the agent would help to increase awareness and avoid any possible, even if unlikely, effect of T1 shortening on image interpretation. If fully informed, radiologists observing T1 hyperintensity on unenhanced images would be reminded of the importance of gathering information on the patients that would make them understand the possible cause of the abnormal T1 shortening seen on the images.

Finally, there are still important knowledge gaps on Gd retention and its possible association with harmful effects and potential interaction with disease processes. Accordingly, the collection of additional information through new, well designed animal and clinical studies is warranted.

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