Study Protocol

Exploratory evaluation of the potential for long-term retention of Gadolinium in the bones of patients who have received Gadolinium based Contrast Agents according to their medical history.

A multicentre, retrospective and prospectively interventional, exploratory phase IV study

EudraCT No.: 2012-001439-30

Study No.: ALS-Gd64/001

Version 4.0 Final, 29 April 2016

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PROTOCOL SYNOPSIS

Title	Exploratory evaluation of the potential for long-term retention of Gadolinium (Gd) in the bones of patients who have received Gadolinium based Contrast Agents (GdCAs) according to their medical history.
Study no.	ALS-Gd64/001
EudraCT no.	2012-001439-30
Clinical phase	Post Authorisation Study / Phase IV
Active ingredients of products under evaluation	Gadobutrol, Gadodiamide, Gadopentetic acid, Gadoteric acid, Gadoversetamide, and Gadoxetic acid
Study objective(s)	Primary objective To prospectively explore the potential for long-term retention of Gd in bones in patients who have received a single dose of GdCA or multiple doses of the same GdCA, with moderate or severe renal impairment or stable normal renal function (estimated glomerular filtration rate > 60 ml/min/1.73 m^2) at the time of GdCA injection. Secondary objectives • To evaluate skin samples for concentration of Gd • To evaluate bone and skin samples for concentrations of calcium, phosphorus, sodium, iron, zinc and potassium • To evaluate skin samples for any dermatopathological changes that may be associated with Nephrogenic Systemic Fibrosis (NSF) • To describe potential co-factors for NSF, susceptibility factors and drug treatments with potential impact on bone metabolism
Methods	Gd concentration in bone (trabecular and cortical) and skin tissue will be analysed by inductively coupled plasma mass spectrometry (ICP-MS). Other analytes (calcium, phosphorus, sodium, iron, zinc and potassium) will be analysed also by ICP-MS or alternatively (if this method is not satisfactory during method set-up) by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Histopathology of skin samples will be evaluated by routine light microscopy methods, including immunostaining for CD34, factor XIIIa and CD68.
Design	Multicentre, retrospective and prospectively interventional, exploratory.
Previous treatment (test group only)	Single doses: 0.025 mmol per kg body weight for Gadoxetic acid and 0.1 mmol per kg body weight for all other GdCAs. Multiple doses: each dose (volume) administered at each time point of dosing.

Population	Study population consists of patients undergoing an orthopaedic procedure, provided that the required amount of trans-operatory collection of bone and skin as defined per-protocol is feasible. All inclusion criteria and no exclusion criteria should be met (please refer to sections 5.1.3, 4.1 and 4.2). Patients must have received GdCA at least 1 month prior to surgery (test group). Additionally, a group of patients undergoing hip or knee replacement surgery who have not received GdCA will be enrolled (control group).
Number of patients	At least 99 evaluable patients will be included.
Number of centres	At least 35 centres in America, Europe and Asia.
Statistics	Sample Size: There are six groups for active GdCA ingredients Gadobutrol, Gadodiamide, Gadopentetic acid, Gadoteric acid, Gadoversetamide, and Gadoxetic acid. In each group, three categories of renal function (stable normal renal function (eGFR > 60 ml/min/1.73 m^2), impaired renal function (eGFR <30 ml/min/1.73 m^2 (severe) and ≥30 to <=60 ml/min/1.73 m^2 (moderate)) and two subgroups of dosing of GdCA (single / multiple) are to be evaluated. Per active GdCA ingredient the recruitment targets are defined as follows • 5 patients for stable normal renal function (eGFR > 60 ml/min/1.73 m2) and single dose • at least 3 patients and up to 5 patients for stable normal renal function and multiple doses • 5 patients for impaired renal function (eGFR <=60 ml/min/1.73 m^2) and single dose • up to 5 patients for impaired renal function (eGFR <=60 ml/min/1.73 m^2) and multiple doses. For Gadobutrol and Gadoteric acid, at least 3 patients are required. For Gadodiamide, Gadopentetic acid, Gadoversetamide, and Gadoxetic acid, there is no requirement to reach a specific number of patients to achieve study completion. The total number of patients enrolled in these groups will be at least 84. The number of subgroups in the GdCA-naïve population is 3 according to renal function category (i.e. severe or moderate impairment, and stable normal renal function). Five patients will be enrolled per each sub-group. Therefore, the total number of GdCA-naïve patients enrolled will be at least 15. Consequently, a minimum of 99 patients in total shall be included in the study.

Statistical analysis	Primary and secondary variables will be analysed descriptively. No formal hypothesis testing is planned. All statistical tables will compare all GdCA groups pooled as well as each GdCA group separately versus the control group. They will be further stratified according to renal function category (3 groups), single / multiple doses of all patients (2 groups), and the combination of "renal function" and "single / multiple doses". The elapsed time from dosing of GdCA until surgery will be classified and used for stratification. Safety data will be tabulated. No further subgroup analyses are planned.
Interim analysis	An interim analysis will be carried out on all patients enrolled in the single dose subgroups for Gadobutrol, Gadoteric acid, Gadodiamide, and Gadopentetic acid once at least three patients have been recruited to the single-dose subgroups for the 4 agents. The descriptive analysis encompasses the evaluation of Gd concentration in trabecular and cortical bone (primary analysis) and the Gd concentration in skin (secondary analysis) within in the subgroups defined for evaluation above.
Planned time schedule	First patient first visit (FPFV): March 2013 Last patient last visit (LPLV) : October 2017.

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A complete list of all participating centres, investigators (including names, titles, addresses and telephone numbers), and all required signature documents, will be filed in the trial master file (TMF).

SERIOUS ADVERSE EVENT REPORTING

All Serious Adverse Events (SAEs) must be reported immediately (within one working day) to:

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SAE reporting will be covered on a 24 hours / 7 days basis.

SIGNATURES

The signatories are obliged to comply in all respects with

- this clinical study protocol
- the standards of Good Clinical Practice (GCP) as defined in the "Note for Good Clinical Practice" (CPMP/ICH 135/95) and related guidelines
- the "Declaration of Helsinki" current valid version according to applicable national regulatory and legal requirements
- all applicable regulatory requirements including national drug and data protection laws

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DECLARATION OF THE PRINCIPAL INVESTIGATOR

I have read this study protocol and agree that it contains all the information required to conduct the study. I agree to conduct the study as set forth in this protocol. In particular, I agree to adhere to the moral, ethical and scientific principles governing clinical research as set forth in the current, valid versions of the "Declaration of Helsinki", all applicable national regulatory and legal requirements, and the guidelines for Good Clinical Practice.

Name	Affiliation / Address	Dated Signature

ABBREVIATIONS

AE	Adverse Event
C	Celsius
CHMP	Committee for Medicinal Products for Human Use
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CPMP	Committee for Proprietary Medicinal Products
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organisation
CV	Coefficient of Variation
DCF	Data Clarification Form
EAG	Ecron Acunova GmbH (CRO)
eCRF	electronic Case Report Form
eGFR	estimated Glomerular Filtration Rate
EMA	European Medicines Agency
ESRD	End Stage Renal Disease
FAS	Full Analysis Population
FDA	Food and Drug Administration
FPFV	First Patient First Visit
GCP	Good Clinical Practice
Gd	Gadolinium
GdCA	Gadolinium based Contrast Agent
GFR	Glomerular Filtration Rate
ICH	International Conference on Harmonisation
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
IEC	Independent Ethics Committee
IF	Investigator File
IRB	Institutional Review Board
i.v.	Intravenous
IWRS	Interactive Web Response System
LPLV	Last Patient Last Visit
LLOQ	Lower Limit of Quantification
MAH	Marketing Authorisation Holder
MDRD	Modification of Diet in Renal Disease

MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic Resonance Imaging
N	Number of non-missing observations
NSF	Nephrogenic Systemic Fibrosis
PP	Per Protocol
PT	Preferred Term
RDC	Remote Data Capture
SADR	Serious Adverse Drug Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
SmPC	Summary of Product Characteristics
SOC	System Organ Class
SOP	Standard Operating Procedure
TMF	Trial Master File
V	Visit
WHO-DD	World Health Organization – Drug Dictionary

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1 INTRODUCTION

1.1 Background

Rationale for the Study

This study was requested by the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA). The potential for long-term retention of Gd in human bone and skin after administration of GdCAs and co-factors that may increase the risk of nephrogenic systemic fibrosis (NSF) warrant further study. The analysis of bone and skin samples from patients undergoing hip or knee replacement surgery was recommended initially and has been widened to other suitable orthopaedic surgeries.

Nephrogenic Systemic Fibrosis

Nephrogenic systemic fibrosis was first reported in 2000 by Cowper et al. (1), who noted that since 1997, 15 dialysis patients presented with a disease that closely resembled scleromyxoedema, yet had significant enough clinical and histopathological differences to warrant its designation as a new clinicopathological entity. A possible association between the administration of GdCAs to patients with severe kidney impairment and NSF was first reported by Grobner in 2006 (2;3). NSF has been diagnosed in particular in patients with severe renal impairment (estimated glomerular filtration rate (eGFR) < 30 mL/ min/1.73 m²). Patients with acute renal insufficiency due to hepato-renal syndrome and perioperative liver transplantation patients were also implicated (for review see (4;5)).

Signs and symptoms of NSF may include progressive thickening and induration of the skin, contractures around the joints impairing mobility, swelling, redness, pruritus, and a burning sensation (4;6). On a microscopic level increased skin cellularity with fibroblast-like cells and collagen deposition were demonstrated (4;7;8). Systemic involvement of organs, including the lungs and heart may also progress rapidly, ultimately resulting in death in approximately 5% of patients (4;5).

Risk Factors in the Development of NSF

Although the precise pathogenesis of NSF remains unknown, its causes are likely to be multifactorial. Risk factors include the type and dosage of GdCAs administered (9), repeated dosing, high cumulative dose (10), and degree of renal impairment (e.g. stage 4 or 5 chronic renal failure) at the time of contrast administration (9;11) (for review see (12)).

NSF patients frequently have numerous co-morbidities and many co-factors may contribute to development of NSF. Among these, coagulation abnormalities, history of deep vein thrombosis (13), systemic and pre-existing inflammation (due to major surgery, infection, vascular events, etc.) (11), erythropoietin administration (10;14), hypothyroidism (13), and disorders of iron metabolism (15) have been evaluated. Additionally, high serum calcium levels (10) and high serum phosphate levels (10;16), renal dialysis equipment, unidentified microorganisms or pathogens, and unidentified “triggers” within the body have been reported as contributing factors.

Deposition of Gd in Skin and Bone

Abraham et al. (17) reported in 2008 that Gd was detected in skin lesions of 20 patients with NSF, whereas Gd was undetectable in a NSF-negative patient. Skin biopsies were obtained from 16 days to 1991 days after Gd contrast administration.

Gibby et al. (18) directly measured Gd in samples taken from surgical bone specimens removed from patients undergoing hip arthroplasty between 3 and 8 days after GdCA administration. White et al. (19) confirmed that Gd deposition reached measurable levels in

bone within days following administration of GdCAs. Weinmann et al. (20) analysed specimens of cranial bone obtained 1 to 21 days after GdCA administration and observed 0.1 to 0.8 µg Gd/g dry weight in specimens versus less than 0.1 µg Gd/g dry weight in untreated control specimens. Darrah et al. (21) reported measurable retention of Gd in the femoral heads of patients who had received GdCA up to 8 years prior to bone sampling.

Clinical and Toxicological Significance of Gd in Bone

A clinical study aimed at the assessment of Gd retained in healthy bone after GdCA exposure may give data on the quantification of the retained Gd. However, similarly as in the case of the Gibby (18), White (19) and Darrah (21) studies, such a study will neither be able to give information on the form of Gd, nor will it yield any information about potential future release, and its kinetics. No information will be obtained on a potential link between Gd storage and the development of NSF.

There are no data as yet, showing whether there is any clinical significance / toxicity of potential release of Gd from bones. Such release would probably occur over years and in small amounts per time unit (e.g. per year), paralleling the slow processes of bone demineralisation (e.g. osteoporosis, or, potentially, of renal osteodystrophy). However, this result does not provide information on the clinical and toxicological significance of the retained Gd. There also are no data regarding what threshold (if any) of gadolinium released from bone tissue might be necessary to trigger NSF in patients.

While the precise pathogenesis of NSF has not been definitively determined, none of the current hypotheses are premised on any direct link between the long-term retention of Gd in bone tissue translating into an increased risk of NSF in patients who subsequently develop severe renal impairment. Indeed, if retained Gd in bone were a long-term risk factor or more direct trigger for NSF, then new cases of NSF would be expected among the many patients with renal impairment who received GdCAs and did not initially develop NSF. On the contrary, the very low incidence of NSF since 2007 (22) suggests that contributing factors other than retention of Gd in bone are important. The virtual disappearance of new onset cases of NSF in recent years is hypothesised to be a result of the label changes and risk management activities of MAHs.

Therefore, this study shall assess the potential of long-term retention of Gd in patients with varying degrees of renal function, who have received single or multiple doses of GdCAs for diagnostic imaging purposes. Various linear and macrocyclic chelates of GdCAs will be studied, and the concentration of Gd in bone will be compared with respective concentrations of Gd in skin tissue and correlated with histopathological changes of the skin.

1.2 Risk-benefit assessment

This study may contribute further insight into the long-term retention of Gd in human tissues.

In this study, the administration of GdCA to study patients must have occurred at least one month prior to a planned surgery, and all study evaluations will be performed retrospectively with regard to GdCA administration. Therefore, the administration of GdCAs does not constitute a study-related risk.

The surgical intervention from which bone specimens will be obtained (i.e. an orthopaedic procedure provided that the required amount of trans-operatory collection of bone and skin as defined per-protocol is feasible and all inclusion criteria and no exclusion criteria are met) will take place for reasons unrelated to the conduct of the study. Two specimens will be taken from surgically removed bone, and such harvesting does not constitute any additional risk to the patient. The skin biopsy is a study initiated intervention and may carry minor risks, including infection, persistent bleeding, or scar formation at the biopsy site.

Overall, the risk-benefit assessment for the study is considered positive, based on the new data to be gained in relation to the non-significant added risk to patients.

2 STUDY OBJECTIVES AND PURPOSE

Primary objective:

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

Secondary objectives:

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

3 OVERALL STUDY DESIGN AND PLAN DESCRIPTION

This is a multicentre, retrospective, prospectively interventional and exploratory, post authorisation study which will be conducted globally evaluating the potential for long-term retention of Gd in bones of patients who received single or multiple doses of GdCAs. The study is retrospective with respect to GdCA administration (no GdCA will be administered to patients during the study by the investigator), prospective with respect to bone sample collection (taken from surgically removed bone) and both, prospective and interventional regarding skin sample collection. Skin sampling is required for both dermatopathological assessment and quantification of Gd.

Patients aged at least 18 years (or older if required by local regulations) who are scheduled for an orthopaedic surgery procedure (provided that the required amount of trans-operatory collection of bone and skin as defined in section 5.1.3 is feasible and all inclusion and no exclusion criteria are met) and agree to provide bone and skin samples at the time of the procedure will be included in the study after they sign a written informed consent form. In this study, a minimum of 99 evaluable patients will be enrolled, from which at least 84 patients have received GdCA (test group) and 15 patients have never received GdCA (control group) prior the orthopaedic surgery procedure.

The products assessed are GdCAs which include one of the active ingredients Gadobutrol, Gadodiamide, Gadopentetic acid, Gadoteric acid, Gadoversetamide or Gadoxetic acid. Figure 1 and Table 1 describe enrolment targets per GdCA and the stratification of patients that will be prospectively controlled (see 5.5).

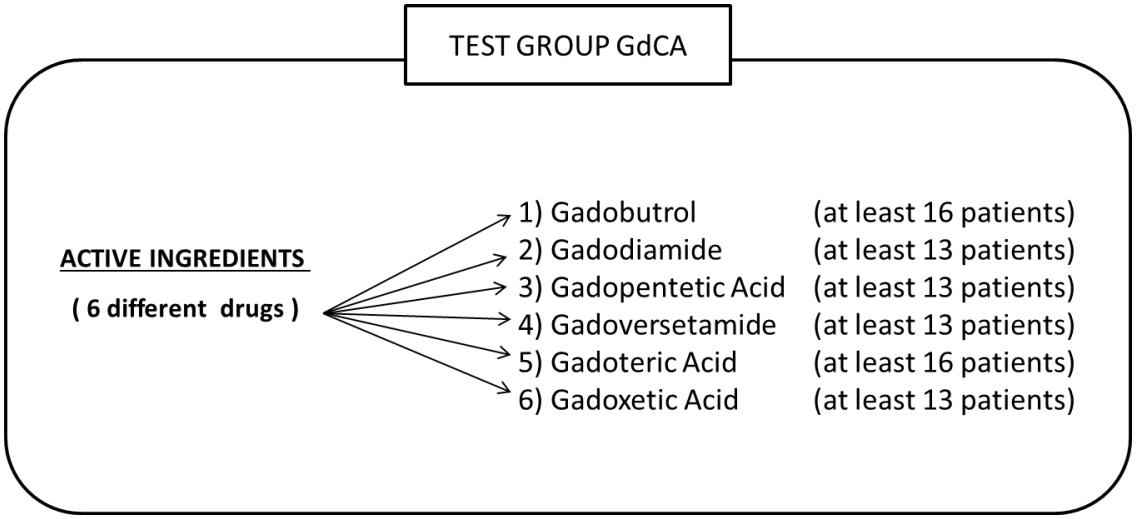


Figure 1: Target number of patients per GdCA

For the test group, GdCA products must have been administered intravenously at least one month prior to the orthopaedic surgery procedure. Only patients for whom information regarding the active ingredient of the specific GdCA, the name of the product, the date(s) of administration and the dose(s) of the administered GdCA are available will be enrolled. Patients who received GdCAs containing any other active ingredient will not be enrolled. Furthermore, documentation of stable renal function prior to administration of the GdCA is required, and renal function must have been assessed at least once within approximately 3 months prior to GdCA injection.

In the group of patients who have received GdCA (test group), at least 13 patients will be included per GdCA active ingredient group for Gadodiamide, Gadopentetic acid, Gadoversetamide, and Gadoxetic acid and at least 16 patients will be included per GdCA active ingredient group for Gadobutrol and Gadoteric acid with the following distribution:

Table 1: Target number of patients per GdCA and renal function status

GdCA	Impaired renal function (at least moderate impairment, eGFR ≤ 60 ml/min)		Stable normal renal function	
	Single dose	Multiple dose	Single dose	Multiple dose
Gadobutrol	5	3 to 5*	5	3 to 5**
Gadoteric acid	5	3 to 5*	5	3 to 5**
Gadodiamide	5	0 to 5*	5	3 to 5**
Gadopentetic acid	5	0 to 5*	5	3 to 5**
Gadoversetamide	5	0 to 5*	5	3 to 5**
Gadoxetic acid	5	0 to 5*	5	3 to 5**

* Patients should be recruited to the Gadodiamide, Gadopentetic acid, Gadoversetamide, and Gadoxetic acid subgroups throughout the study duration up to a maximum of 5, but there is no minimum requirement to reach a specific number of patients in this subgroup to achieve study completion. At least 3 patients and a maximum of 5 patients are required for Gadobutrol and Gadoteric acid. If the target of 3 patients is reached, but the study has not yet completed, then recruitment to this subgroup should be continued during the remaining study time.

** At least 3 patients are required for this subgroup. If the target of 3 patients is reached, but the study has not yet completed, then recruitment to this subgroup should be continued during the remaining study time, stopping when until 5 patients have been recruited to this subgroup, or when all other subgroups have reached their minimum target for recruitment, whichever comes first.

Table 1 shows patient enrolment targets (see chapter 8 for statistical evaluation).

Figure 2 shows the enrolment by renal functional status and GdCA dosing:

a) 5 patients with stable impaired renal function (at least moderate impairment, eGFR ≤ 60 ml/min/1.73 m^2) who have received a single GdCA,

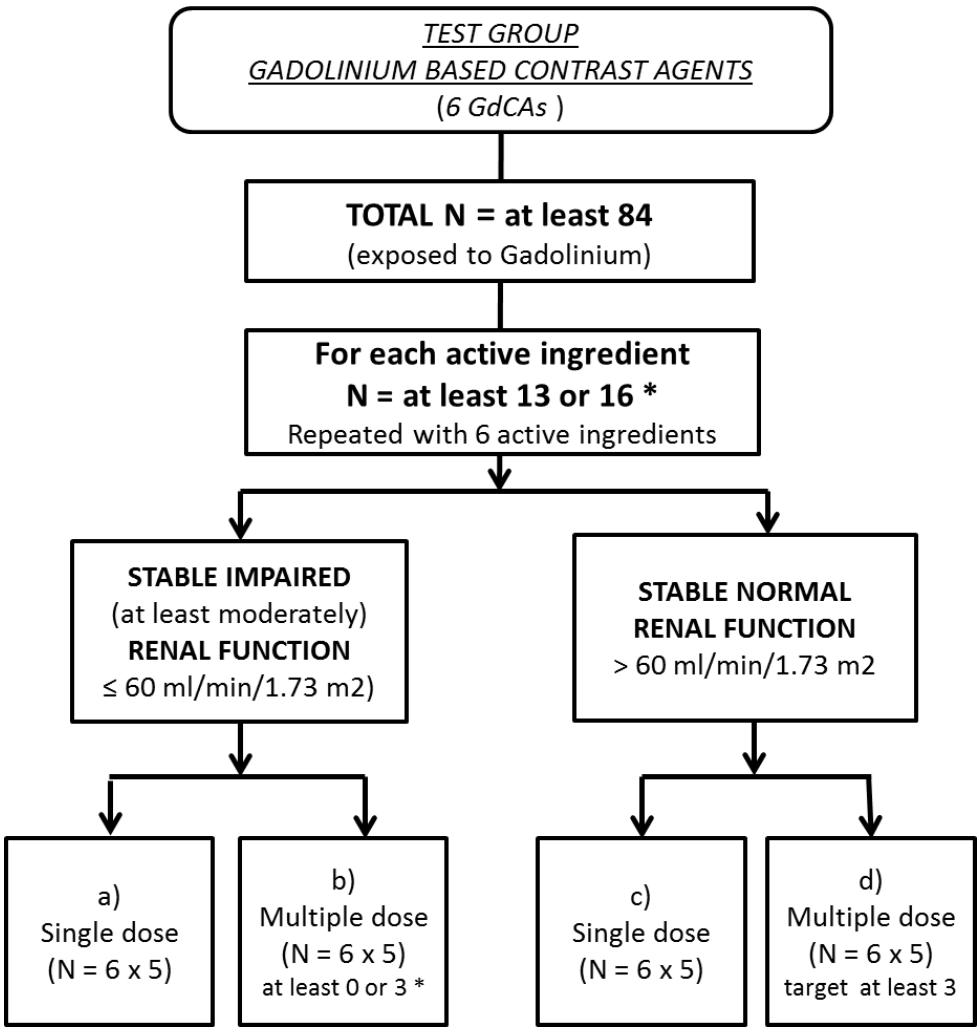
b) Up to 5 patients with stable impaired renal function (at least moderate impairment, eGFR ≤ 60 ml/min/1.73 m^2) who have received multiple doses of the same GdCA:

- Gadodiamide, Gadopentetic acid, Gadoversetamide, and Gadoxetic acid subgroups: no target number. Patients should be recruited to this subgroup throughout the study duration, but there is no requirement to reach a specific number of patients in this subgroup to achieve study completion.
- Gadobutrol and Gadoteric acid subgroups: at least 3 patients. If the target of 3 patients is reached, but the study has not yet completed, then recruitment to this subgroup should be continued during the remaining study time.

c) 5 patients with stable normal renal function (eGFR > 60 ml/min/1.73 m^2) who have received a single GdCA dose,

d) Up to 5 patients (target at least 3) with stable normal renal function (eGFR > 60 ml/min/1.73 m^2) who have received multiple doses of the same GdCA. If the target of 3 patients is reached, but the study has not yet completed, then recruitment to this subgroup should be continued during the remaining study time, stopping when 5

patients have been recruited to this subgroup, or when all other subgroups have reached their minimum target for recruitment, whichever comes first.



* **Depending on the GdCA subgroup**

Figure 2: Stratification of patients for recruitment purposes by renal functional status and GdCA dosing

Out of 15 patients in the control group, 5 patients shall be included in each of the following 3 categories (Figure 3):

e) patients with stable severe renal impairment (eGFR < 30 ml/min/1.73 m^2)

f) patients with stable moderate renal impairment (eGFR within the range 30 to 60 ml/min/1.73 m^2)

g) patients with stable normal renal function (eGFR > 60 ml/min/1.73 m^2)

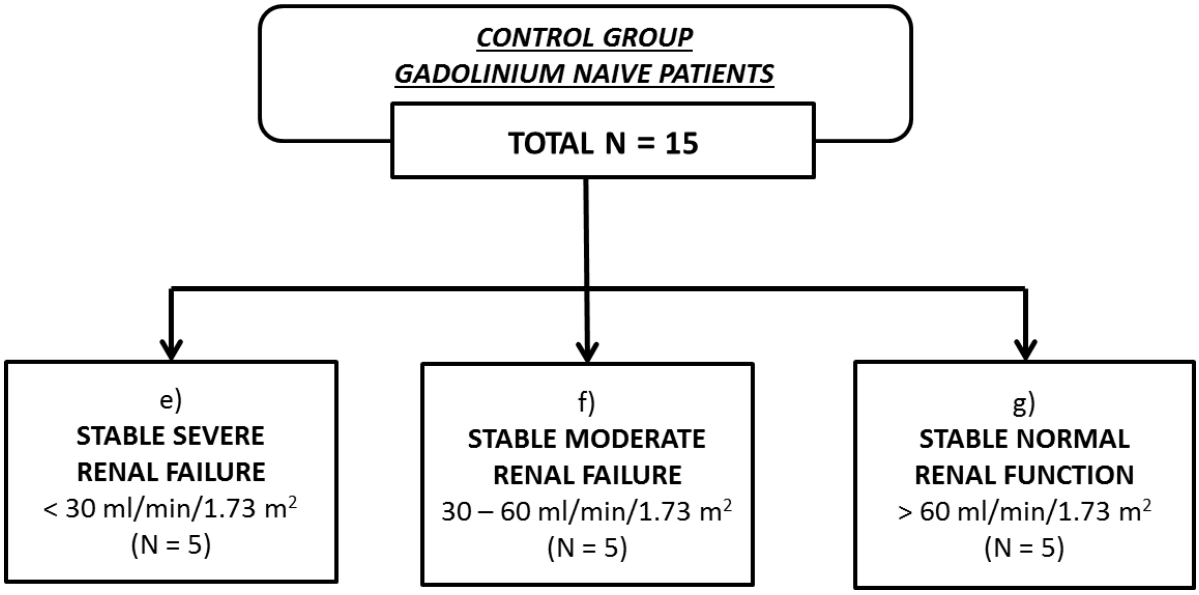


Figure 3: Stratification of control group patients by renal function

The course of the study is depicted in Figure 4, showing the sequential relationship of GdCA administration prior to patient enrolment and the collection of bone and skin tissue samples during the orthopaedic surgery procedure (e.g. hip or knee replacement surgery).

End of study for the individual patient is follow-up visit 2 (visit 4)[1] taking place, approximately within 10-14 days after the orthopaedic surgery procedure (e.g. hip or knee replacement surgery) at the time of potential stitch removal under normal healing conditions[2]. A detailed schedule of study procedures and assessments is presented in section 7.1 (Flow Chart).

[1] Note: If the patient is scheduled for a second orthopaedic surgery procedure (e.g. hip or knee replacement surgery), end of study is the follow-up visit 4 (visit 8).

[2] Note: Any other wound closure technique used according to the local clinical practice is acceptable.

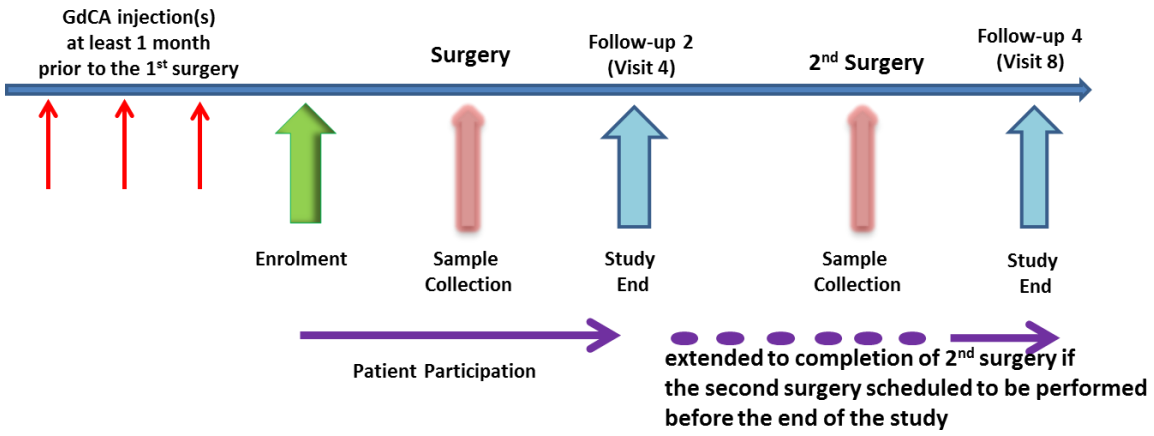


Figure 4: Course of the study

The overall study period is expected to be approximately 4 ½ years from FPFV to end of study. This period includes the scheduled bone and skin sample collection in conjunction with a medically indicated orthopaedic surgery procedure. If the patient is scheduled for a second orthopaedic surgery procedure (e.g. hip or knee replacement surgery) to be performed within the study period, the patient can continue to participate in the study, provided that the required amount of trans-operatory collection of bone and skin as defined per-protocol is feasible during the second procedure. A second bone and skin sample shall be collected during the second scheduled orthopaedic procedure. The overall study period will not be extended in order to obtain the second bone and skin sample collection.

Based on medical experience, it is assumed that approximately 10% of enrolled patients will undergo a second hip or knee replacement surgery of e.g. the contra lateral joint. During both surgeries (if applicable), portions of the femoral or tibial bones will be removed in the course of the respective procedure. From these ex-vivo remains, two samples (trabecular and cortical bone) will be harvested for analysis in the central bioanalytical laboratory. In addition, a skin sample will be collected from the edge of the surgical incision during each joint replacement procedure.

Handling and analysis of trabecular and cortical bone and skin samples will be performed under blinded conditions at central sites. The dermatopathological examinations will be done at the Yale Dermatopathology, New Haven, USA, the bioanalytical examinations (quantification of Gd and other analytes from skin and bone samples) will be conducted by the Bioanalytical Laboratory of Manipal Acunova, Bangalore, India.

3.1 Discussion of study design

The current study design is explorative in nature and will deliver descriptive data on the potential for long-term retention of gadolinium in human bone. Restricting the study population to patients with stable normal renal function is unlikely to provide further insight into the mechanism of Gd retention and the potential development of NSF. Therefore, subgroups with different degrees of renal decrement are included.

A limitation of the study is the retrospective nature of data associated with the single or multiple administration of the GdCAs. It is anticipated that some of the specified analytical clinical and laboratory parameters will not be available within the patient records. However, it would be impossible to prospectively assess potential Gd retention in patients with moderate and

severe decrement in renal function, because the administration of GdCAs to such patients is now limited due to regulation.

The current study, therefore, will enrol patients who received a specified GdCA at least 1 month prior to an orthopaedic surgery procedure and will prospectively collect bone and skin samples for analysis. Conducting the study in patients who plan to undergo a medically indicated orthopaedic surgery procedure provides an accessible model for collecting samples without adding a significant interventional load onto the patients. The collection of bone and skin biopsies are required to correlate Gd content in bone and skin with potential histopathological findings associated with NSF.

GdCA-naïve patients will be included as controls for any background concentration of Gd in bone, e.g. from the environment. For example, Gd has been found in skin biopsies of NSF patients who were considered to be “gadolinium-free” (23), as well as in bone samples from patients with no previous exposure to GdCA (21)

In summary, the study design is adequately selected to contribute to the understanding of long-term Gd retention in patients who receive GdCAs. The study design was developed through scientific discussions with the EMA/ CHMP.

4 SELECTION OF STUDY POPULATION

In this study a minimum of 99 evaluable patients (see 8.1.1) will be recruited, according to the distribution per Assessed GdCA/Renal function status depicted in table 1.

The patients will be recruited in at least 35 centres in America, Europe and Asia. Each patient must meet all inclusion criteria and not present any exclusion criteria.

4.1 Patient inclusion criteria

Each patient must fulfil the following criteria to be eligible for the study:

1. Patient must be at least 18 years of age (or older if required by local regulations)
2. Patient scheduled for an orthopaedic surgical procedure provided that the required amount of trans-operatory collection of bone and skin as defined per-protocol in section 5.1.3 is feasible and all inclusion and no exclusion criteria are met.
3. Patient is scheduled to have sufficient bone removed during the orthopaedic surgical procedure and sufficient non-scarred skin tissue from the edge of the surgical incision or amputated part to permit sample collection, in the investigator’s medical opinion
4. Patient agreed to have bone and skin samples collected at the time of the surgical procedure(s)
5. Patient’s surgical procedure and subsequent remission will not be altered by study-specific skin biopsies, in the investigator’s medical opinion
6. Patient is fully informed about the study and has signed the informed consent form

Patients having received GdCA

7. Patient belongs to one of the following subgroups with respect to the number of GdCA doses received and the status of their renal function:
   a) patient (total to enrol = 5) has **stable impaired renal function** (at least moderate impairment, eGFR ≤ 60 ml/min/1.73 m^2) and has received **one GdCA injection** at the standard dose (0.025 mmol per kg body weight for Gadoxetic acid and 0.1 mmol per kg body weight for all other agents)

or

b) Up to 5 patients with **stable impaired renal function** (at least moderate impairment, eGFR ≤ 60 ml/min/1.73 m^2) and has received **more than one injection** of the same GdCA.

- Gadodiamide, Gadopentetic acid, Gadoversetamide, and Gadoxetic acid groups: no target number. Patients should be recruited to this subgroup throughout the study duration, but there is no requirement to reach a specific number of patients in this subgroup to achieve study completion.
- Gadobutrol and Gadoteric acid groups: at least 3 patients. If the target of 3 patients is reached, but the study has not yet completed, then recruitment to this group should be continued during the remaining study time.

or

c) patient (total to enrol = 5) has **stable normal renal function** (eGFR > 60 ml/min /1.73 m^2) and has received **one GdCA injection** at the standard dose (0.025 mmol per kg body weight for Gadoxetic acid and 0.1 mmol per kg body weight for all other agents)

or

d) patient (total to enrol = 3 to 5) with **stable normal renal function** (eGFR > 60 ml/min/1.73 m^2) who have received **more than one injection** of the same GdCA. If the target of 3 patients is reached, but the study has not yet been completed, then recruitment to this subgroup should be continued during the remaining study time, stopping when 5 patients have been recruited to this subgroup, or when all other subgroups have reached their minimum target for recruitment, whichever comes first.

8. A minimum of 1 month have elapsed between GdCA dose and the scheduled orthopaedic surgical procedure.
9. Patient GdCA history, including dose(s), date(s), and product(s) administered is complete and accurate.

GdCA naïve patients (control group)

10. Patient has never received GdCA before the scheduled orthopaedic surgical procedure.
11. Patient belongs to one of the following renal function categories:

e) patient (total to enrol = 5) has stable severe renal impairment (eGFR < 30 ml/ min/1.73 m^2);

f) patient (total to enrol = 5) has stable moderate renal impairment (eGFR within the range 30 to 60 ml/min/1.73 m^2);

g) patient (total to enrol = 5) has stable normal renal function (eGFR > 60 ml/min /1.73 m^2).

4.2 Patient exclusion criteria

No patient may enter the study if any of the following exclusion criteria are fulfilled:

1. Patient has received different GdCAs.
2. Patient has received intra-articular GdCA or per any other non-i.v. route.

3. Patient has received or is scheduled to receive GdCA within 1 month prior to the date of the scheduled orthopaedic surgical and study sample collection procedures.
4. Patient has received any investigational product or has participated in any other clinical trial within 30 days prior to enrolling in this study.
5. Patient suspected of, or diagnosed with, tumour of the skeletal system (i.e. tumour, metastatic bone, and bone marrow disease in the region from where the skin and bone will be harvested).
   Note: Patients shall not be excluded for other bone diseases with the exception of those with bone cancer as specified in exclusion criterion 5.
6. Patient has diagnosed or suspected NSF at time of enrolment.
   Note: Patients shall not be excluded for diagnosis of NSF subsequent to enrolment.
7. Patient presents with scarring in the region(s) of the scheduled surgical procedure(s) or of the skin sampling location, to the extent that collection of an unscarred skin sample is not feasible.
8. Patient has a close affiliation with the investigational site; e.g., a relative of the investigator, dependent person (e.g., employee or student of the investigational site).

4.3 Patient withdrawal criteria

An enrolled patient must be withdrawn from the study immediately if any of the following occurs:

- During screening: if the enrolment group for which the patient qualifies, has already been closed.
- Patient requires a prohibited concomitant medication, including the administration of a GdCA after enrolment.
- Patient withdraws consent.
- An illness occurs during the study period that is likely to influence the assessment of the study in the investigator's medical opinion (i.e. patient is suspected of, or diagnosed with, tumour of the skeletal system (e.g. in knee / hip bone, metastatic bone and bone marrow disease in knee / hip or other location where the orthopaedic surgery is to be performed)).

Furthermore, an enrolled patient may be withdrawn prematurely from the study, at the investigator's discretion, under the following circumstances:

- One of the inclusion criteria listed in section 4.1 does not apply.
- One of the exclusion criteria listed in section 4.2 applies.
- Patient does not cooperate adequately to permit study procedures and assessments.
- Any adverse event (AE) after which a continuation in the study would constitute an unacceptably high risk for the patient.
- Investigator determines it is in the best interest of the patient to withdraw from the study.

The Sponsor should be informed of any decision to withdraw an individual patient. If possible, a complete final examination according to the visit schedule should be carried out and documented on the corresponding pages of the electronic case report form (eCRF) for

those patients prematurely discontinuing the study. Although patients may withdraw from the study at any time without giving any reasons and without prejudice to future treatment, efforts should be made to document the reason for withdrawal in each case.

Instructions for patient follow-up in case of withdrawal due to an AE are described in section 6.2.4.3.

Withdrawal criterion for patients undergoing multiple GdCA doses:

- If a patient undergoes a second orthopaedic surgical procedure that would qualify for the collection of bone and skin as described elsewhere in the protocol section 5.1.3 (e.g. hip or knee surgery) during the study period, and the patient receives any additional dose of a GdCA between the first and the second surgical procedure, the patient must be withdrawn from the study. No additional bone and skin samples shall be collected during the second surgery for the study. However, the data and tissue samples obtained in connection with the first surgery, prior to administration of disqualifying GdCAs, will be used for the statistical evaluation.

5 STUDY TREATMENTS. PRODUCTS AND INTERVENTIONS

5.1 Products administered

5.1.1 Products administered prior to enrolment

5.1.1.1 Products under evaluation

Patients in the test groups (a to d) must have received only one of the following GdCAs, at least 1 month prior to scheduled orthopaedic surgery on study:

1. Gadobutrol (Gadovist®, Gadavist® & Gadograf®);
2. Gadodiamide (Omniscan®);
3. Gadopentetic acid (Magnevist®);
4. Gadoteric acid (Dotarem® & Magnescope®);
5. Gadoversetamide (Optimark®);
6. Gadoxetic acid (Primovist®, Eovist® & EOB-Primovist®)

Patients in the control groups e), f) and g) must be treatment-naïve regarding any GdCA.

5.1.1.2 Dosage of products under evaluation

- 0.1 mmol per kg body weight for the products 1–5: GdCAs with the active ingredients Gadobutrol, Gadodiamide, Gadopentetic acid, Gadoteric acid, and Gadoversetamide; subgroups a) and c).
- 0.025 mmol per kg body weight for the product 6: GdCAs with the active ingredient Gadoxetic acid; subgroups a) and c).
- Actual doses injected for all multiple doses subgroups for all products 1–6; subgroups b) and d).

5.1.1.3 Dosing frequency

- Single GdCA dose (one injection) at least 1 month prior to the scheduled orthopaedic surgical procedure; products 1–6: subgroups a) and c).

- Multiple doses (more than one injection) of the same GdCA, regardless of frequency. Last GdCA dose at least 1 month prior to the scheduled orthopaedic surgical procedure; products 1–6: subgroups b) and d).

5.1.1.4 Route of administration

- Intravenous only

5.1.2 Products administered after enrolment

None

Note: No GdCA will be administered to the patients during the study by the investigators.

5.1.3 Prospective study interventions

Patients must be scheduled for orthopaedic surgery (e.g. hip or knee replacement surgery, limb amputations and shoulder replacement) before enrolment in the study. This surgical intervention for the patient's underlying condition will be completed during the study; however, the surgical procedure itself is not a study-driven intervention.

The scheduled skin biopsy is the only interventional procedure to be performed to patients participating in this study (i.e. the only procedure performed for study purposes and not part of the otherwise necessary care of the patients). This biopsy will be performed during the scheduled orthopaedic surgical procedure. Bone samples will be collected from a surgically removed bone specimen and therefore not considered being a study-related intervention. If a patient is scheduled for a second orthopaedic surgical procedure that would qualify for the collection of bone and skin as described in this section (e.g. hip or knee surgery), additional bone and skin samples will be collected.

Bone tissue sampling:

Bone tissue will be collected from bone removed during the scheduled orthopaedic surgical procedure. A portion of the removed bone (e.g. the heads of femoral or tibial bone, whichever is removed during surgery), will be further processed ex-vivo. At surgical site, two aliquots will be taken, each with a minimum weight of 15 g. Further bone sample handling will be done in the bioanalytical central laboratory to harvest two samples from each aliquot, one consisting of trabecular and the other of cortical bone. Bone samples will precisely be weighed and analysed in the central bioanalytical laboratory (for the concentration of Gd, Ca, P, Na, Zn, K and Fe).

Skin tissue sampling:

Skin samples will be collected from the incisional site made for the scheduled orthopaedic surgical procedure or amputated part if applicable. A parallel linear sample of tissue will be cut from one edge of the incision wound to the other. It should be 2 mm thick, and as long as the incision (typically at least 10 cm of length, according to surgical practice). Depth should be the depth of the skin to the fascia (4 mm is a conservative estimate; the linear incision will be no longer or deeper than it would have been without removing a sample). Since thickness and depth of skin tissue slices are kept constant, the maximum amount of skin tissue collected is defined by the length of the surgical incision. The wound is closed in accordance to the sites' clinical practice.

From the linear sample, two aliquots of 5 mm length each are required for later histopathological analysis and will be evaluated in the central histopathology laboratory by an experienced and independent (blinded) dermatopathologist in order to identify any skin changes associated with NSF.

The remaining linear sample will be divided into two aliquots for later bioanalytical analysis (yielding approximately 320 mg of fresh tissue per aliquot). The aliquots will be weighted and analysed in the central bioanalytical laboratory for the concentration of Gd, Ca, P, Na, Zn, K and Fe.

5.2 Method of assigning patients to treatment groups

No randomisation of patients to treatments applies. However, ad-hoc stratification of patients to the enrolment groups will be managed centrally (see 5.5).

5.3 Selection of dose and timing for each patient

This protocol focuses on the exploration of long-term Gd-retention in patients exposed to GdCAs. There is no upper limit for the time-interval between GdCA administration and sample collection during the scheduled orthopaedic surgical procedure, based on prior evidence that Gd may be detectable in bone 8 years after injection of GdCA (21). In this protocol, the time interval between the last dose of GdCA and surgery must be at least 1 month. The enrolment of patients with a wide range of times between GdCA injection and time of tissue sample collection during surgery may support the evaluation of long-term Gd-retention.

The study will evaluate for the subgroups with a history of single GdCA dose administration only patients who received the approved standard dose for each of the GdCAs. For the products with the active ingredients Gadobutrol, Gadodiamide, Gadopentetic acid, Gadoteric acid, and Gadoversetamide this is 0.1 mmol per kg body weight. For Gadoxetic acid, a single dose is 0.025 mmol per kg body weight.

Patients in the respective multiple doses subgroups will have received either repeated doses or a single or multiple higher than the approved standard dose, and thus higher cumulative concentrations of the same GdCA. Any higher dose than 120% of the standard dose of GdCA will be analysed as part of the multiple doses subgroup. In both dose subgroups, the individual and total combined doses administered will be documented and evaluated.

5.4 Supply, packaging and labelling

Since qualifying GdCA was administered prior to patient enrolment, no supply of any study medication is required in the present study. Therefore, neither storage, shipment, packaging, nor labelling are applicable.

5.5 Blinding and randomisation

There will be no randomisation. The analysis of bone and skin tissue samples will be done in a blinded manner.

Patients will be registered centrally via an interactive web response system (IWRS). This system will be connected with the eCRF and will be accessible 24 hours/7 days a week. Each patient will be assigned to a stratified enrolment group defined by the GdCA ingredient administered (or control), renal function category (moderate impairment, severe impairment or stable normal renal function eGFR > 60 ml/min/1.73 m^2) and dosing number (single / multiple). Confirmation of patient enrolment will be provided to the enrolling centre and shall be retained by the investigator.

Once a stratification group is complete, no further enrolment into that group will be allowed. All participating centres will be notified upon the completion of each enrolment group.

The data of all patients on study will be de-identified by allocation of a patient number to each patient enrolled. No identifying information will be contained in the study eCRF. Patient numbers will be issued sequentially to each centre upon patient enrolment.

5.6 Prior and concomitant therapy

Concomitant therapies and medications administered during the study period are to be documented. The recording should be limited to diseases, medications that are relevant to the setting, e.g. osteoporosis, hormone therapy, etc. (see 7.2.2). This also includes all medical and non-medicinal treatment and hormonal contraception. The following details are to be recorded in the source documentation: disease indication, date of diagnosis, name of each medicine (including active ingredient(s)) or specification of measures, dosage, route of administration and the dates of the start and end of treatment.

The investigator should advise each patient to undergo other medical treatment only after having consulted the investigator - except for emergencies.

5.6.1 Prohibited previous treatments

- GdCA within 1 month prior to the date of the scheduled orthopaedic surgical procedure.
- Intra-articular administrated GdCAs or per any other non-i.v. route.
- Combination of different GdCAs.

5.6.2 Admissible concomitant treatments

- Medically indicated, non-GdCA treatments

5.6.3 Prohibited concomitant treatments

- GdCA re-administration prior to completion of all surgeries and tissue collection procedures on study

5.7 Treatment compliance

Only patients who have received one of the qualifying GdCAs and for whom a full history of GdCA administration and dosage is available, are considered for enrolment into one of the respective test groups of this study. In this regard treatment compliance reflects the adherence to the protocol inclusion criteria and not to be assessed separately.

5.8 Management of drug overdose

Not applicable

6 VARIABLES AND METHODS

6.1 Evaluation

6.1.1 Primary evaluation variable(s)

- Concentration of total Gd in trabecular bone (determined by ICP-MS).
- Concentration of total Gd in cortical bone (determined by ICP-MS).

6.1.2 Secondary evaluation variable(s)

- Concentration of total Gd in skin tissue samples (determined by ICP-MS), collected at the time of the scheduled orthopaedic surgical procedure, from a biopsy from the edge of the surgical wound or the amputated part.

- Concentrations of calcium, phosphorus, sodium, iron, zinc and potassium in bone (both trabecular and cortical) and skin tissue samples (determined by ICP-MS or alternatively ICP-AES if the feasibility evaluation of ICP-MS does not show reliable results).
- Histopathological evaluation of skin samples with regard to the possibility of findings associated with NSF (determined by an experienced dermatopathologist).
- Description of potential co-factors for NSF, susceptibility factors and drug treatments with potential impact on bone metabolism.

Please note: Sample handling, analysis and dosage will be performed under blinded conditions at the central sites.

6.1.3 Evaluation methods – Bioanalytics

6.1.3.1 Sample collection and handling:

Sample collection for the bioanalytical quantification of Gd and the other analytes will be prepared with a sample collection kit. Precise description of the collection procedures, including sample harvesting, sample size, transfer of specimens into collection tubes, and sample storage until transport to the central bioanalytical laboratory will be provided. The Sponsor will take care of secure transportation of the samples under adequate transport conditions. The transport conditions will be adjusted according to the test results obtained by the bioanalytical central lab during method set-up. All bone and skin tissue samples will be identified by a numbering system that does not reveal any information on the patient's identity or history to the blinded investigators in the central bioanalytical laboratory (Manipal Acunova, Bangalore, India).

- Sample collection: Samples of trabecular and cortical bone must be harvested from bone removed during the scheduled orthopaedic surgical procedure, according to the type of surgery performed on the individual patient. Skin will be collected during surgery by a biopsy of the edge of the surgical incision or the amputated part. If the patient is scheduled for a second orthopaedic surgical procedure within the study period, additional bone and skin samples will be harvested.
- Tissue samples will be kept frozen at at least -20 °C during storage at sites and during the transport to the central bioanalytical laboratory. The weight of the samples will be measured in the central bioanalytical laboratory.
- Bone and skin sample processing and analysis will be performed under blinded conditions at the central bioanalytical laboratory.
- Central laboratory sample storage: tissue samples are kept frozen at at least –20°C until analysis.
- A detailed manual of sampling handling procedures and transport logistics will be provided.

6.1.3.2 Method description

A ICP-MS bioanalytical method (based on (19;24;25)) will be developed and validated according to EMA and FDA requirements (26;27) for the quantification of Gd and other specified analytes, including calcium, phosphorus, sodium, iron, zinc and potassium in tissue samples. The validated methods will be employed for the study sample analysis. In case that the reliable analysis of all planned analytes is not possible with the ICP-MS (based on the method setup results), alternative methods for analysis of some of the parameters will be done with an ICP-AES technique (18;28).

6.1.3.3 Validation protocol

The bioanalytical method to quantify Gd, Ca, P, Na, Zn, K and Fe in skin and bone tissue will be developed and validated according to current regulatory requirements and guidelines (26;27).

6.1.4 Evaluation methods – Histopathology

Sample collection for the histopathological analysis will be performed using a sample collection kit. Precise description of the collection procedures, including sample harvesting, transfer of specimens into collection tubes, and sample storage until transport to the central bioanalytical laboratory will be provided. The Sponsor will take care of secure sample transportation under adequate transport conditions. The skin tissue samples will be identified by a numbering system that does not reveal any information on the patient's identity or history to the blinded investigators in the central histopathology laboratory Yale Dermatopathology, New Haven, USA.

6.1.4.1 Sample collection and handling:

- Sample collection: Skin will be collected during the orthopaedic surgical procedure by a biopsy from the edge of the surgical incision or the amputated part. If the patient is scheduled for a second orthopaedic surgical procedure within the study period, an additional skin sample will be harvested.
- Skin specimens will be fixed and preserved in formaldehyde solution for later paraffin embedding and histopathological examination at the central histopathology laboratory. Samples will shipped under ambient conditions to the central histopathology laboratory. There the paraffin embedding will be completed, further stabilising the samples until analysis.
- A detailed manual of sampling handling procedures and transport logistics will be provided.

6.1.4.2 Method description

The histopathological analysis will be completed by an experienced dermatopathologist in the central laboratory at the Yale Dermatopathology, New Haven, USA. Methods will include routine light microscopy evaluation of skin biopsy samples (stained e.g. for mucin and elastic fibres) and immunostaining for CD34-positive "circulating" fibrocytes (4;25;29). Electron microscopy for ultra-structural analysis and in situ localisation of Gd will not be performed (29).

6.1.4.3 Reporting of results

The results of the individual histopathology parameters, including a dermatopathological expert statement, will be reported to the respective study centres and to EA, allowing assessment of potential AEs, medical review and further data handling. The results of the individual histopathology parameters will be transferred to EA data management electronically, considered for descriptive statistics and included in the data listing of the study report. However, the study centres will not enter the results into the patient specific eCRF.

6.1.5 GFR estimate

Various methods have been previously established for the estimate of GFR. Most commonly, the Cockgroft/Gault method, the Modification of Diet in Renal Disease (MDRD) or the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) methods are used (30). In the present study, any of the clinically accepted, serum creatinine-based methods of GFR

estimation will be accepted as indicator of renal function. In case eGFR was not computed prior to GdCA administration, eGFR will be estimated by the investigator at enrolment based on serum creatinine, age, sex, biometric and/or other laboratory data required for the application of one of the above-mentioned eGFR formulas.

6.2 Safety

6.2.1 Vital signs

No vital signs will be measured for this study.

6.2.2 Laboratory variables

No routine clinical laboratory tests will be performed in this study.

6.2.3 Additional safety assessments

It will be left to the investigator's discretion whether safety assessments in addition to the documentation of AEs as outlined below (6.2.4) are required. Unless useful for specific purposes (e.g. individual serious adverse events), the data gained from these additional safety assessments will not be part of the study's data base. However, if the investigator considers the finding to be clinically significant and of importance regarding safety assessment (e.g. severe bleeding following surgery), it must be documented in the eCRF as an adverse event.

6.2.4 Adverse events

After signature of the informed consent form the investigator will ask the patient to report any significant clinical abnormality and will monitor the patient's medical records. Any AE occurring from the date the patient enrols in the study until follow-up visit 2 (visit 4) taking place approximately within 10-14 days post-surgery (at the time of potential stitch removal[3] under normal healing conditions) after the scheduled orthopaedic surgical procedure, must be documented by the investigator in the eCRF. Complications related to the operation wound and its healing will be followed-up as an adverse event until consolidation. In case the patient is scheduled for a second orthopaedic surgical procedure that would qualify for the collection of bone and skin as described in this protocol in section 5.1.3, AE monitoring will be handled like the AE monitoring of the first surgery.

AEs of interest are: NSF-like events after enrolment, wound related problems, and Serious Adverse Drug Reactions (SADRs).

All AEs, including intercurrent illnesses, must be documented in detail, including the date and time of occurrence, the date of event resolution and the outcome (6.2.4.2). The related treatments of the event will be documented in the eCRF as well as event intensity, potential causal relationship to any of the categories specified below (6.2.4.2) and seriousness of the AE (6.2.4.2).

6.2.4.1 Definition of adverse events

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can, therefore, be any unfavourable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

[3] Note: Any other wound closure technique used according to the local practice is acceptable.

6.2.4.2 Assessment of adverse events

Each AE will be assessed by the investigator regarding potential causal relationship with one or more of the following categories:

- (1) related to the orthopaedic surgery (bone resection included).
- (2) related to the skin biopsy.
- (3) related to GdCA exposure in the past (e.g. NSF signs after enrolment).
- (4) related to other health conditions of the patient.

The investigator must assess each AE as either **unrelated** or causally **related** to any of the categories (1) – (4). Related means possibly, probably or certainly related.

Each AE will be characterised by the investigator according to intensity of the event and whether any of the seriousness criteria apply. The definitions are given below:

Intensity

Regardless of the classification of an AE as serious or non-serious (see below), its intensity must be assessed according to medical criteria alone (taking into account the patient's limitations in routine activities due to the surgical indication before or status after joint replacement) using the following categories:

Mild: does not interfere with routine activities of the patient in the review or opinion of the investigator

Moderate: interferes with routine activities of the patient in the review or opinion of the investigator

Severe: impossible to perform routine activities of the patient in the review or opinion of the investigator

It should be noted that a severe AE does not necessarily have to be serious in nature and that a serious adverse event need not be severe.

Seriousness

A serious adverse event (SAE) is an AE that, at any dose,:

- results in death;
- is life-threatening;
- requires in-patient hospitalisation or prolongation of existing hospitalisation unless it lasts less than 12 hours, or hospitalisation is pre-planned (e.g. standard of care or for respite care);
- results in persistent or significant disability/incapacity;
- is a congenital anomaly or birth defect

or

- is an important medical event that does not have to be immediately life-threatening or result in death or hospitalisation, but which may jeopardise the patient or may require medical intervention to prevent one of the outcomes listed above.

NSF-like events will be considered medically important events, and thus are serious AEs.

All SAEs must be reported by the investigator immediately (within one working day) to the pharmacovigilance team of EA (page 4). Unreported SAEs (e.g. not noticed by the investigator) must be reported by the Clinical Research Associate (CRA) directly to the pharmacovigilance team of EA. A study-specific SAE reporting form is to be used.

All AEs that do not fall into any of the above categories are defined as non-serious.

Expectedness

Expectedness for potential AEs in response to GdCA exposure in the past (e.g. NSF signs after enrolment), category 3 of the list above: The Summary of Product Characteristics (SmPC) of the respective GdCA as applicable locally and at the time of the event is considered as reference document of expectedness.

Expectedness for potential AEs related to the interventional part of the study (skin biopsy), category 2 of the list above, or in a broader sense to the participation in the study: Medical judgement will be applied by the investigator. If the event is considered related to the skin biopsy - or in a broader sense to the participation in the study - and it is reasonably expected according to the medical knowledge, then it will be considered expected for the study.

Outcome

The outcome of the AE is to be documented as follows:

- Recovered.
- Recovered with sequelae.
- Not recovered yet.
- Fatal.
- Unknown.

6.2.4.3 Follow-up of adverse events

Regardless of the duration of the study, each AE must be followed up until it resolves, the cause is documented, or an adequate final assessment can be given. If follow-up is not performed or possible, justification must be given by the investigator.

6.2.4.4 Documentation and reporting of adverse events

Any AE occurring while a patient is in the study (from informed consent until follow-up visit 2 (visit 4)[4] taking place approximately within 10-14 days post-surgery (at the time of potential stitch removal under normal healing conditions[5]) after the surgical intervention must be documented and reported by the investigator. Complications related to the surgical wound and its healing will be followed-up as an adverse event until consolidation. Each AE must be documented in the eCRF according to the requirements set forth in section 6.2.4.2.

[4] Note: If the patient is scheduled for a second orthopaedic surgery procedure that would qualify for the collection of bone and skin as described in the protocol, end of study is the follow-up visit 4 (visit 8).

[5] Note: Any other wound closure technique used according to the local clinical practice is acceptable.

7 STUDY CONDUCT

7.1 Flow-chart

Table 1: Schedule of Procedures and Assessments

	Visit 0 Screening Visit	**Visit 1 (V1) Baseline** (within 3 days prior to surgery)	**Visit 2 (V2) 1st surgery** (day 0)	**Visit 3 (V3) Follow-up 1** (3-5 days after surgery)	**Visit 4 (V4) Follow-up 2** (approximately within 10-14 days after surgery)	**If applicable: Patients receiving a 2nd surgery only**			
						Visit 5 (V5)* Baseline (within 3 days prior to surgery)	**Visit 6 (V6)* 2nd Surgery** (within study timeframe)	**Visit 7 (V7)* Follow-up 3** (3-5 days after surgery)	**Visit 8 (V8)* Follow-up 4** (approximately within 10-14 days after surgery)
Patient information and signature on Medical Data Release Form	X								
Patient information, written informed consent		X							
Written informed re-consent, if applicable						X			
Demographic data	X								
Diagnosis and medical history		X				X			
Inclusion and exclusion criteria	X	X				X			
Renal status (retrospective with regard to GdCA administrations)	X								
GdCA history / verification of GdCA history	X	X	X			X	X		
Connection to IWRS for stratified enrolment		X							
Concomitant diseases and therapies	X	X	X	X	X	X	X	X	X
Study-specific parameters:									
Bone sample collection			X				X		
Skin sample collection			X				X		
Visual check of wound/scar				X	X			X	X
Adverse events		X	X	X	X	X	X	X	X

* Additional visit for patients scheduled for second orthopaedic surgical procedure within study timeframe In case no second surgery is planned at completion of visit 2, the study ends with the end of the follow-up visit 2 (V4)

7.2 Evaluations and procedures by visits

7.2.1 Screening Visit (Visit 0)

The investigator will use the inclusion and exclusion criteria (section 4.1 and 4.2) to determine the eligibility of each candidate patient for enrolment in the study. Patients who are eligible and willing to participate in the study will receive appropriate information and sign a Medical Data Release Form allowing the screening of out-clinic patient files prior to enrolment.

At the screening examination the following will be carried out and/or documented:

- date and time of visit;
- check of inclusion and exclusion criteria;
- demographic data (height, weight, age, sex);
- diagnosis of qualifying study indication (*specify duration, severity as requested)*;
- retrospective renal status (eGFR value(s) at time point of first and (if applicable) subsequent GdCA administration, including date(s) of sampling);
- GdCA history, i.e. prior GdCA administration (brand name of product(s), date(s) of administration, dosing, and route of administration and patient's body weight determined within 4 weeks prior to GdCA administration);

Patients meeting the study entry criteria will complete the baseline visit of the study.

Note: *The screening visit 0 can be combined with the baseline examination (Visit 1) if feasible for the site / patient and if in accordance with local regulations*

7.2.2 Baseline examination (Visit 1)

At the baseline examination the patient will sign the Informed Consent Form when it has been validated that the patient is suitable for study participation and ample time has been ensured for the subject to decide. Afterwards, the following will be carried out and/or documented:

- date and time of visit;
- verification of inclusion and exclusion criteria (if baseline visit is performed separately from screening visit 0);
- GdCA history: verification that no GdCA has been given between the visits (not applicable if Visit 0 and Visit 1 are combined);
- connection to IWRS (via eCRF system) for stratified enrolment;
  Note: In cases where the applicable stratified enrolment group is completed, the patient must be withdrawn from the study.
- relevant medical history and concomitant disease(s) (including date of diagnoses), concomitant therapies (brand name, dose, route, frequency, start and stop dates);
- other relevant medical history and past therapies since first GdCA administration(s), where possible, including:
  - vascular, thrombotic and/or pro-inflammatory events;
  - serum concentrations of calcium, phosphorus, sodium, iron, potassium and zinc;
  - any event which may have led to iron mobilization, including concomitant iron drug;
  - cumulative dose of erythropoietin;
  - T4 and TSH;
  - Drug treatments with potential impact on bone metabolism, including long-term chronic drug treatments (i.e. biphosphonates, estrogens, calcitonin, calcitriol, fluoride salts, vitamin D, corticosteroids);
- AEs (from informed consent on);

- a physical examination of the skin for any clinical signs of NSF according to clinical routine (4).

In addition to the general documentation of concomitant diseases, bone-related diseases in the medical history should especially be focussed on (e.g. osteoporosis, fractures, menopausal effects on bone, etc.) with the exception of suspected or diagnosed tumour of the skeletal system , metastatic bone and bone marrow disease (which are exclusionary).

7.2.3 Scheduled orthopaedic surgical procedure (Visit 2)

On the day of surgery the following will be carried out and/or documented:

- date and time of visit;
- check for withdrawal criteria;
- GdCA history: verification that no GdCA has been given between the visits;
- relevant changes in concomitant disease(s) (including date of diagnoses), relevant changes in concomitant therapies (brand name, dose, route, frequency, start and stop date)*;
- AEs;
- bone sample collection;
- skin sample collection;
- schedule next appointment or study visit date.

7.2.4 Follow-up visit 1 (Visit 3)

This visit should be performed in connection with a routine wound inspection (about 3-5 days after surgery). The following will be carried out and/or documented:

- date and time of visit;
- relevant changes in concomitant disease(s) (including date of diagnoses), relevant changes in concomitant therapies (brand name, dose, route, frequency, start and stop date)*;
- inspection of the surgery wound in order to assess if the wound healing process can be assessed as normal (any finding needs to be documented as AE);
- AEs;
- schedule next appointment or study visit date.

7.2.5 Follow-up visit 2 (Visit 4)

This visit should be performed approximately within 10-14 days (at the time of potential stitch removal under normal healing conditions[6]) but not later than 18 days post-surgery.

The following will be carried out and/or documented:

- date and time of visit;
- relevant changes in concomitant disease(s) (including date of diagnoses), relevant changes in concomitant therapies (brand name, dose, route, frequency, start and stop date)*;
- inspection of the surgery wound in order to assess if the wound healing process can be assessed as normal (any finding needs to be documented as AE);
- AEs;

*AE related changes or bone-disease related changes should be considered as relevant

[6] Note: Any other wound closure technique used according to the local practice is acceptable.

- end of study documentation or in case that a second orthopaedic surgical procedure is planned surgery date and appointment for the 2nd baseline visit.

7.2.6 2nd surgery baseline examination (Visit 5) – if applicable

For patients scheduled to receive a 2nd orthopaedic surgical procedure (that would qualify for the collection of bone and skin as described in this protocol) within the study period, the following will be carried out and/or documented:

- date and time of visit;
- re-consent: the patient should be asked to confirm with actual date and signature that the provided written informed consent is still valid;
  (Note: If the patient does not agree to re-consent, the patient must be withdrawn from the study.)
- verification of inclusion and exclusion criteria;
- GdCA history: verification that no GdCA has been given between the visits;
- relevant changes in concomitant disease(s) (including date of diagnoses), relevant changes in concomitant therapies (brand name, dose, route, frequency, start and stop date) *;
- AEs;
- a physical check of skin for any clinical signs of NSF according to clinical routine (4).

In addition to the general documentation of concomitant diseases, bone-related diseases other than the study indication shall especially be focussed on.

7.2.7 2nd orthopaedic surgical procedure (Visit 6) – if applicable

On the day of the second surgery the following will be carried out and/or documented:

- date and time of visit;
- check for withdrawal criteria;
- GdCA history: verification that no GdCA has been given between the visits;
- relevant changes in concomitant disease(s) (including date of diagnoses), relevant changes in concomitant therapies (brand name, dose, route, frequency, start and stop date)*;
- AEs;
- bone sample collection;
- skin sample collection;
- schedule next appointment or study visit date.

7.2.8 Follow-up visit 3 (Visit 7) – if applicable

This visit should be performed in connection with a routine wound inspection (about 3-5 days post-surgery). The following will be carried out and/or documented:

- date and time of visit;
- relevant changes in concomitant disease(s) (including date of diagnoses), relevant changes in concomitant therapies (brand name, dose, route, frequency, start and stop date)*;
- check of the surgery wound in order to assess if the wound healing process can be assessed as normal (any finding needs to be documented as AEs);
- AEs;
- schedule next appointment or study visit date.

* AE related changes or bone-disease related changes should be considered as relevant

7.2.9 Follow-up visit 4 (Visit 8) – if applicable

This visit should be performed approximately within 10-14 days (at the time of potential stitch removal under normal healing conditions[7]) but not later than 18 days post-surgery.

The following will be carried out and/or documented:

- date and time of visit;
- relevant changes in concomitant disease(s) (including date of diagnoses), relevant changes in concomitant therapies (brand name, dose, route, frequency, start and stop date)*;
- inspection of the surgery site in order to assess if the wound healing process can be assessed as normal (any finding needs to be documented as AE);
- AEs;
- end of study documentation.

7.3 Duration of the study

The study is scheduled to start in March 2013 and will be completed with the LPLV in October 2017. Enrolment will be competitive over all study centres and per stratified enrolment group. As soon as a stratified enrolment group is completed, centres will be informed that the respective group is closed, and enrolment shall continue for the remaining open enrolment groups.

7.3.1 Planned duration for the individual patient

For each participating patient, the study lasts until the second follow-up visit (visit 4) is completed. If a patient is scheduled for a second orthopaedic surgical procedure (to be performed within the overall study period), the duration of participation will be extended for that patient until completion of the last follow-up visit (visit 8).

7.3.2 Premature termination

Study

At any time, the study as a whole may be terminated prematurely by the Sponsor, at the Sponsor's discretion, if important reasons call for this step.

Centre

At any time, an individual centre participating in the study may be excluded, at the Sponsor's discretion, for the following reasons:

- The centre fails to comply with the requirements of the protocol.
- The centre fails to comply with GCP standards.
- No patients are enrolled by the centre within a reasonable period after initiation of the centre.

In addition, each centre shall have the right to discontinue (reasonable written notice expected) participation if the study can no longer be carried out for organisational or other reasons.

Patient

Individual patients shall be withdrawn from the study according to the criteria specified in section 4.3, including termination due to suspected breach.

[7] Note: Any other wound closure technique used according to the local practice is acceptable.

8 STATISTICS

8.1 Statistical and analytical plans

8.1.1 Analysis populations

All patients who have undergone the planned orthopaedic surgical procedure will be included in the safety analysis (safety population).

The primary analysis will be performed on the full analysis population (FAS) and the per protocol (PP) population (see definitions below).

Definition of analysis populations

Population	Description
Screening population	All patients who entered the study
Safety population	All patients who have undergone the planned orthopaedic surgical procedure
Full analysis population (FAS)	All patients in the safety population for whom Gd measurements from the bone are available
Per protocol (PP) population	All patients in the full analysis population who meet the defined requirements of GdCA treatment history, all inclusion and exclusion criteria, and whose tissue samples yield data for Gd in bone (trabecular as well as cortical) and skin. The evaluability of patients /samples shall be assessed by a blinded data review prior to final unblinding and analysis.

The primary analysis will be conducted on the per protocol population.

The full analysis population will be used for sensitivity analyses, as the FAS could potentially encompass patients not fulfilling the requirements for addressing study objectives.

8.1.2 Primary and secondary analyses

Statistical methods:

Study data will be analysed descriptively. No formal hypothesis testing is planned.

All parameters will be analysed for the per protocol population using descriptive statistics including confidence intervals whenever appropriate. Details will be defined in a statistical analysis plan (SAP), which will be signed prior to unblinding of the samples.

Descriptive statistical methods will be used to summarise the results of the study. The following descriptive statistics will be calculated for continuously distributed data and for ordered categorical data (ordinal data) if applicable:

- N (number of non-missing measurements)
- Arithmetic mean
- Standard deviation
- Coefficient of variation (CV),
- Geometric mean

- Geometric standard deviation (re-transformed standard deviation of the logarithms)
- Corresponding CV
- Minimum
- Median
- Maximum

For ordered categorical data and nominal data, absolute and relative frequencies (in %) will be calculated.

Group or stratification variables due to the study design are:

- GdCA test group or control
- Renal function category (moderate impairment, severe impairment or stable normal renal function eGFR > 60 ml/min/1.73 m^2, Figure 3)
- Single (one injection of standard dose) or multiple (more than one injection or one injection with higher than standard) doses of GdCA administered

The elapsed time from dosing of GdCA until surgery will be classified and will also be used for stratification.

Statistical tables will compare all GdCA groups pooled as well as each GdCA group separately versus the control group. They will be further stratified according to renal function category (3 groups), single / multiple doses of all patients (2 groups), and the combination of "renal function with "single / multiple doses".

The primary analysis encompasses the evaluation of Gd concentration in trabecular and cortical bone. The secondary analysis deals with Gd concentration in skin as well as the parameters specified below.

Descriptive analyses include

- Gd concentration in bone (trabecular and cortical) and skin will be expressed as nmol Gd / g bone or skin.
- Gd concentration in bone (trabecular and cortical) and skin as a function of time elapsed between GdCA injection and surgery. Additional stratification classifications of time after dosing of Gd will be specified in the SAP.
  ➢ Analyses will be performed separately for trabecular bone, cortical bone, and skin.
- Time elapsed between GdCA injection and surgery, cumulative GdCA dose, number of doses and time intervals of dosing.
- eGFR.
- Potential co-factors for NSF and susceptibility factors, based on the following data and in the limit of their availability in medical history of the patients:
  - eGFR;
  - cumulative dose of GdCA,
  - co-existing vascular, thrombotic and/or pro-inflammatory events;
  - serum concentrations of calcium, phosphorus, sodium, iron, potassium and zinc;
  - any event which may have led to iron mobilization, including concomitant iron drug;
  - cumulative dose of erythropoietin;
  - T4 and TSH.
- Concomitant drug treatments with potential impact on bone metabolism: biphosphonates, estrogens, calcitonin, calcitriol, fluoride salts, vitamin D, corticosteroids.

- Concentration of Ca, P, Na, Zn, K and Fe in skin and in tissue of both trabecular and cortical bone, determined by ICP-MS (or ICP-AES if the feasibility evaluation of ICP-MS does not show reliable results).
- Histopathological evaluation of skin samples (taken at time of surgery / surgeries) with regard to the possibility of findings associated with NSF by an experienced dermatopathologist under blinded conditions at a central site.

8.1.3 Analysis of safety data

The AEs will be encoded using the MedDRA thesaurus in the most recent version available at study start. This version will be used throughout the entire study without subsequent update. Frequency tables for the preferred terms (PTs) will be compiled, based on patients experiencing an AE and based on the number of AEs. These frequency tables will also be stratified by system organ class (SOC).

Concomitant diseases and medical history will also be encoded using MedDRA. These data will be presented applying frequency tables.

Previous and concomitant medication will be encoded using the WHO-DD dictionary. Frequency tables will be compiled based on medication coding.

8.1.4 Missing data

Missing values will not be replaced.

8.1.5 Multicentre study

This study will be performed as a multinational multicentre study. Due to the small sample size of each stratification group, results will not be further stratified by country or centre.

8.1.6 Subgroup analyses

Due to the small number of patients in each stratification subgroup, special subgroup analyses are not planned.

The patients who have undergone two surgeries on study will be analysed as a subgroup, at least for Gd concentration in bone and skin. Descriptive statistics will be calculated for the difference of Gd concentration in bone (trabecular and cortical) and in skin at the time of first and the time of second surgery. Stratification of the results will depend on the number of patients in this subgroup (8.1.2).

8.1.7 Patient data listings

All recorded data will be presented in patient data listings.

8.1.8 Deviations from the planned statistical analysis

Any deviations from the planned statistical analysis shall be defined in the statistical analysis plan prior to unblinding the sample results. They shall also be described and justified in the final study report. If a deviation has an impact on the primary analysis, the Sponsor may elect to document such deviation in a protocol amendment.

8.1.9 Interim analysis

An interim analysis will be carried out on all patients enrolled in the single dose subgroups for Gadobutrol, Gadoteric acid, Gadodiamide and Gadopentetic acid once at least three patients have been recruited to all the single dose exposure subgroups for the 4 agents.

The interim analysis will be based on the PP population to determine the Gd concentration in bone (trabecular and cortical) and skin (expressed as nmol Gd / g). Gd concentrations will be descriptively evaluated (as defined in section 8.1.2) by:

- Renal function status: Stable normal renal function (eGFR > 60 ml/min/1.73 m2), impaired renal function (eGFR <30 ml/min/1.73 m2 (severe) and ≥30 to <=60 ml/min/1.73 m2 (moderate)) versus control
- GdCA: Each of the 4 GdCAs (Gadobutrol, Gadoteric acid, Gadodiamide, and Gadopentetic acid) versus control

No statistical tests are to be calculated.

All data used in this interim analysis will be listed. All listings will include patient identifier, GdCA type, renal function, and will be sorted by GdCA type, renal function, and patient identifier.

Study Investigational sites and analytical laboratories will be blinded to the Interim Analysis results. Details on the interim analysis evaluation will be described in the Statistical Analysis Plan (SAP).

8.1.10 Software used for statistical analysis

The SAS software version 9.2 or higher will be used for the statistical analysis and for the reporting of this study.

8.1.11 Determination of sample size

There are no prior expectations or assumptions relative to the measured concentrations of Gd in the bone (primary objective) and in skin (secondary objective). Due to the exploratory nature of the study, the sample size was not determined based on formal statistical consideration, but rather on the feasibility of conducting the study as a whole. The study is intended to be analysed descriptively. No formal hypothesis testing is planned.

There are six groups for active GdCA ingredients (Gadobutrol, Gadodiamide, Gadopentetic acid, Gadoteric acid, Gadoversetamide, and Gadoxetic acid).

In each group, three categories of renal function (stable (normal) renal function (eGFR > 60 ml/min/1.73 m^2) versus impaired renal function (eGFR <30 ml/min/1.73 m^2 (severe) and ≥30 to <=60 ml/min/1.73 m^2 (moderate)) and two subgroups of dosing of GdCA (single / multiple) are to be evaluated.

Per active GdCA ingredient the recruitment targets are defined as follows

- 5 patients for stable normal renal function (eGFR > 60 ml/min/1.73 m^2) and single dose
- at least 3 patients and up to 5 patients for stable normal renal function and multiple doses
- 5 patients for impaired renal function (eGFR <=60 ml/min/1.73 m^2) and single dose
- up to 5 patients for impaired renal function (eGFR <=60 ml/min/1.73 m^2) and multiple doses. For Gadobutrol and Gadoteric acid, at least 3 patients are required. For Gadodiamide, Gadopentetic acid, Gadoversetamide, and Gadoxetic acid, there is no requirement to reach a specific number of patients to achieve study completion.

The total number of patients enrolled in these groups will be at least 84.

The number of subgroups in the GdCA-naïve population is 3 according to renal function category (i.e. severe or moderate impairment, and stable normal renal function). Five patients will be enrolled per each sub-group. Therefore, the total number of GdCA-naïve patients enrolled will be at least 15.

Consequently, a minimum of 99 patients in total shall be included in the study.

8.2 Data management

The data management will be performed using Oracle Clinical version 4.5.3 or later. Details will be defined in the data management manual, which will be signed by the Sponsor prior to start of the data entry.

9 DATA HANDLING AND DATA QUALITY ASSURANCE

9.1 Documentation

9.1.1 Patient identification list

All patients who have given informed consent to study participation – regardless if the patient has received any product under evaluation or not – shall be entered on the patient identification list by the assigned study staff, giving full name, initials, date of birth and patient number. The patient identification list will be kept in the Investigator's File (IF).

9.1.2 Source data and patient records

All data entered into the eCRF shall have corresponding source documentation available at the site.

9.1.3 Case report forms

This study will use an eCRF for data capture. The Oracle Clinical Remote Data Capture (RDC) System version 4.5.3 or later will be applied. An eCRF completion manual for the use of the RDC system will be provided prior to study start.

The system used shall be validated and is compliant with Food and Drug Association (FDA) Title 21 CFR part 11 and the requirements of ICH GCP.

For each patient, the investigator must enter all requested data and findings as they occur during the study in the eCRF. The eCRFs should be made available at all times for monitoring visits (even for visits arranged at short notice). The investigator must keep the eCRFs in good order and up to date so that they always reflect the latest observations on the patients enrolled in the study.

Any discrepancies in the eCRF data detected by the study personnel are to be resolved via data clarification forms (DCFs).

9.2 Direct access to source data/documents

Monitoring the study progress requires checking the eCRFs for completeness and clarity as well as their cross-checking with source documents. The CRAs are entitled to compare eCRF entries to source data and to inform the investigator about any errors and omissions. The investigator will provide direct access to source data/documents for the Sponsor's designated representatives (CRAs and auditors) as well as IRB/IRC members and regulatory inspections.

Regulatory authorities and/or the Sponsor's Clinical Quality Assurance Group may also carry out such source data checks and/or on-site audit inspections. They will be carried out giving

due consideration to data protection and medical confidentiality. The investigator is to give the Sponsor whatever support is necessary.

9.3 Monitoring

Monitoring will be conducted by Sponsor CRAs in accordance with the stipulations of chapter 5.18 of the ICH GCP Guideline.

During the course of the study, a CRA will visit each centre to review protocol compliance, compare eCRFs to corresponding patient medical records, and ensure that the study is being conducted according to pertinent regulatory requirements and the protocol. eCRF entries will be verified against source documentation in accordance with the applicable Standard Operating Procedures (SOPs) of the Sponsor. CRAs will maintain and protect the confidentiality of personal data of patients.

Data monitoring will include 100% source data verification of every patient's documentation.

For patients who are withdrawn from the study prior to surgery, only the written informed consent form shall be verified.

The CRA will determine whether all AEs and SAEs have been appropriately reported.

The Sponsor reserves the right to order special examinations of laboratory samples in case of doubt concerning the validity of individual patient data or the accuracy of data generation at an investigational centre.

9.4 Quality assurance

Pre-study site visits will be performed by representatives of the Sponsor in order to assure the eligibility of the study centres to implement the protocol, with specific focus on staff qualification and/or technical equipment requirements.

Furthermore it is planned that a member of the Sponsor's quality assurance unit will perform on-site audits (approximately one audit per active country). The auditor(s) will usually be accompanied by a CRA. The investigator will be informed about the outcome of any audit of their respective centre.

In addition, inspections by health authority representatives - including foreign authorities - and IEC(s) / IRB(s) are possible at any time. The investigator should immediately notify the Sponsor of any such inspection announcement.

As other planned quality measures:

- A protocol audit
- A TMF audit at the end of the study
- An audit of the final study report.

will be performed.

10 ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

10.1 General considerations

The procedures regarding the conduct, evaluation, and documentation of this study as described in this protocol are designed to ensure that the study is conducted according to GCP guidelines and under the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in accordance with FDA Title 21 CFR part 11 and in accordance with applicable local laws and regulations.

Each investigators of this study shall ensure that this clinical investigation is conducted in accordance with the signed investigator agreement, the study protocol, and all applicable regulations. The investigator is responsible ensuring that the rights, safety, and welfare of the study patients are protected at all times.

10.2 Approval procedures

Before the start of the study, the study protocol and/or other relevant documents will be approved by the appropriate ethics committee.

The relevant authorities, in accordance with local legal requirements will be notified/asked for approval of the intended study.

10.3 Protocol amendments

After initiation of the study, any change in this protocol will require a formal amendment. The amendment must be signed by all of the signatories to the original protocol. Once the study has started, amendments will be made only in exceptional cases.

All protocol amendments will be submitted to ethics committee(s) and the regulatory authorities, as required by national law.

Changes to the protocol may only be implemented after all appropriate requirements listed above (ethics, regulatory approval of all responsible personnel) have been fulfilled.

10.4 Informed consent

Before any patient can be enrolled or admitted into the study, their informed consent will be obtained according to the national regulatory and legal requirements. To this end, the investigator or an authorised designee will explain the nature, purpose, significance and scope of the study, including its potential risks, to the patient. In addition to this oral information, the patient will receive for his/her own records a written patient information sheet summarising the relevant information. Sufficient time will be allowed to discuss any questions raised by the patient. Only after this information has been provided can informed consent for participation be given. The consent form must be personally signed and dated by the patient giving consent, and it must be retained by the investigator as part of the study records. A copy of the signed informed consent form will be given to the patient.

The investigator will not undertake any investigation required for the clinical study until informed consent has been obtained. The terms of the consent and the date when it was obtained should also be entered in the eCRF.

After releasing an amendment to the protocol, the contents of which might influence the patient's decision for participation, the patient information sheet and the informed consent form must be amended accordingly. They must be submitted to the relevant ethics committee and the relevant authority as requested by local law. Depending on the nature of the amendment it might be necessary that patients already enrolled must confirm their informed consent on the basis of the new information by reviewing and signing the amended informed consent form.

10.5 Confidentiality

All local legal requirements regarding protection of personal patient data will be adhered to.

The anonymity of study patients will be maintained. Throughout documentation and evaluation, the patients will be identified on eCRFs and other documents by an identification (patient) number. Documents which identify the patient (e.g. the signed informed consent) must

be maintained in confidence by the investigator. The patients will be informed that all study findings will be stored on computer and handled in the strictest confidence.

Any results derived from the study and documents will also be regarded as confidential. The investigators and members of their research teams will be not be allowed to disclose such information without prior written approval from the Sponsor.

10.6 Liability and insurance

Where required by the laws and regulations of the country in which the study is performed, insurance of patients against health impairment occurring as a result of participation in the study will be obtained in accordance with the applicable laws and regulations. All relevant documentation regarding such insurance will be filed in the TMF and/or IF, as appropriate.

The General Insurance Conditions will be kept in the investigator's file and shall be made available for patients at any time that the centre is open.

10.7 Publication and use of study findings

By signing the study protocol, the investigator agrees with the use of results of the study for the purposes of national and international registration, publication and information for medical and pharmaceutical professionals. If necessary, the authorities will be notified of the investigator's name, address, qualifications and extent of involvement.

An integrated study report covering clinical and biometrical aspects will be prepared by the Sponsor.

All relevant aspects regarding publication will be part of the contract between the Sponsor and the investigator/institution.

10.8 Archiving of study records

Essential documents as listed in ICH GCP, chapter 8, will be retained for at least 15 years after study end. The documents may be retained for a longer period if required by other applicable regulatory requirements or by a separate agreement between the Sponsor and the investigator.

Patient medical records will be archived according to local regulations and in accordance with the maximum period of time permitted by the hospital, institution, or private practice.

The investigator’s file is not to be destroyed without the Sponsor’s approval. The investigator’s contract will contain all regulations relevant for the study centre. The Sponsor will inform the investigator when the prescribed period for archiving study documents has elapsed and the investigator no longer needs to retain the records relating to the study.

11 LIST OF REFERENCES

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12 APPENDICES

None.