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Datasheet for the decision of 16 June 2016

Case Number: T 0772/12 - 3.3.02

Application Number: 04758356.2

Publication Number: 1616184

IPC: G01N33/68

Language of the proceedings: EN

Title of invention:
A METHOD AND KIT FOR DETECTING THE EARLY ONSET OF RENAL TUBULAR CELL INJURY

Patent Proprietors:
Children's Hospital Medical Center
The Trustees of Columbia University

Opponent:
Dr Kilger Ute

Headword:
Detection of renal tubular cell injury/CHILDREN'S HOSPITAL AND COLUMBIA UNIVERSITY

Relevant legal provisions:
EPC Art. 123(2), 123(3), 56, 83, 107
EPC R. 115(2)
RPBA Art. 15(3), 13

Keyword:
Summons to oral proceedings - non-attendance of party
Amendments - allowable (yes)
Sufficiency of disclosure - (yes)
Inventive step - (yes)

Decisions cited:
T 0789/89, T 0629/90

Catchword:

Europäisches Patentamt
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Case Number: T 0772/12 - 3.3.02

D E C I S I O N
of Technical Board of Appeal 3.3.02
of 16 June 2016

Appellants: (Patent Proprietors)	Children's Hospital Medical Center 3333 Burnet Avenue Cincinnati, Ohio 45229 (US) The Trustees of Columbia University 500 West 120th Street New York NY 10027 (US)
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Respondent: (Opponent)	Dr Kilger Ute Meinekestrasse 26 10719 Berlin (DE)
Representative:	Kilger, Ute Boehmert & Boehmert Anwaltspartnerschaft mbB Patentanwälte Rechtsanwälte Pettenkoferstrasse 20-22 80336 München (DE)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 31 January 2012 revoking European patent No. 1616184 pursuant to Article 101(3)(b) EPC.**

Composition of the Board:

Chairman	L. Bühler
Members:	T. Sommerfeld
	K. Giebeler

Summary of Facts and Submissions

I. European patent No. 1616184, based on application No. 04758356.2, entitled "A method and kit for detecting the early onset of renal tubular cell injury" and published as international application WO 2004/088276, was granted with 29 claims.

II. Two oppositions were filed against the granted patent, both opponents requesting revocation of the patent in its entirety on the grounds of lack of novelty and inventive step (Articles 54(2) and 56 EPC and Article 100(a) EPC), lack of sufficiency of disclosure (Article 100(b) EPC) and added subject-matter (Article 100(c) EPC).

III. During the proceedings before the opposition division, the patent proprietors requested that the patent be maintained on the basis of a main request filed with letter of 29 December 2010 or, alternatively, on the basis of auxiliary requests 1 to 9 (filed as auxiliary requests 1A to 1D and 2 to 6) submitted with letter of 6 October 2011.

IV. The documents cited during the proceedings before the opposition division and the board of appeal include the following:

D1a Matthaeus et al. 2001, J.Am.Soc.Nephrol.12:787A abstract A4112
D1b Matthaeus et al. 2001, Kidney Blood Press. Res. 24:342; abstract P268
D2 Bläser et al. 1995, Clin.Chim. Acta 235:137-145
D5 Monier et al. 2000, Clin.Chim. Acta 299:11-23
D12 Yan et al. 2001, J.Biol.Chem. 276(40):37258-65
D13 WO 97/44460

D14 Haase et al. 2011, J.Am.Coll.Cardiol.57:1752-61
D15 Muramatsu et al. 2002, Kidney Int. 62:1601-1610
D16 Grenier et al. 2010, Clin.Biochem.43(6):615-620
D18 Emami et al. 1991, Am.J.Physiol. 260:F479-F485
D25 Daemen et al. 1999, J.Clin.Invest. 104:541-549
D26 US 6136526
D28 Mishra et al. 2005, Lancet 365:1231-1238
D29 Soni et al. 2010, Int.Urol.Nephrol. 42:141-150

V. By its decision pronounced at oral proceedings, the opposition division revoked the patent under Article 101(3)(b) EPC.

The opposition division decided that all claim sets fulfilled the requirements of Articles 123, 84, 83 and 54 EPC as well as of Rule 80 EPC, but not those of Article 56 EPC.

VI. The patent proprietors (hereinafter: the appellants) lodged an appeal against that decision. With the statement of the grounds of appeal, the appellants requested inter alia that the decision be set aside and that the patent be maintained according to the main request of 29 December 2010.

VII. With their replies to the grounds of appeal, the opponents requested that the appeal be dismissed and that the patent be revoked. Both opponents provided arguments as regards sufficiency of disclosure and inventive step. Additionally, opponent 2 objected to the patentability of the claimed subject-matter under Articles 54 and 123(2) EPC but did not further substantiate these objections.

VIII. Further letters from both opponents followed: opponent 1 withdrew its opposition and is thus no

longer a party to the proceedings, while opponent 2 (now the sole respondent) filed new documents.

IX. The board issued a summons for oral proceedings, scheduled for 16 June 2016. In the accompanying communication, the board summarised the case and raised objections under Article 123(2) EPC.

X. With letter dated 15 April 2016, the respondent announced that it would not be attending the oral proceedings.

XI. With letter dated 18 April 2016, the appellants re-submitted the main request and submitted new auxiliary requests 1 to 10.

XII. Oral proceedings before the board took place as scheduled, in the absence of the duly summoned respondent. During oral proceedings, the appellant withdrew the main request and auxiliary request 1, and pursued its auxiliary request 2 as main request.

The **main request** is thus identical to auxiliary request 2 as filed with letter of 18 April 2016. Independent claims 1, 5, 8, 20, 22 and 23 read as follows:

"1. A method for the detection of a renal tubular cell injury which is an ischemic renal injury in a human patient, comprising the steps of:
1) contacting a urine sample obtained from a human patient with an antibody for a biomarker consisting of NGAL, appearing within the first 24 hours of the onset of the ischemic renal injury, to allow formation of a complex of the antibody and NGAL; and
2) detecting the antibody-NGAL complex."

"5. A method according to claim 1 for further monitoring the effectiveness of a treatment for the renal tubular cell injury, comprising the further steps of:
3) contacting at least one post-treatment urine sample from the human patient experiencing the renal tubular cell injury and receiving a treatment therefore, with a capture antibody for NGAL to allow formation of a complex of the antibody and NGAL; and
4) detecting for the presence of NGAL in the post-treatment urine sample by detecting the antibody-NGAL complex."

"8. Use of a kit, comprising:
1) a means for acquiring a quantity of a urine sample from the human patient;
2) a media having affixed thereto a capture antibody capable of complexing with NGAL;
3) an assay for the detection of a complex of NGAL and the capture antibody, in a method according to claim 1."

"20. A method of identifying the extent of a renal tubular cell injury, which is an ischemic renal injury, caused by an event, comprising the steps of:
1) detecting in at least one urine sample obtained from a human patient the presence of a biomarker consisting of NGAL, appearing within the first 24 hours of the onset of the ischemic renal injury; and
2) determining the extent of the renal tubular cell injury based on the time for onset of the presence of NGAL in the urine sample, relative to the time of the event,
wherein the event is a surgical procedure."

"22. The method according to Claim 1 for the detection of ischemic renal injury wherein the urinary NGAL, measured within two hours of kidney transplantation, is predictive of acute renal failure."

"23. The method according to Claim 1 for the detection of post-operative acute renal failure in human patients after open heart heart surgery, wherein the urinary NGAL measured within two hours after surgery is predictive of acute renal failure."

Claims 2 to 4, 21 and 24 are dependent on claim 1, claims 6 and 7 are dependent on claim 5, and claims 9 to 19 are (directly or indirectly) dependent on claim 8.

XIII. The appellants' submissions may be summarised as follows:

Article 83 EPC

As stated by the opposition division in its decision, the claimed method could be readily put into effect by a skilled person in the light of the teaching of the patent and of common general knowledge in the art. Post-published documents D14, D16, D28 and D29 all demonstrated that NGAL acted as an early indicator of acute kidney ischemia.

Article 56 EPC

Document D15 was the closest prior art and differed from the claimed subject-matter in that Cyr61 rather than NGAL was used as biomarker. The fact that NGAL could be detected already in the first two hours of renal ischemia was an advantage over D15. The biomarkers of the prior art either appeared too late after the injury (e.g. serum creatinine and KIM-1) or their detection required the use of a time-consuming method. The technical problem should thus be the provision of an improved method for the early detection of an ischemic renal injury (IRI). There was however no requirement that the claim recited a time point at which the detection was to be made, which would imply that the skilled person had to know, before performing the method of the invention, that an ischemic renal injury had occurred. The method was advantageous because it provided for detection not only in those cases that would also be detected using Cyr61 but also in those cases that would not (i.e. those occurring only 2 hours after an ischemic injury). The method thus increased the likelihood that any ischemic injury would be detected (longer "detection window").

XIV. The arguments put forward in the written proceedings by the former opponent 1 and by the respondent (opponent 2) may be summarised as follows:

Article 83 EPC

The respondent argued that there was no guidance for the skilled person on how to detect or diagnose a renal tubular cell injury (RTCI) as an ischemic renal tubular cell injury (IRTCI), since in fact NGAL could also be present in the urine of subjects who were healthy or

had other diseases (e.g. D2, D5, D12, D29). NGAL was also an established diagnostic marker to distinguish bacterial from viral infection and known to increase with inflammation due to activation of neutrophils (D26: HNL being synonymous to NGAL). There was also no indication of the threshold levels for a diagnosis, which was particularly relevant for claim 20, directed to determining the extent of an IRTCI. The method could not be conducted over the entire breadth of the claims, because these were not restricted to any time point after the ischemic injury when the sample was to be taken. Such a time point was important as evidenced by D28 (Figure 1A and 1B, Figure 2), and should be between 2 and 4 hours after the ischemic event in order to have diagnostic value.

Former opponent 1 also considered that claim 1 included non-working embodiments, since both healthy individuals and individuals suffering from diseases other than IRTCI had NGAL in their urine (D2, D5, D12, D16) and the patent offered no reference value to distinguish such individuals from patients with IRTCI. Example 6 of the patent was evidence that the mere appearance of NGAL was not indicative of ischemic injury. Example 5 of the patent stated that NGAL was undetectable in healthy patients, but this could be due to the method used, as admitted by the inventor in D14 (page 1754, column 1, lines 11 to 15). Since claim 1 encompassed a single measurement, a cut-off value was needed, but this was not disclosed in the patent. The patent itself stated that multiple serial measurements of NGAL were necessary (paragraph [0032] last sentence).

Article 56 EPC

The respondent argued that the fact that the effect could not be obtained over the entire breadth of the claims was also detrimental for inventive step. Moreover it was known in the art that kidney tissue proteinuria could serve as a pool for potential biomarkers for IRTCI. The closest prior art was document D1. D1 disclosed overexpression of NGAL protein in a rat model for IRI in proximal tubuli tissue derived from a rat kidney, and disclosed that its mRNA and protein were more than 20-fold enhanced at 24 and 48 hours after IRI. The skilled person would thus also consider checking for NGAL in urine within the first 24 hours of onset of IRI, in particular in view of D15, disclosing that Cyr61 (also of proximal tubuli tissue origin) was present in urine within 3 to 6 hours after renal injury. NGAL was known as an inflammatory marker (D26), and since D25 showed that the inflammation process was the earliest event after the ischemia occurred and before IRTCI happened, the skilled person would hypothesise that NGAL was a very early marker. As regards the appellants' arguments concerning the need for a simple and rapid method for NGAL detection in urine, D26 had already disclosed an immunoassay to detect NGAL in liquid samples, with the only difference that NGAL was to be used as a bacterial inflammation marker.

Former opponent 1 argued that the opposed patent did not solve a technical problem, because detection of NGAL in the urine did not allow a diagnosis, since NGAL was a normal constituent of the urine. D1 was the closest prior art as it disclosed the upregulation of NGAL in IRTCI and was sufficient itself, in view of the common general knowledge at the priority date of the

patent, to destroy inventiveness of the claimed subject-matter. D13 or D15 could be taken as closest prior art as well. The presence of increased amounts of proteins in the urine was known to occur also in healthy subjects and had been used for decades to diagnose kidney disorders, including those caused by ischemia. D1 disclosed upregulation of NGAL following renal ischemia and detection of IRTCI with an antibody against NGAL. Although 24 and 48 hours were mentioned, the skilled person would know that de novo synthesis of proteins in kidney in response to ischemia was a rapid event (D18). The difference of the claimed subject-matter to D1 was that human urine samples were used. The technical problem could thus be formulated as the provision of a simpler, less invasive method of detecting IRTCI in humans employing the biomarker NGAL. In view of D1's data that high NGAL protein expression was detected in the most extensively damaged areas of the kidney, the skilled person would expect NGAL to leak into the urine, since cells disintegrated due to necrosis. D13, disclosing use of KIM-1 as a biomarker for renal injury, could also be regarded as closest prior art, the difference being the use of an antibody against another biomarker; the objective problem would thus be the provision of an alternative biomarker for the detection of IRTCI. D13 taught that any protein which was upregulated following ischemic injury was an IRTCI biomarker, and thus the skilled person would consider NGAL of D1. D15 could also be the closest prior art; it disclosed that Cyr61 was identified as an immediate early gene in a differential cDNA analysis taken 2 hours after induction of ischemic injury. In D15 it was hypothesised that genes induced very early following renal injury could serve as markers of IRTCI and that secreted proteins originating from such genes could be detected in the urine; the same teaching was

in the patent, paragraph [0034]. The skilled person would thus look into D1 and consider NGAL as a suitable candidate.

XV. The appellants (patent proprietors) requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request filed as auxiliary request 2 with letter of 18 April 2016, or, alternatively, on the basis of one of auxiliary requests 3 to 10, all filed with letter of 18 April 2016.

The respondent (opponent 2) had requested in writing that the appeal be dismissed.

Reasons for the Decision

1. The appeal is admissible.

2. The oral proceedings before the board took place in the absence of the respondent, who had been duly summoned but decided not to attend.

According to Rule 115(2) EPC, if a party duly summoned to oral proceedings does not appear, the proceedings may continue without that party. As stipulated by Article 15(3) RPBA, the board shall not be obliged to delay any step in the proceedings, including its decision, by reason only of the absence at the oral proceedings of any party duly summoned who may then be treated as relying on its written case.

3. Opponent 1 withdrew its opposition during the appeal proceedings. A respondent to an appeal and former

opponent who withdraws its opposition ceases to be party to the appeal proceedings except insofar as the question of apportionment of costs under Article 104 EPC is at issue (T 789/89, OJ 1994, 482). However, withdrawal of the opposition by a respondent does not affect the appeal proceedings. The board has still to examine the substance of the opposition division's decision on the basis of the appellant's requests and may take into account the submissions and evidence filed by the respondent before the opposition was withdrawn (T 629/90, OJ 1992, 654).

4. Main request

4.1 Admission into the proceedings

4.1.1 The present main request was filed as auxiliary request 2 with letter of 18 April 2016. Although it was filed at a late stage of the appeal proceedings, namely after oral proceedings had been arranged, the board can accept the appellants' arguments that this request in fact corresponds to an auxiliary request which had already been considered by the opposition division, namely auxiliary request 1B filed with letter of 6 October 2011, and renumbered as auxiliary request 2 in the decision of the opposition division. No objections were raised by the opponents to its admission, and the board has none either. The main request is thus admitted into the proceedings (Article 13 RPBA).

4.2 Article 123(2) and (3) EPC

4.2.1 In the communication accompanying the summons to oral proceedings, the board raised objections under Article 123(2) EPC against claims 5, 24 and 25 of the then

pending main request (corresponding respectively to claims 5, 22 and 23 of the present main request).

However, in view of the appellants' arguments, the board is satisfied that the present claims comply with Article 123(2) EPC. The basis for claims 5, 22 and 23 is as follows:

4.2.2 Claim 5 is based on the combination of originally filed claims 6, 7 and 9, which were directed to a method of monitoring the effectiveness of a treatment for a renal tubular cell injury comprising the steps as claimed. Although originally filed claims 6, 7 and 9 did not refer back to claim 1 and thus did not provide a basis for the now claimed method of detecting a renal tubular cell injury (according to claim 1) followed by a method of monitoring the effectiveness of treatment (as in original claims 6, 7 and 9), the board nevertheless considers that paragraphs [0040] and [0044] do provide a basis for the combination of the two methods: in fact, these paragraphs explicitly state that "The method of the invention can be used to detect the onset of renal tubular cell injury, and to monitor the treatment thereof" (paragraph [0040]) and that "Once an indication of renal tubular cell injury or acute renal failure has been detected, and intervention and treatment of the disease or condition has commenced, the clinician can employ the method and kit of the invention to monitor the progress of the treatment or intervention" (paragraph [0044]).

4.2.3 Claims 22 and 23 have no counterpart in the originally filed claims, but find a basis in paragraphs [0098] and [0099] which state, respectively, that "urinary NGAL measured within two hours of transplantation was predictive of ARF" and that "urinary NGAL measured

within two hours of cardiac surgery was predictive of ARF", ARF being acute renal failure. Although these passages are taken from examples, the board notes that these examples make use of the method of detection of claim 1, namely a method wherein urinary NGAL is measured in urine samples of human patients by Western blot and ELISA (i.e. immunological assays involving the detection of an antibody-NGAL complex). The further details included in the examples are not considered as necessary features to carry out these particular embodiments of the invention and can thus be omitted. In fact, it is readily apparent that the above-mentioned statements of paragraphs [0098] and [0099] are not limited to the examples but generally applicable to the invention as defined in claim 1.

4.2.4 As regards Article 123(3) EPC, the present main request differs from the claims as granted in that claims 22 and 26 to 28 as granted have been deleted and in that independent claim 20 has been combined with dependent claim 21. There is thus no extension of protection in relation to the granted subject-matter.

4.3 Article 83 EPC

4.3.1 Article 83 EPC stipulates that the application shall disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. Common general knowledge available in the field at the effective filing date may be used to complement the disclosure of the application. On the other hand, post-published evidence may be used to support sufficiency of disclosure but not to establish it on its own. In general, the burden of proof lies with the party raising the objection, which should be based on "serious doubts substantiated by verifiable

facts": evidence for lack of enablement may also be present in post-published documents.

4.3.2 Claim 1 is directed to a method for detecting a renal tubular cell injury which is an ischemic renal injury by contacting a urine sample of the patient with an antibody against the biomarker NGAL and then detecting the antibody-NGAL complex. It was not disputed that such a method for immunological detection of the known protein NGAL in a urine sample could be routinely performed and that all necessary tools including anti-NGAL antibodies were available and/or could be readily prepared (see e.g. paragraph [0045] of the patent application). Moreover, the patent provides a number of examples which demonstrate that NGAL is detected in the urine following a renal ischemic injury. Using well-established murine and rat models of renal ischemia-reperfusion injury (paragraphs [0064], [0067] and [0083]), the patent shows that NGAL protein was detected in the urine "within 2 h of the injury (in the very first urine output following ischemia)" (mouse model) and "within 3 h of the injury (in the very first urine output following ischemia)" (rat model) and "persisted for the entire duration examined (24 hours of reperfusion)"; in both cases, NGAL was absent from the urine prior to ischemia (quotations from paragraph [0087] for the mouse model and from paragraph [0089] for the rat model). Example 5 (paragraph [0098]) further demonstrates that increased urinary NGAL in patients two hours after kidney transplantation ("which is a predictable human model of ischemic renal injury") was predictive of acute renal failure. Similar conclusions could be drawn from example 6 (paragraph [0099]) in the context of open-heart surgery. The board is hence satisfied that the patent renders it plausible that NGAL is indeed a biomarker for ischemic renal

injury, and can thus be used in a method of detection as claimed in claims 1 to 7 and 20 to 24, as well as in the uses of claims 8 to 19. These claims are thus considered to relate to subject-matter which is sufficiently disclosed in the patent application.

4.3.3 The respondent's arguments were mainly that NGAL is not a specific biomarker for renal ischemia, because it is also present in the urine of healthy individuals as well as those suffering from other diseases such as cancer, inflammation and heart failure. A number of prior art and post-published documents were cited in support of this argument (e.g. D2, D5, D12, D29).

The board does not find this argument persuasive. The fact that NGAL may also occur in other disease states (or even in healthy subjects) does not impair its usefulness as a diagnostic marker for renal ischemia: as for many well-known and established diagnostic markers, NGAL's diagnostic value depends on the clinical context and its presence does not by itself necessarily lead to a diagnosis. The claims are not directed to a method for differential diagnosis between e.g. cancer, inflammation or chronic heart failure versus renal injury but rather to a method for detection of renal ischemic injury. Such a method clearly is only to be used when there is reason to suspect that a renal ischemic injury may have taken place, i.e. when the whole clinical situation suggests that possibility. While of course it cannot be excluded that in a given patient other underlying pathologies such as cancer, inflammation or chronic heart failure may be present which would presumably reduce the diagnostic value of the method for renal ischemic injury, it is the role of the physician to interpret the results accordingly. The patent certainly provides

convincing data which support the contention that NGAL can be used as an early biomarker for renal ischemic injury, and these conclusions are further confirmed by post-published documents D14, D16, D28 and D29. In fact, D29, published 6 years after the patent, and cited by the respondent as evidence that NGAL is not specific to renal ischemic injury, while acknowledging that NGAL is a biomarker not only for renal ischemia (e.g. page 147, right column, last 2 lines to page 148, left column, line 1), still emphasises its importance as a biomarker for acute kidney injury.

4.3.4 The respondent and former opponent 1 further argued that a cut-off value was necessary and that multiple measurements were required in order to establish the diagnosis. The board however notes that the patent supports the general concept that NGAL can be used as an early urine biomarker for acute renal ischemia. The establishment of a cut-off value goes beyond the requirements for enablement under Article 83 EPC in the present case. The board is also not convinced by the respondent's and former opponent 1's arguments that determining a cut-off value is particularly relevant for claim 20, directed to a method to measure the extent of renal ischemia injury. In fact, the method of claim 20 does not rely on the amount of NGAL detected but rather on the time point at which NGAL is detected following the ischemic event (the surgical procedure).

4.3.5 A further argument of the respondent was that the lack of restriction of the claimed method to a given time point of detection in relation to the onset of the injury also resulted in lack of enablement because, as evidenced by D28 (page 1233, Figure 1A and 1B and page 1234, Figure 2), said time point was important for making the diagnosis. The board however notes that it

is in fact quite apparent from the cited figures of D28 that, while higher levels of NGAL are detected within 2 to 4 hours of the onset of the injury, NGAL is nevertheless significantly increased also at later time points, at up to 120 hours (which was the latest time point measured). D28 thus provides evidence that NGAL is not only an early biomarker for ischemic kidney injury but also a long-lasting one. It is furthermore noted that to restrict the claim to a method practised within a given time frame would require the skilled person to be aware of the moment when said injury took place: this is however not necessarily the case (see also below, section 4.4.5) and would not make technical sense considering that the purpose of the method according to claim 1 is to detect such an injury.

4.4 Article 56 EPC

4.4.1 The patent aims at identifying "a reliable, early biomarker for a renal tubular cell injury" which "would be useful to facilitate early therapeutic intervention" (paragraph [0003]). After discussing a number of indicators of acute renal failure, including serum creatinine as the "gold standard" (paragraph [0004]), the patent states at paragraph [0007] that "there remains an urgent need to identify improved biomarkers for early ischemic renal injuries". Paragraph [0010] of the application as filed - which text was replaced in the patent by a reference to the claims (paragraph [0009]) - describes as an embodiment of the invention "a kit for use in detecting the presence of an immediate or early onset biomarker for renal tubular cell injury in the urinary fluid of a subject". It is thus apparent that the aim of the patent is to provide a method of early detection of renal tubular cell injury.

4.4.2 Document D15, which is also directed to providing means for early detection of renal ischemic injury in urine, is the closest prior art. D15 discloses the use of Cyr61/CCN1 as an early biomarker of renal ischemic injury. The difference to claim 1 is thus that a different biomarker is used.

4.4.3 According to the patent, detection of Cyr61 in the urine "is problematic with respect to specificity as well as the cumbersome nature of the procedure", because it "required a bioaffinity purification and concentration step with heparin-sepharose beads, followed by a Western blotting protocol" and "Even after bioaffinity purification several nonspecific cross-reacting peptides were apparent" (paragraph [0006]). From the examples of the patent, it is apparent that also NGAL is detected in the urine using Western blot (paragraph [0070]), but there are no details concerning the purification method; moreover the claim is also not limited to any specific method of detection other than by using antibodies: accordingly it is not clear that this is indeed an advantage of the claimed method over D15. The appellant further argued that the fact that NGAL can be detected already in the first two hours of renal ischemia is also an improvement over D15. The board however notes that detection at 2 hours is described only for the mouse model (paragraph [0083]) while for the rat model it is 3 hours (paragraph [0085]) and thus identical to the time point when Cyr61 is detected in D15, which also uses a rat model. In fact both the patent and D15 disclose that NGAL and Cyr61, respectively, are detected in the first urine output after reperfusion (patent, paragraphs [0083] and [0085], and D15, page

1608, left column, last 3 lines to right column, line 3 and lines 19 and 20 of the legend to Figure 8).

Thus, in the absence of any shown advantages of the method of the patent over the method of D15 (at the filing date), the technical problem is formulated as the provision of an alternative method of early detection of renal ischemia by means of a urine biomarker. In view of the examples of the patent (see also above, section 4.3.2) the board is satisfied that the problem as formulated is plausibly solved by the subject-matter of claim 1.

4.4.4 While NGAL was well known as a biomarker for a variety of conditions, there was no disclosure in the prior art teaching or even suggesting that it could also serve as an early urine biomarker for renal ischemic injury. The only documents in the prior art establishing a link between renal ischemic injury and NGAL are the meeting abstracts D1a and D1b (of almost identical content) which disclose that "in the postischemic kidney NGAL mRNA synthesis was enhanced more than 20-fold at 24 and 48 hours after the ischemic event (Northern blot analysis)" and that "Correspondingly, NGAL protein expression was upregulated **after** 24 and 48 hours (Western blot analysis)" (lines 9 to 12 of D1a, emphasis added by the board; an almost identical disclosure is present in lines 13 to 18 of D1b). Neither of these two documents discloses however that the NGAL protein is detected in the urine, let alone already in the first urine output after the ischemic event. Hence the board considers that D1a and D1b may provide a hint that NGAL might possibly be useful as a biomarker for renal ischemic injury but they do not render it obvious that NGAL is an early urine biomarker. As such the skilled person would not

consider NGAL as an obvious alternative to the early urine biomarker Cyr61 of D15.

The board thus comes to the conclusion that the subject-matter of claim 1 involves an inventive step. The same is true for claims 2 to 24.

4.4.5 Both the respondent and former opponent 1 argued that the early detection time should not be taken into account in the analysis of inventive step because it was not recited in the claims.

The board however notes that claim 1 is directed to a method of detecting renal tubular ischemic injury, which, like any other alternative method for the same purpose, can be performed at any time following the injury. In comparison with other established methods (such as serum creatinine measurement) the claimed method has a larger "detection window" because, as shown in the patent, NGAL does appear in the urine at an early time point after the occurrence of the ischemic event. The clinician, who on the basis of his clinical evaluation of the patient, suspects that an ischemic event may have occurred but does not know whether and when it took place, may use this method to establish whether there is in fact a renal ischemic injury, with the knowledge that the method will detect such injury when other methods are still negative and will keep on detecting it when the other methods become positive. This is thus an inherent advantage of the method, which however does not imply that the claims have to recite it.

4.4.6 As regards the choice of the closest prior art, both the respondent and the former opponent 1 selected document D1a/D1b, and argued that this document was by

itself detrimental for inventive step because, based on its teachings, the skilled person would expect NGAL to be increased in the urine of patients with renal ischemic injury.

The board disagrees. D1a/D1b is not at all concerned with detection of renal ischemic injury but rather with the identification of genes which are involved in the molecular response of the kidney to ischemic injury, and does not provide any hint that the increased NGAL protein expression which is seen in the postischemic kidney would also be detectable in the urine. Additionally, D1a/D1b does not hint at providing an early biomarker for acute renal ischemia (cf. section 4.4.4. above).

4.4.7 A further argument from the respondent and former opponent 1 was that, based on the teachings of D1a/D1b, the skilled person would expect NGAL to leak into the urine and also that this would happen soon after renal ischemia, since it was known that de novo synthesis of proteins in the kidney in response to ischemia was a rapid event (D18). Moreover NGAL was known as an inflammatory marker (D26) and D25 showed that inflammation was the earliest event after ischemia.

The board does not find this argument convincing. While some proteins may indeed appear very rapidly after ischemia, this is not the case for all proteins, as exemplified by KIM-1 (D13): D18 merely discloses HSP72 as a cryoprotectant appearing early in the kidney upon transient ischemia or heat stress; D18's observations concern this specific protein and cannot be extrapolated to any other protein and in any case do not even disclose that HSP72 is detected in the urine at all. Thus the skilled person would not be in a

position to predict that NGAL would be such an early-appearing protein based on the only available information which was contained in D1a and D1b and which showed detection of NGAL mRNA and protein in the kidney at a much later time point than is shown in D15 for the early biomarker Cyr61 (page 1604, left column, first full paragraph, describing that the "dramatic up-regulation of rat *Cyr61* mRNA abundance in the whole kidney" was detected "at two hours following bilateral renal ischemia" and returned to "normal at 24 to 72 hours of reperfusion"). Likewise D25 does indeed teach that inflammation is an early event occurring in renal ischemia but it does not teach that any inflammatory markers, let alone NGAL, can be detected early in the urine. Also D26, which teaches that NGAL is an inflammatory marker, does not provide any hint that it can be used as an early urine biomarker for renal ischemia, nor does it disclose renal ischemia as an example of an inflammatory state.

4.4.8 The respondent and former opponent 1 further argued that the claimed subject-matter did not solve the problem it purported to solve, because, due to the lack of specificity of NGAL as a biomarker, it did not allow detection of a renal ischemic injury. This argument has already been dealt with in the context of Article 83 EPC, and the board arrives at the same conclusions, mutatis mutandis, in the context of Article 56 EPC.

4.5 The board thus comes to the conclusion that none of the grounds of opposition raised during the appeal proceedings prejudices the maintenance of the patent in amended form on the basis of the present main request.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance with the order to maintain the patent with the following claims and a description to be adapted thereto:

Claims 1 to 24 of the main request filed as auxiliary request 2 with letter of 18 April 2016.

The Registrar: The Chairman:

N.Schneider L. Bühler

Decision electronically authenticated