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Datasheet for the decision of 21 January 2022

Case Number: T 0029/19 - 3.3.08

Application Number: 11752546.9

Publication Number: 2611935

IPC: C12Q1/68

Language of the proceedings: EN

Title of invention:
ANALYTICAL METHODS FOR CELL FREE NUCLEIC ACIDS AND APPLICATIONS

Applicant:
Centre National de la Recherche Scientifique (C.N.R.S.)

Headword:
Cell free nucleic acids cancer/CNRS

Relevant legal provisions:
EPC Art. 83, 84, 123(2)
RPBA Art. 12(4)
RPBA 2020 Art. 12(2)

Keyword:
Admission - New main request (yes), new auxiliary requests 1 and 2 (no);
New main request - added matter (yes);
Auxiliary request 3 - clarity and support by description (no);

Decisions cited:
G 0010/93

Catchword:

Europäisches Patentamt
European Patent Office
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Beschwerdekammern

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Case Number: T 0029/19 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 21 January 2022

Appellant: (Applicant)	Centre National de la Recherche Scientifique (C.N.R.S.) 3, rue Michel-Ange 75016 Paris (FR)
Representative:	Cabinet Becker et Associés 25, rue Louis le Grand 75002 Paris (FR)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 30 July 2018 refusing European patent application No. 11752546.9 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman	B. Stolz
Members:	P. Julià
	A. Bacchin

Summary of Facts and Submissions

I. European patent application no. 11 752 546.9 (EP 2 611 935), originally filed under the PCT and published as WO 2012/028746 (hereinafter "the patent application"), was refused by an examining division of the EPO. Basis for the refusal were a main request and auxiliary request 3 filed on 28 June 2018, and auxiliary requests 1 and 2 filed on 31 May 2018. The main request and auxiliary requests 1 and 2 were considered to contravene Article 123(2) EPC and auxiliary request 3 not to fulfil the requirements of Article 56 EPC.

II. An appeal was lodged by the applicant (appellant). With the statement setting out the grounds of appeal, the appellant filed a new main request, new auxiliary requests 1 and 2, and auxiliary request 3. Oral proceedings were requested as an auxiliary measure.

III. The board summoned the appellant to oral proceedings scheduled for 14 December 2021. In a communication pursuant to Article 17 of the Rules of Procedure of the Boards of Appeal (RPBA 2020), the appellant was informed of the board's provisional opinion on the issues of the case.

IV. On 13 December 2021, upon an inquiry made by the board's registrar, the board was informed that the appellant would not attend the oral proceedings.

V. The board cancelled the oral proceedings and informed the appellant that it intended to issue a decision in line with the provisional opinion as summarised in the

conclusions set out in the communication pursuant to Article 17 RPBA 2020.

VI. Claims 1 and 10 of the main request underlying the decision under appeal read as follows:

"1. A method for the diagnosis, prognosis, or for assessing the evolution of a cancer in an individual, said method comprising calculating the index of integrity of cell free DNA in a body fluid sample obtained from said individual,

wherein the index of integrity is calculated by a method comprising (i) determining from an amplification by PCR the concentration or amount of a short sequence from cell free DNA in said body fluid sample, wherein said short sequence has a length inferior to 100 bp, (ii) determining from an amplification by PCR the concentration or amount of a long sequence from cell free DNA in said body fluid sample, wherein said long sequence has a length between 180-450 bp, and (iii) calculating the ratio of the concentration or the amount of said cell free DNA of long size and of short size, and

wherein the ratio long/short size is indicative of the presence of a tumor.

10. A method for the diagnosis, prognosis, or for assessing the evolution of a cancer in an individual, said method comprising:

a) calculating the size fraction ratio ("SFR") of cell free DNA in a body fluid sample obtained from said individual, and

b) comparing the SFR obtained to that of a healthy individual,

wherein a decreased SFR is indicative of the presence of cancer, and

wherein the SFR is calculated by a method comprising (i) calculating from amplification by PCR from said body fluid sample the concentration or amount of a range of short size cell free DNA in said body fluid sample, (ii) calculating from amplification by PCR from said body fluid sample the concentration or amount of a range of long size cell free DNA in said body fluid sample, and (iii) calculating the ratio of the concentration or the amount of said cell free DNA of long size range and of short size range, and wherein:

the short size cell free DNA range is within 60-100bp and the long size cell free DNA range is within 145-409, or

the short size cell free DNA range is within 60-100bp or 43-100bp or 60-145bp, and the long size cell free DNA range is higher than 200bp, or

the short size cell free DNA range is below 100bp, and the long size cell free DNA range is within 180-450bp, or

the short size cell free DNA range is below 100bp, and the long size cell free DNA range is within 249-409bp, preferably the short size cell free DNA range is within 73-100bp, and the long size cell free DNA range is within 300-357bp."

Claims 2 to 9 and claims 11 to 15 were directed to particular embodiments of the methods of claim 1 and claim 10, respectively.

VII. The examining division considered that claims 1 and 2 and claims 10 and 11 of the main request contravened Article 123(2) EPC. As regards claim 1, the examining division considered that there was no basis in the patent application for a method that could be indicative of the presence of a tumor without indicating a threshold value for the ratio long/short (amplicon) size. Moreover, the length of the short (amplicon) sequence cited in claim 2 ("between 50-100 bp") was not in line with the range cited in claim 1 ("inferior to 100 bp"). As regards claim 10, the examining division considered that there was no basis in the patent application for a decreased size fraction ratio (SFR) in general, but only for a SFR decrease inferior to 0.5. There was also no basis in the patent application for the combination/selection in claim 11 of saliva as a body fluid with the specific short/long size cell free DNA ranges cited in claim 10. Claims 2 to 9 and claims 11 to 15 were dependent on claims 1 and 10, respectively, and therefore, they also were considered to contravene Article 123(2) EPC.

VIII. The new main request filed in appeal proceedings read as the main request underlying the decision under appeal, except for steps (i), (ii) and (iii) of claim 1 which read as follows:

"1. ... (i) determining from an amplification by PCR of a short sequence from cell free DNA in said body fluid sample, the concentration or amount of a short amplicon, wherein said short sequence has a length inferior to 100 bp, (ii) determining from an

amplification by PCR of a long sequence from cell free DNA in said body fluid sample, the concentration or amount of a long amplicon, wherein said long sequence has a length between 180-450 bp, and (iii) calculating the ratio of the concentration or the amount of said DNA amplicons of long size and of short size, and ... [as in claim 1 of the main request underlying the decision under appeal]."

IX. Claim 1 of the new auxiliary request 1 filed in appeal proceedings read as claim 1 of the new main request except for four amendments, namely the length of the short sequence was defined as being "inferior or equal to about 60 bp" in step (i) of the claim, the length of the long sequence was defined as being "superior to 100 bp" in step (ii) of the claim, the ratio of the concentration or the amount was of the amplicon (instead of "cell free DNA" in the main request) in step (iii) of the claim, and the ratio long/short (the term "size" was deleted here) was indicative of the presence of "a cance" [sic] instead of a tumor, in the last sentence of claim 1.

Claims 2 to 9 were directed to particular embodiments of the method of claim 1. Claims 10 to 15 of the new main request were deleted in new auxiliary request 1.

X. Claims 1 and 2 of the new auxiliary request 2 filed in appeal proceedings read as follows:

"1. A method to identify or to analyse body fluid of cancer patient from body fluid from healthy individual wherein said method comprises the steps of:

a) quantifying in the two body fluid samples by a method using PCR, cfDNA of size within the 50-100 bp range and of size superior to 101 bp;

b) comparing the ratio obtained between the level of these two fragment ranges for each of the two body fluid samples and wherein the ratio long/short size range <1, and preferably <0.75 is indicative of presence of a tumor.

2. A method to identify whether a body fluid sample of an individual is from a cancer patient or from healthy individual wherein said method comprises the steps of:

a) quantifying in the body fluid sample by a method using PCR, cfDNA of size within the 50-100 bp range and of size superior to 101 bp;

b) calculating the ratio obtained between the level of these two fragment ranges for said body fluid sample and wherein a ratio long/short size range <1, and preferably <0.75 is indicative of presence of a tumor."

Claims 3 to 5 were directed to particular embodiments of the method of claim 1.

XI. The auxiliary request 3 filed in appeal proceedings is identical to auxiliary request 3 underlying the decision under appeal and the claims of this request read as follows:

"1. A method for determining the DNA Size Fraction Ratio (SFR) of cell free nucleic acid in a body fluid sample, said method comprising:

a) identifying a subject of interest, and

b) determining from a body fluid from said subject the Size Fraction Ratio (SFR) of cell free nucleic acid,

wherein said SFR is calculated as the ratio of the amount of ctDNA having a specific range of specific sizes to the amount of ctDNA having another specific range of specific sizes.

2. The method of claim 1, wherein said SFR is calculated as the ratio of the amount of ctDNA size higher than 200 to the amount of ctDNA size between 60 bp and 100 bp or between 43 bp and 100 bp or between 60 bp and 145 bp."

XII. The following documents are cited in this decision:

(7): J. Ellinger *et al.*, The Journal of Urology, January 2009, Vol. 181, pages 363 to 371;

(8): J. Ellinger *et al.*, BJU International, July 2009, Vol. 104, pages 48 to 52.

XIII. The arguments of the appellant, insofar as relevant to the present decision, may be summarised as follows:

Admission of the main request and of auxiliary requests 1 and 2 into the appeal proceedings

No reasons were given why these requests were filed only in appeal proceedings and why they could not have been filed at an earlier stage of the proceedings before the first instance.

Main request
Article 123(2) EPC

In the statement of grounds of appeal, the appellant argued that the patent application provided a broad description and support for methods of calculating and determining an index of integrity or an SFR (claims 1 to 9 and 10 to 15, respectively) without limitation to any specific (range of) size or ratios, using any fragment or range of fragments below 100 bp and above 100 bp for the short and long sequences, respectively. Support for claims 1 and 10 could be found on page 1, lines 11 to 5 and page 26, lines 13 to 30, as well as on claims 16 and 17 of the patent application. Further reference was also made to claim 1, page 14, lines 27 to 30, and page 31, lines 18 to 25 of the patent application. Support in the patent application was also indicated for dependent claims 2 to 9 and claims 12 to 15.

Auxiliary request 3
Article 84 EPC alone and/or in combination with Article 83 EPC

The appellant did not reply to any of the objections raised by the board under these articles in the communication pursuant to Article 17 RPBA 2020.

XIV. The appellant (applicant) requested that the decision under appeal be set aside and that a patent be granted on the basis of the main request or, in the alternative, any one of auxiliary requests 1 to 3, all filed with the appellant's statement of grounds of appeal.

Reasons for the Decision

1. The present decision is based on the same grounds, arguments and evidence on which the board's provisional opinion was based. They were neither questioned by the appellant, nor did other aspects come up that would require their re-consideration.

Admission of the new main request and new auxiliary requests 1 and 2 into the appeal proceedings

2. Article 12(2) RPBA 2020 states that, in view of the primary object of the appeal proceedings to review the decision under appeal in a judicial manner, a party's appeal case shall be directed to, *inter alia*, the requests on which the decision under appeal was based. In the present case the new main request and the new auxiliary requests 1 and 2 filed in appeal proceedings, to the extent that they introduce subject-matter which was not part of the appealed decision, are subject to the board's discretion as to their admission into the appeal proceedings.

3. Since the statement of grounds of appeal was filed on 4 December 2018 and thus, before the date of entry into force of the RPBA 2020, Article 12(4) RPBA 2007 applies to the present case for the purpose of establishing admittance (Article 25(2) RPBA 2020). According to Article 12(4) RPBA 2007, the board may hold inadmissible requests which should have been presented in the first instance proceedings.

The requests in appeal proceedings

4. The new main request, new auxiliary requests 1 and 2 and auxiliary request 3 were filed by the appellant

with the statement of grounds of appeal. Except for auxiliary request 3, which is identical to auxiliary request 3 at first instance, all other requests have been amended and are thus different from the corresponding requests at first instance.

4.1 The new main request is identical to the main request at first instance, except for claim 1 which has been amended to define the (PCR amplified) short and long sequences in the method for calculating the index of integrity as short and long amplicons, respectively (*supra*).

4.2 The new auxiliary request 1 is different from auxiliary request 1 at first instance. It is based on the new main request filed in appeal but has the following amendments: i) claims 10 to 15 of the new main request have been deleted; ii) the length of the short and long sequences in claim 1 has been amended to "inferior or equal to 60 bp" and "superior to 100 bp", respectively; iii) the terms "size" and "tumor" in the last sentence of claim 1 have been deleted, the latter term being replaced by the term "cance" [sic]; and iv) dependent claims 2 and 3 of the new main request have been replaced by claims 2 to 4 which define the length of the short and long sequences with values different from those present in these claims of the main request.

4.3 The new auxiliary request 2 is identical to auxiliary request 2 at first instance, except for the deletion of claim 6.

The proceedings in examination

5. According to the documents on file, the course of events of the proceedings in examination was as follows:

5.1 In a first communication issued on 2 January 2014, the examining division informed the appellant that the set of 16 claims filed on 28 March 2013 at the entry into the European regional phase contravened Articles 123(2) and 82 EPC. In response thereto, appellant filed a set of 19 claims on 14 October 2014.

5.2 In a second communication issued on 6 May 2015, the examining division raised objections under Articles 123(2), 53(c), 54, 56 and 84 EPC. The appellant replied thereto and filed a set of 16 claims on 19 February 2016.

5.3 In a third communication issued on 19 December 2016, the examining division raised objections under Article 123(2) EPC. In response thereto, the appellant filed a set of 20 claims on 22 June 2017.

5.4 With the Summons to attend oral proceedings issued on 6 February 2018, the examining division raised objections under Articles 123(2), 54, 56 and 84 EPC. With submissions dated 31 May 2018, the appellant filed a main request and an auxiliary request.

5.5 In a first telephone consultation on 21 June 2018, the examining division informed the appellant that the main request and claim 6 of the auxiliary request contravened Article 123(2) EPC and that the auxiliary request did not fulfil the requirements of Article 56 EPC. On 28 June 2018, the appellant filed a

new main request and auxiliary request 3, making its former main request and auxiliary request its auxiliary requests 1 and 2, respectively.

5.6 In a second telephone consultation on 29 June 2018, the examining division informed the appellant that the main request, auxiliary request 1 and claim 6 of auxiliary request 2 contravened Article 123(2) EPC, and that auxiliary request 3 was not inventive.

5.7 Oral proceedings were held on 2 July 2018 in the absence of the appellant. The examining division decided that the main request, auxiliary request 1 and (claim 6 of the) auxiliary request 2 contravened Article 123(2) EPC, and that auxiliary request 3 was not inventive (Article 56 EPC). Reasons for this decision were given in the decision under appeal.

Reasons given by the appellant for introducing the new main request and new auxiliary requests 1 and 2 into the appeal proceedings

6. In the statement of grounds of appeal, the appellant stated that the amendments introduced into the new main request were only formal and made in order to clarify the invention. No reasons were given why these amendments could not have been made at the first instance and why the new auxiliary requests 1 and 2 could not have been filed at the first instance.

7. Nor were any reasons provided by the appellant in response to the board's communication pursuant to Article 17 RPBA 2020, wherein the appellant was informed of this deficiency and of the fact that the board was minded not to admit these new requests into the appeal proceedings.

The amendments in the new main request and new auxiliary requests 1 and 2 could and should have been made at the first instance

8. Whilst the amendments introduced into the new main request filed in appeal are mainly formal, this is not the case for those introduced into new auxiliary requests 1 and 2 concerning the length of the short and long (amplicon) sequences (new auxiliary request 1) and the deletion of a claim previously objected to by the examining division (new auxiliary request 2).

New auxiliary request 1

8.1 The length of the long and short (amplicon) sequences in claim 1 of the new auxiliary request 1 filed in appeal have been amended in comparison to claim 1 of auxiliary request 1 underlying the decision under appeal; for the long sequence: "superior to 100 bp" *vs.* "superior or equal to 100 bp", and for the short sequence: "inferior or equal to about 60 bp" *vs.* "inferior to 100 bp". The length of the long and short (amplicon) sequences in the dependent claims of new auxiliary request 1 are also different from those given in the dependent claims of auxiliary request 1 underlying the decision under appeal. It is also the first time in the proceedings that the term "about" is used for defining the length of any of these sequences. Claims 12 to 21 of the auxiliary request 1 underlying the decision under appeal have been deleted in new auxiliary request 1 filed in appeal.

8.2 In the board's view, the appellant had ample opportunity at first instance to introduce amendments for defining and/or limiting the length of the short

and long (amplicon) sequences, either by introducing subject-matter of dependent claims and/or by selecting other values disclosed in, and supported by, the patent application. The introduction of these amendments in appeal proceedings - and thus, of subject-matter not examined at first instance - is not in line with the primary object and function of an appeal as expressed in Article 12(2) RPBA 2020, namely to review the decision under appeal in a judicial manner, but not to re-open or continue the examination as if it was at first instance (cf. "Case Law of the Boards of Appeal of the EPO", 9th edition 2019, in the following "Case Law", V.A.1, 1133).

New auxiliary request 2

8.3 In the first telephone conversation, the examining division informed the appellant that claim 6 of the auxiliary request filed in response to the Summons to attend oral proceedings contravened Article 123(2) EPC. This objection was maintained in the second telephone conversation, wherein this auxiliary request was made by the appellant its auxiliary request 2.

8.4 Although oral proceedings were held by the examining division, the appellant, by not attending the oral proceedings, waived also this opportunity for arguing and filing an amended auxiliary request 2 - or any other request - before the examining division at first instance. In light of this course of events, the board considers that the appellant had ample opportunity to file at first instance the new auxiliary request 2, instead of filing it for the first time in appeal proceedings.

Conclusion on the admission of the new requests filed in appeal

9. Thus, in the communication pursuant to Article 17 RPBA 2020, the board informed the appellant that, whilst the admission of the new main request appeared to be questionable, the board, in the exercise of its discretion, was minded not to admit new auxiliary requests 1 and 2 into the appeal proceedings. Auxiliary request 3 already forms part of the appeal proceedings.

10. Although in this communication, the admission of the new main request into the appeal proceedings was questioned, the board, for reasons of procedural efficiency, drew appellant's attention to several issues that were considered to be relevant under Article 123(2) EPC.

11. In view of the course of events at first instance and, since the appellant has not replied to the communication pursuant to Article 17 RPBA 2020 and provided reasons why these new requests could not have been filed at the proceedings before the first instance, the board decides, in the exercise of its discretion under Article 12(4) RPBA 2007, to admit the new main request into the appeal proceedings but not new auxiliary requests 1 and 2.

New main request
Article 123(2) EPC

12. In the communication pursuant to Article 17 RPBA 2020, the board drew the appellant's attention to the following issues:

12.1 According to the established case law, the content of the application is not a reservoir from which features pertaining to separate embodiments of the application can be combined in order to artificially create a particular embodiment (cf. "Case Law", *supra*, II.E. 1.6.1, 459). The gold standard for assessing compliance with Article 123(2) EPC of an amendment is that it must be directly and unambiguously derivable from the whole of the patent application, using common general knowledge. A distinction is also made between subject-matter which is, either implicitly or explicitly, disclosed in the patent application and subject-matter which is rendered obvious by the content of the application. Whilst the former complies with Article 123(2) EPC, this is not the case for the latter (cf. "Case Law", *supra*, II.E.1.3 *et seq*., 436).

12.2 The method of claim 1 is directed to several purposes, namely the diagnosis, prognosis, or assessment of cancer evolution in an individual (first technical feature). This method relies on a calculation of the index of integrity of a cell free DNA in a body fluid sample obtained from said individual, wherein a (PCR) amplification of short and long (amplicon) sequences (second and third technical features) of cell free DNA in said body fluid sample is performed. The ratio long/ short size is indicative of the presence of a tumor (fourth technical feature). The second and third features in claim 1 have specific values, namely "a length inferior to 100 bp" and "a length between 180-450 bp", respectively. It is the combination of all these features that must be supported by, and have a basis in, the patent application.

12.3 Methods for the diagnosis, prognosis, or for assessing the evolution of a physiological state of an

individual, such as cancer, are disclosed on page 31, lines 1 to 16 of the patent application. A particular aspect of these methods for diagnosis or prognosis of tumor progression in a patient is disclosed on page 31, lines 18 to 24 of the patent application. Claims 29 and 30 of the patent application are directed to a method for the diagnosis, prognosis, or evolution of a specific physiological state of an individual. All these methods comprise two steps which are defined as: "repeatedly calculating during an interval of time the index of integrity or SFR ..." (step (a)), and "comparing the indexes of integrity or SFR ... had been varied over this interval of time" (step (b)). There is no basis in the patent application for a method directed to the purposes indicated in claim 1 without said steps (a) and (b) (see "Case Law", *supra*, II.E.1.4 *et seq*., 446; for the removal of features from a claim).

12.4 All methods for diagnosis, prognosis or for assessing the evolution of a specific physiological state (cancer) of an individual disclosed in the patent application rely on the calculation of the index of integrity or SFR. Methods for a calculation of said index of integrity or SFR are disclosed in the patent application (cf. page 14, line 4 to page 19, line 14; see also claims 8 to 12 of the patent application), which also discloses the use of these calculation methods in methods "to identify or analyse body fluid (...) of cancer patient from body fluid (...) from healthy individual" (cf. page 26, line 13 to page 29, line 2; see also claims 23 to 26 of the patent application). Although identification/analysis methods are related to the method of claim 1 and rely also on index of integrity or SFR calculation methods as the method of claim 1, they are different from the method

of claim 1 both in the purpose (identification/analysis *vs*. diagnosis/prognosis/assessment of evolution) and in the steps of these methods (single sample *vs*. repeated samples and comparison of values). Therefore, these identification/analysis methods do not provide a basis for the method of claim 1.

12.5 Since claim 10 is directed to the same purposes as claim 1 (diagnosis/prognosis/assessment of evolution), the objections raised against claim 1 apply also to claim 10.

12.6 The method of claim 1 refers to a specific method for calculating the index of integrity of cell free DNA in a body fluid sample. This method relies on a short (amplicon) sequence with a length "inferior to 100 bp" and a long (amplicon) sequence with a length "between 180-450 bp". A method using a combination of short and long (amplicon) sequences with the specific lengths defined in claim 1, is disclosed on page 14, lines 4 to 14 of the patent application (see also claim 8 when in combination with claim 1 of the patent application). Claim 2 further defines the length of the short sequence and claim 3 defines the length of both short and long sequences. As a result of the dependency of these two claims on claim 1, the claimed subject-matter comprises specific combinations of particular (short and long sequence) lengths for which there is no basis in the patent application, such as for a short sequence of a length "between 50-100 bp" with a long sequence of a length "between 180-450 bp" (see "Case Law", *supra*, II.E.1.5 *et seq*., 452; on compliance with Article 123(2) EPC for combinations of ranges and combinations of upper and lower end points of ranges).

12.7 Likewise, there is no basis in the patent application for all specific combinations of the particular short and long sequence lengths cited in claim 10 such as, for instance, (short) "within 60-100 bp" and (long) "within 145-409 bp"; and (short) "within 60-145 bp" and (long) "higher than 200 bp".

12.8 Claim 1 is directed to "the diagnosis, prognosis, or for assessing the evaluation of a cancer" and further requires that "the ratio long/short size is indicative of the presence of a tumor". The method disclosed on page 31, lines 1 to 16 of the patent application refers to a variation of the indexes of integrity or SFR over an interval of time, wherein cancer is only one physiological state out of seven states disclosed as preferred embodiments. For tumor or cancer progression, reference is made therein to "a decreased of index of integrity or SFR" which, in a preferred embodiment, is to "a value inferior to 0.5, preferably inferior to 0.1" (cf. page 31, lines 18 to 24 of the patent application). Likewise, whilst claim 29 of the patent application refers only to a specific physiological state in general and to a variation of the index of integrity or SFR, claim 30 of the patent application refers specifically to the progression of cancer and to "a decreased of index of integrity to a value inferior to 0.5, preferably inferior to 0.1". Thus, there is no basis in the patent application for "the ratio long/ short size" in general - not limited to a decreased ratio but also including an increased ratio, and regardless of the specific value of said decrease/ increase - as being "indicative of the presence of a tumor".

12.9 Claim 10 refers to "a decreased SFR is indicative of the presence of cancer". A possible basis is the

disclosure on page 31, lines 18 to 22 of the patent application wherein reference is made to such a decreased SFR without indicating any value. However, this disclosure requires the nucleic acid for which the index of integrity or SFR is calculated to be "associated ... to said tumor". This feature is not present in claim 10 which refers only to short and long size cell free DNA in general.

12.10 Indeed, the disclosures on page 31 and of claims 29 and 30 of the patent application concern a method that requires the short and long (amplicon) sequences and the short and long size cell free DNA to be associated to the specific physiological state (cancer/tumor) of the individual from which the body fluid sample is taken. However, neither claim 1 nor claim 10 contain such feature. Thus, for this reason alone, there is no basis in the patent application for the methods of any of claims 1 and 10 and for all other claims of the main request due to their dependency on claims 1 and 10 (see "Case Law", *supra*, II.E.1.4 et seq., 446; for removal of features from a claim).

12.11 The appellant has not addressed the objection raised by the examining division against claim 11, namely lack of a basis in the patent application for a combination of "saliva" as body fluid sample with a method for the diagnosis, prognosis, or for assessing the evolution of cancer in an individual when relying on a SFR calculation of cell free DNA with the particular short and long size cell free DNA ranges indicated in claim 10.

13. Therefore, the new main request contravenes Article 123(2) EPC.

Auxiliary request 3

14. In the decision under appeal, the examining division raised an objection under Article 56 EPC against the subject-matter of this request. No other objections were raised.

15. According to decision G 10/93 (OJ EPO, 1995, 172), where the examining division has refused an application, the board has the power to examine whether the application or the invention to which it relates meets the requirements of the EPC. This also holds good for requirements that the examining division had not considered in the examination proceedings or had regarded as fulfilled. In the present case, as stated in the communication pursuant to Article 17 RPBA 2020, the board considers the subject-matter of auxiliary request 3 not to fulfil the requirements of Article 84 EPC alone and/or in combination with those of Article 83 EPC.

Article 84 EPC alone and/or in combination with Article 83 EPC

16. Article 84 EPC requires that the claims define the matter for which protection is sought, that they are clear and concise, and supported by the description. According to the established case law, this provision requires a claim not only to be comprehensible from a technical point of view but also to indicate all the essential features needed to define the invention (cf. "Case Law", *supra*, II.A.3.2, 292).

16.1 Claim 1 relates to a method for determining the DNA SFR of cell free nucleic acid in a body fluid sample of a subject of interest, wherein said SFR is calculated as the ratio of the amount of two cell free nucleic acids.

The two cell free nucleic acids are defined in claim 1 in the same manner, namely "having a specific range of specific sizes", wherein neither the "specific ranges" nor the "specific sizes" are defined in the claim. Thus, both ranges and sizes are completely open and may have any value and property, regardless of their possible biological and/or physiological relevance. However, in the light of the whole content of the patent application, both the "specific ranges" and "specific sizes", in particular a size range of nucleic acids having a length <100 bp, are essential technical features of the claimed method. Therefore, in line with the referred to case law, they should be clearly and unambiguously defined in the claim.

16.2 There is no indication in claim 1 of the method used for measuring or determining "the amount of ctDNA". The amount of cell free, circulating DNA is known in the art to be very low (few nanograms) and difficult to measure (cf. *inter alia*, page 49, left-hand column of document (8)). All relevant prior art cited in the patent application refers to a (PCR) amplification (cf. page 4, line 25 to page 6, line 10). Since, according to the patent application, not all methods appear to be appropriate (cf. page 66, line 22 to page 67, line 4, for electrophoresis) and the sole method exemplified in the patent application is a (PCR) amplification of short and long (amplicon) sequences, such a (PCR) amplification is an essential technical feature of the claimed method and thus, should be clearly and unambiguously defined in the claim.

17. It is also established case law that, in some cases, the use of trademarks, symbols and abbreviations in a claim may result in the introduction of ambiguity or

lack of clarity in the claim (cf. "Case Law", *supra*, II.A.3.1, 289).

17.1 The abbreviation "ctDNA" in claims 1 and 2 has no antecedent defining the meaning of this abbreviation. The meaning of the abbreviation should be spelled out and clearly defined in the claim 1. The patent application refers to the relevance of several nucleic acids and uses different abbreviations for each of them, namely "cfDNA" for cell free or extracellular nucleic acids (cf. page 2, lines 4 to 8) and "cirDNA" for circulating DNA (cf. page 2, lines 9 to 16). On page 1, line 2 of the patent application, the abbreviation "ctDNA" is disclosed as referring also to circulating DNA and thus, appears to be identical to "cirDNA" in general.

17.2 However, although all these abbreviations are used in the patent application, it is questionable whether all of them are interchangeable and refer to the same type and/or species of nucleic acids. Whilst circulating DNA refers, and is limited, to DNA circulating in blood (cf. page 2, line 9), extracellular or cell free nucleic acids are not limited thereto but refer to nucleic acids present in any body fluid, including blood (cf. page 2, lines 4 to 8). Therefore, if the abbreviation "ctDNA" in claim 1 is identical to circulating DNA ("cirDNA") in a narrow or limited meaning, i.e. DNA circulating in blood, the references in the preamble of claim 1 to "cell free nucleic acid" and "body fluid sample" in general appear to be broader and thus, to introduce ambiguity and lack of clarity in the claim.

17.3 Although there appears to be no clear reference thereto in the patent application, the abbreviation "ctDNA" may

also be understood as not being identical to circulating DNA in general ("cirDNA"), but to refer to a particular subgroup of circulating DNA, namely circulating tumor DNA or tumor circulating DNA (only in blood?). However, in this case, the (short and large) nucleic acids used for defining the specific ranges of sizes of the SFR would not be any (arbitrary) nucleic acid but a nucleic acid necessarily associated with a tumor marker gene known in the art, i.e. a circulating tumor genomic DNA, such as the RAS (*KRAS* and *NRAS*) gene exemplified in the patent application. Indeed, all examples described in the patent application relate to, and are based on, circulating tumor genomic DNA. Thus, it is not clear from the wording of claim 1 - not even in the light of the description of the patent application - whether the abbreviation "ctDNA" is limited to, and must be read as, said circulating tumor genomic DNA. In any case, the abbreviation "ctDNA" introduces ambiguity and a lack of clarity in the claim.

18. This unclear technical teaching of the claim results also in a lack of clarity arising from an inconsistency or disagreement between the disclosure of the patent application and the scope of claim 1, in particular, as regards both, the size of the cell free nucleic acid (ctDNA) used for determining the SFR and the method used for determining said SFR.

18.1 As regards the size of the cell free nucleic acid used for determining the SFR, the board considers the following issues to be relevant:

18.1.1 According to the disclosure of the patent application, "the inventors, for the first time, have demonstrated the presence of a higher proportion of cirDNA of a size

<100 bp" (cf. *inter alia*, page 8, lines 16 to 25). Indeed, the short length (amplicon) sequence of a size <100 bp is disclosed in the patent application as the actual contribution of the patent application over the prior art (cf. *inter alia*, page 5, lines 8 and 9 and lines 19 to 22; and page 14, lines 17 to 19). Since, as stated above, there is no limitation in claim 1 as regards the size of any of the ctDNA used for calculating the SFR, this contribution is not reflected in claim 1. The size of the two ctDNAs referred to in claim 1 is not defined and they could be, both of them, >100 bp; such a value would be inconsistent and in disagreement with the content of the patent application. Indeed, claim 1 does not even require the SFR to be a ratio of long/short size ranges, but it may also be a SFR ratio of short/long size ranges similar to the calculation of the apoptosis rate (cf. page 59, lines 4 to 9). In this context, it is worth noting that, according to the case law, it cannot be relied on the description to read into the claim a restrictive feature not suggested by the explicit wording of the claim, even though present in the description of the patent application (cf. "Case Law", *supra*, II.A.6.3.4, 312; and I.C.4.8, 122). In other words, when assessing clarity, if the claims are clear in themselves, their clarity is not affected if the description contains subject-matter which is not claimed.

18.1.2 The indexes of integrity or SFR obtained by using short cell free nucleic acid sequences of a length <100 bp are disclosed in the patent application as being (biological/physiological) relevant for several purposes, such as the diagnosis, prognosis or assessment of the evolution of a specific physiological state of an individual, wherein said state may be a disease, such as cancer or diabetes, but also sunburn

or an intense effort production (cf. page 31, lines 13 to 16). Although there is no indication of purpose in the claims of auxiliary request 3, the question arises as regards the (physiological/ biological) relevance of methods for determining the SFR wherein none of the cell free nucleic acid sequences used has a length <100 bp. Methods which do not rely on short cell free nucleic acid sequences of a length <100 bp are not supported by the patent application.

18.1.3 Moreover, references to semi-open size ranges introduce ambiguity in the actual scope of the claims.

A reference to a specific semi-open size range for a long amplicon sequence, such as >101 bp or >200 bp, without further defining any specific size of the cell free nucleic acid (ctDNA) fragments used within this range, renders the calculation of SFR ambiguous and lacks clarity, because it comprises (very) long cell free nucleic acids originated not only from apoptotic cells (180-200 bp) but also from necrotic cells (rarely smaller than 1000 bp) (cf. page 3, lines 16 to 22; see the calculation of the apoptosis rate, i.e. proportion of apoptotic and of necrotic origin, on page 59, lines 4 to 9). Moreover, there is evidence on file referring to increased levels of large cell free nucleic acid fragments (>300 bp) in certain types of cancer (such as breast, colon and ovarian), as well as increased levels of middle size cell free nucleic acid fragments (180-200 bp) related to an increased apoptosis in other cancers (see page 364, left-hand column, second paragraph, and paragraph bridging pages 369 and 370 of document (7)).

A reference to a specific semi-open size range for a short amplicon sequence, such as <100 bp, without

further defining any specific size of the cell free nucleic acid (ctDNA) fragments used within this range, renders the calculation of SFR ambiguous and lacks clarity, because it comprises (very) short cell free nucleic acids originating not from genomic DNA, as exemplified in the patent application, but from other sources, such as the cell free nucleic acid originating from mitochondria (mtDNA) described in document (8) (circulating mtDNA is known in the art to be in the range of 30-80 bp with peaks in 42-60 bp). There is no reason for not considering a circulating mtDNA which increases/decreases in the presence of a cancer/tumor, a circulating tumor DNA in a broadest sense.

The effects of these (very) long and (very) short cell free nucleic acid fragments in the determination of the SFR and their relevance on the ratio long/short size range (<1, <0.75?) for the indication of a tumor are not described in the patent application. Indeed, there is no experimental data in the patent application - and in the additional data submitted to the examining division and referred to by the appellant in the statement of grounds of appeal - for methods using amplicons with a length longer than 409 bp and shorter than 60 bp, let alone for the possible relevance, if any, of a SFR obtained with such (very) long and (very) short size amplicons in the identification/analysis of cancer patients.

18.1.4 Thus, methods in the claim which do not rely on short cell free nucleic acid sequences of a length <100 bp are not supported by the patent application and methods in the claim relying on semi-open ranges (<100 bp; >101 bp or >200 bp) may be related to, and comprise a large area of, non-working embodiments (cf. "Case Law", *supra*, II.A.5, 303; II.C.7 *et seq.*, 371; and II.C.5. *et*

seq., 355). These objections are relevant under both Articles 84 and 83 EPC (cf. "Case Law", *supra*, II.C.8 *et seq.*, 385).

18.2 As regards the method for determining the SFR, the board considers the following issues to be relevant:

18.2.1 Whilst the DNA integrity index (DII) corresponds to the amount of cell free nucleic acid longer than a given length to the amount of cell free nucleic acid shorter than a given length, the DNA size fraction ratio (SFR) corresponds to the ratio of two size fractions. DII and SFR are both nucleic acid fragmentation indexes (cf. page 14, lines 19 to 26; see also page 14, line 27 to page 15, line 17).

18.2.2 According to the patent application, electrophoresis is not an appropriate analytical method to appreciate cell free or circulating nucleic acid in the ranges of interest, i.e. <100 bp (cf. page 66, line 19 to page 67, line 4). The analysis and amount of cell free or circulating nucleic acid is carried out by using pairs of primers to amplify several amplicons of different (long and short) length or size. On page 46, Example I refers to the design and use of 9 pairs of primers that allow the amplification of amplicons of 60, 73, 101, 145, 185, 249, 300, 357 and 409 bp. Whilst all these primer sets are used in Example VI to calculate cirDNA concentration profiles (cf. page 63), only three amplicons are used in Example VIII, namely the 73 bp, 145 bp and 300 bp (cf. page 66, line 13). Example XI reports the calculation of SFR using these set of primers (cf. page 69). Example XII reports the determination of DII and SFR using the sets of primers shown in Tables 7 and 8 (cf. page 70, and paragraph bridging pages 71 and 72).

18.2.3 Both DII and SFR rely on the amplification of (amplicon) sequences of different (long and short) length or size. Whilst, for the calculation of a DII, it is enough to carry out or perform a (PCR) amplification of one long and one short (amplicon) sequence, for the calculation of a specific size range (used for determining the SFR), several (PCR) amplifications of long and short (amplicon) sequences of different length or size (falling within said specific size range) may be carried out - even though it is not necessary to carry out a (PCR) amplification for each and every long and short (amplicon) sequence of a length or size falling within said specific size range. Indeed, in the absence of any further indication in the claim, if only one long (amplicon) sequence and only one short (amplicon) sequence - falling within a specific size range - are (PCR) amplified, the determination of a SFR using only these two (amplicon) sequences appears to be identical to a calculation of a DII using these two (amplicon) sequences. Thus, for a meaningful technical definition of the SFR, it is not enough to define the specific size ranges of said SFR but it is also necessary to further specify the length or size of the long and short (amplicon) sequences used in said determination, i.e. the number of (amplicon) sequences used within the specific (long and short) size range and, preferably also, the specific length or size of these (amplicon) sequences on which the determination of SFR is based. In other words, it is necessary to further define the actual method used for determining said SFR (cf. "Case Law", *supra*, II.A.3.5, 298, characterisation by a parameter).

19. Thus, auxiliary request 3 does not fulfil the requirements of Article 84 EPC alone and/or in combination with Article 83 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated