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(71) Applicant: **OXFORD UNIVERSITY INNOVATION LIMITED** [GB/GB]; Buxton Court, 3 West Way, Botley, Oxford OX2 0SZ (GB).

(72) Inventor: **EMIR, Uzay Emrah**; John Radcliffe Hospital, Headington, Oxford OX3 9DU (GB).

(74) Agent: **J A Kemp & Co**; MERRYWEATHER, Colin Henry, 14 South Square, Gray's Inn, London Greater London WC1R 5JJ (GB).

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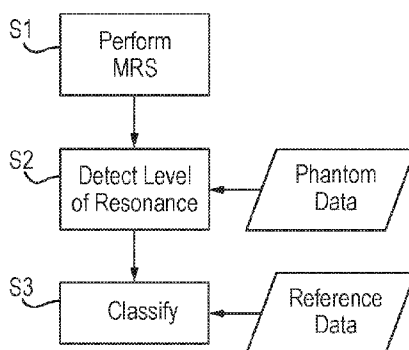
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(54) Title: In Vivo MRS Discrimination of Types of IDH

Fig. 3



(57) Abstract: In Vivo MRS Discrimination of Types of IDH H-magnetic resonance spectroscopy is performed in vivo on a voxel of tissue of a subject and the level of a resonance associated with 2-hydroxyglutarate in the derived magnetic resonance spectrum is determined. The tissue is classified as containing either (i) wild type isocitrate dehydrogenase, (ii) mutant isocitrate dehydrogenase 1 (IDH1), or (iii) mutant isocitrate dehydrogenase 2 (IDH2), on the basis of at least the determined level of the resonance associated with 2-hydroxyglutarate. This provides discrimination between IDH and IDH 2, which is useful in the diagnosis of cancer. The H-magnetic resonance spectroscopy is controlled to shape the magnetic resonance spectrum to provide the resonance associated with 2-hydroxyglutarate and to distinguish it from resonances associated with other biochemical molecules that overlap therewith. The classification may also be performed on the basis of the determined level of the resonance associated with other biochemical molecules including lactate. Fig. 3

In Vivo MRS Discrimination of Types of IDH

The present invention relates to in vivo analysis of tissue of a subject using magnetic resonance spectroscopy (MRS) to provide detection of 2-HG (2-hydroxyglutarate) for discriminating the presence of wild type isocitrate dehydrogenase (IDH), mutant isocitrate dehydrogenase 1 (IDH1) and mutant isocitrate dehydrogenase 2 (IDH2).

2-HG is a biomarker for brain cancer, acute myelogenous leukemia (AML) and other cancers in which mutant IDH1 or IDH2 occurs. Mutations in IDH1 and IDH2 occur in over 80% of low-grade gliomas and secondary glioblastomas (Parsons et al. (2008), Watanabe et al. (2009), Yan et al. (2009)). IDH catalyzes the conversion of isocitrate to α -ketoglutarate (α -KG). IDH1 (cytosolic) and IDH2 (mitochondrial) mutant tumors accumulate 2-HG as a result of a neomorphic enzymatic activity, which further reduces α -KG to 2-HG (Dang et al. (2009), Ward et al. (2013)). The role of 2-HG in gliomagenesis is uncertain, but it is recognised as a tumour-specific biomarker and a potential target for pharmacological intervention (Yen et al. (2010)). Indeed, different subtypes of IDH mutation might be distinguished based on their characteristic neurochemical profile.

To date, immunohistochemical (IHC) and molecular pathological analysis of surgically obtained tumor tissue is required to make the diagnosis of an IDH mutated glioma. Recently, the detection of 2-HG with high-resolution magic angle spinning ^1H -MRS has been demonstrated, followed by in vivo detection of ^1H -MRS at 3T (Andronesi et al. (2012), Andronesi et al. (2013), Choi et al. (2012), WO-2013/049112). Such techniques determine whether the magnetic resonance spectrum has a resonance associated with 2-HG as an indicator of the presence of IDH1 or IDH2 without providing discrimination between IDH1 and IDH2. However, it would be desirable to provide such discrimination between IDH1 and IDH2 as this may provide additional information that is useful towards diagnosis of a cancer, due to IDH1 being associated with the production of 2-HG in the cytoplasm and IDH2 being associated with the production of 2-HG in the mitochondria.

According to the present invention, there is provided a method of in vivo analysis of tissue of a subject for discriminating the presence of wild type isocitrate dehydrogenase, mutant IDH1 and IDH2, the method comprising:

performing ^1H -MRS in vivo on a voxel of the tissue and deriving a magnetic resonance spectrum;

determining the level of a resonance associated with 2-HG in the magnetic resonance spectrum;

classifying the tissue as containing either (i) wild type isocitrate dehydrogenase, (ii)

mutant IDH1, or (iii) mutant IDH2, on the basis of at least the determined level of the resonance associated with 2-HG.

In the experiments reported in detail below, it has been demonstrated that the level of a resonance associated with 2-HG in the magnetic resonance spectrum derived using ¹H-MRS can be used as a basis for classifying tissue as containing either (i) wild type isocitrate dehydrogenase, (ii) mutant IDH1, or (iii) mutant IDH2, in particular providing the capability of providing discrimination between IDH1 and IDH2. Since IDH1 and IDH2 are localized to the cytosol and mitochondria, respectively, this discrimination is also capable of indicating subcellular compartmentalization of 2-HG. This provides information that is useful in making a diagnosis of the presence or absence of cancer, and potentially allows the monitoring of treatment response.

Recent studies have demonstrated that gliomas expressing IDH mutations have prolonged survival and a better response to chemotherapy. Specifically, IDH2 mutations in glioma lead to enhanced sensitivity level to chemotherapy. In addition, it has been reported that novel drug AG-221 (Agiros) by targeting inhibition of mutant IDH2 gene in a small, first-in-man study shows positive response in acute myeloid leukaemia. Similar benefits may be achieved with other cancers, such as breast or prostate cancer. Thus, in general, differential classification of IDH1 and IDH2 may be useful in the prognosis, treatment stratification, and management of cancer patients.

Due to the use of MRS, the method may be performed in vivo without the need to surgically obtain tissue for further analysis.

The method may provide such discrimination between IDH1 and IDH2 using one-dimensional ¹H-MRS. While two-dimensional J-resolved ¹H-MRS may allow separation of the resonance associated with 2-HG, it has the disadvantage of requiring longer acquisition times that are disadvantageous and in some cases impractical in a clinical setting in which (i) motion artefacts are resulted in correctable phase and frequency instabilities and changes in the shimming, (ii) under sampled second dimension, to reduce the acquisition duration, lead to truncation artefacts of the diagonal that obscure cross-peaks. So, achieving discrimination between IDH1 and IDH2 with one-dimensional ¹H-MRS is a significant advantage.

Advantageously, the ¹H-MRS may be performed at a static magnetic field which is relatively high, for example at least 4T, preferably at least 7T. Such high magnetic fields benefits in vivo ¹H-MRS detection of metabolites with substantial gains in signal-to-noise ratio (SNR) and spectral resolution, enabling the detection of subtle changes in metabolite levels from small voxels and higher specificity than at 3T.

It has been appreciated that it is possible for the ^1H -MRS to be controlled to shape the magnetic resonance spectrum to provide the resonance associated with 2-HG and to distinguish it from resonances associated with other biochemical molecules that overlap therewith, for example glutamine or glutamate. This shaping improves the accuracy achievable for determining the level of a resonance associated with 2-HG, which in turn improves the reliability of the classification.

By way of example, the shaping may distinguish the resonance associated with 2-HG from said resonances associated with said other biochemical molecules by suppressing said resonances associated with said other biochemical molecules relative to said resonance associated with 2-HG, or by inverting said resonances associated with said other biochemical molecules with respect to said resonance associated with 2-HG.

The ^1H -MRS may be controlled by applying a MRS pulse sequence that is selected to perform said shaping of the magnetic resonance spectrum, for example by selecting the echo time. Particular advantage is achieved by selecting an echo time in a range from 90ms to 130ms, preferably from 100ms to 120ms, for example 110ms.

The classification may be performed on the basis of other characteristics of the magnetic resonance spectrum in addition to the determined level of 2-HG, for example on the basis of the determined level of a resonance associated with one or more other biochemical molecules. It has been demonstrated in particular that lactate is another biochemical molecule that may be used to classify the tissue in combination with 2-HG. The use of other biochemical molecules that may be used in combination with 2-HG can improve the classification.

To allow better understanding, embodiments of the present invention will now be described by way of non-limitative example with reference to the accompanying drawings, in which:

Fig. 1 is a schematic diagram of a cell showing representative biochemical pathways;

Fig. 2 is a schematic diagram of a system for performing MRS;

Fig. 3 is a flowchart of a method of in vivo analysis performed using the system of Fig. 1;

Fig. 4 is a diagram of an ^1H -MRS pulse sequence using adiabatic slice selective refocusing pulses optimised for use at 7T;

Fig. 5 is a diagram of an ^1H -MRS pulse sequence using adiabatic slice selective refocusing pulses optimised for use at 3T;

Fig. 6 is a set of graphs illustrating optimisation of semi-LASER echo time for the

detection of 2-HG at 3T, wherein Fig. 6A is a graph of signal integral as a function of echo time (TE) for 2-HG, Glu and Gln; Fig. 6B is a set of graphs showing spectral profiles for four different echo times; and Fig. 6C is an expanded view of the 2-HG H3/H3' (1.9 ppm) resonance at 110 ms with overlaps showing a 15 Hz integration window;

5 Fig. 7 is three plots of in vivo ^1H -MRS spectra measured at a static magnetic field of 7T for (a) Glu, (b) Gln and (c) 2-HG, at echo times of 30ms and 110ms;

Fig. 8 is a single plot combining the in vivo ^1H -MRS spectra of Fig. 7 for Glu, Gln and 2-HG at an echo time of 110ms;

10 Fig. 9 is three plots of in vivo ^1H -MRS spectra measured at a static magnetic field of 3T for (a) Glu, (b) Gln and (c) 2-HG, at echo times of 30ms and 110ms; and

Fig. 10 is a single plot combining the in vivo ^1H -MRS spectra of Fig. 9 for Glu, Gln and 2-HG at an echo time of 110ms;

Fig. 11 is a 3D plot of the level of a resonance associated with 2-HG against the inter-pulse delays of a semi-LASER MRS pulse sequence estimated by density matrix simulations;

15 Fig. 12 shows MRS spectra derived from respective phantom solutions containing 2-HG and lactate (Lac);

Fig. 13 shows, for histological samples from three subjects, (a) images of Haematoxylin and eosin (H&E) stains, (b) images of immunohistochemistry with anti-IDH1 R132H, and (c) for the subjects having immunonegative samples (middle and bottom rows),
20 PCR and direct sequencing of codon 132 of IDH1 (middle row) and R172 of IDH2 (lower row);

Fig. 14 shows, for the same three subjects as Fig. 13, (a) MRI images showing voxel placement and (b) in vivo ^1H -MRS spectra, for an immunopositive (top row), an immunonegative with rare mutation (middle row) and a wild type tumor subject (bottom
25 row);

Fig. 15 shows in vivo ^1H -MRS spectra for the same three subjects as Fig. 7;

Fig. 16 is a plot of a Linear Discriminant Analysis (LDA) latent space for measurements of 2-HG from a number of subjects;

Fig. 17 shows neurochemical profiles determined by LCModel fitting;

30 Fig. 18 is a plot of measured concentrations of 2-HG (in $\mu\text{mol/g}$) for different subjects and a control case;

Fig. 19 is a plot of measured concentrations of 2-HG and lactate for six subjects;

Fig. 20 is a plot of a 2D Linear Discriminant Analysis (LDA) latent space for measurements of 2-HG against measurements of lactate from a number of subjects;

Fig. 21 is a set of graphs for the Cramér–Rao lower bound (CRLB) of fitting spectra acquired from six phantoms using three different pulse sequences, with the simulated localised LCModel basis;

Fig. 22 is images of a patient having IDH-mutant (IDH1) (uppermost) showing voxel positioning for MRS; spectra (middle) acquired using semi-LASER (middle, left) and PRESS (middle, right) at 7T; and LCmodel fits (lowermost) for 2- HG, glutamate, glutamine, GABA and Lac shown with CRLBs (%) given below the corresponding spectra; and

Fig. 23 is images of a patient having IDH-mutant (IDH-WT) (uppermost) showing voxel positioning for MRS; spectra (middle) acquired using semi-LASER at 3T (middle, left) and 7T (middle, right) at 7T; and LCmodel fits (lowermost) for 2- HG, glutamate, glutamine, GABA and Lac shown with CRLBs (%) given below the corresponding spectra.

Fig. 1 illustrates the biochemical pathways associated with the production of 2-HG and other metabolites in a cell. IDH catalyzes the conversion of isocitrate to α -KG. In the case of mutation in the cytoplasm, mutant IDH1 further reduces α -KG to 2-HG. In the case of mutation in the mitochondria, mutant IDH2 further reduces α -KG to 2-HG. The chemical pathway for the derivation of lactate in the cytoplasm is also shown.

Fig. 2 illustrates an MRS system 1 for performing in vivo analysis of tissue 2 of a subject 3 by MRS. As IDH1 and IDH2 occurs in gliomas and secondary glioblastomas, the tissue 2 may be brain or spinal tissue.

The MRS system 1 comprises an MRS apparatus 4 arranged to perform ^1H -MRS in vivo on a voxel 5 of the tissue 2. The MRS apparatus 4 may have a conventional construction including, for example, coils including shim coils and gradient coils for providing a static magnetic field and gradient pulses, radio frequency (RF) antenna for providing RF pulses and detecting the magnetic resonance (the free induction decay (FID)) as an output current signal.

The MRS apparatus 4 includes a control section 6 that controls the operation of the MRS apparatus 4 in accordance with user-input. The control effected by the control section includes driving the MRS apparatus 4 to apply a selected MRS pulse sequence and controlling other operational parameters such as the static magnetic field.

The MRS apparatus 4 further includes a signal processor 7 that processes the output current signal to provide the magnetic resonance spectrum 8 as output data. The signal processor 7 may be formed by conventional computing equipment and may be implemented in hardware or software running on a processor, or any combination thereof. The magnetic resonance spectrum may be obtained from the output current signal in a conventional manner, for example by performance of a Fourier Transform.

A computer apparatus 9 may be connected to the signal processor 7 for supply of the magnetic resonance spectrum 8 for further processing. The computer apparatus 9 may have a conventional construction, for example being a personal computer.

Fig. 3 illustrates a method that may be performed by the MRS system 1 shown in Fig. 1 for discriminating between the presence of wild type IDH, mutant IDH1 and mutant IDH2.

In step S1, the MRS apparatus 4 is controlled to perform ^1H -MRS on the voxel 5 of the tissue 2 of the subject 3 and the signal processor 4 derives the magnetic resonance spectrum. In general terms, the ^1H -MRS is performed using conventional MRS techniques, albeit that control to shape the magnetic resonance spectrum 8 as described further below.

Steps S2 and S3 may be performed in the signal processor 7 or the computer apparatus 9 by execution of an appropriate computer program.

In step S2, the level of a resonance associated with 2-HG in the magnetic resonance spectrum 8 is detected. The resonance whose level is detected may be the resonance associated with 2-HG at a chemical shift of 2.25 parts per million. Chemical shift may be defined as the relative difference in resonant frequency compared to a standard signal. For ^1H -NMR, this standard signal may be the signal of the compound tetramethylsilane (TMS)

Optionally in step S2, the level of a resonance associated with one or more other biochemical molecules may be detected. One such other biochemical marker is lactate. A resonance whose level is detected may be the resonance associated with lactate at a chemical shift of 1.33 parts per million, which resonance is a doublet. Other such biochemical markers may include any one or any combination of: lactate; myo-inositol; total choline; glucose and taurine combined; glutamate; glutathione; and total N-acetylaspartate (N-acetylaspartate and N-acetylaspartylglutamate).

In step S2, the detected levels may be calibrated to represent the detected level as a concentration of the 2-HG or other biochemical molecule by phantom data 10 that has been obtained from a magnetic resonance spectrum derived by controlling the MRS apparatus 4 to perform ^1H -MRS on a phantom containing the 2-HG or other biochemical molecule in a known concentration.

In step S3, the tissue is classified as containing either (i) wild type isocitrate dehydrogenase, (ii) mutant IDH1, or (iii) mutant IDH2. This classification is performed on the basis of at least the determined level of the resonance associated with 2-HG. In the experiments reported in detail below, it has been demonstrated that the level of a resonance associated with 2-HG in the magnetic resonance spectrum derived using ^1H -MRS can be used as a basis for such a classification.

Optionally, the classification in step S3 may be performed on the basis of other characteristics of the magnetic resonance spectrum in addition to the determined level of 2-HG, for example on the basis of the determined level of a resonance associated with one or more other biochemical molecules detected in step S2. It has been demonstrated in particular
5 that lactate is another biochemical molecule that may be used to classify the tissue in combination with 2-HG.

The classification is performed on the basis of comparison with reference data 11. Such reference data 11 may be derived from magnetic resonance spectra of known samples. The reference data 11 may represent a model used to make the classification, or may be used
10 to train the classification model.

The classification may be performed using any type of classifier. Many types of classifier are known and may be applied. For example, the classification may use a fixed model, a parametric model, or may use a non-parametric model. As an example of a parametric model, the classification may use Linear Discriminant Analysis (LDA).

15 There will now be discussed how the MRS apparatus 4 is controlled to perform ^1H -MRS in step S1.

Advantageously, the ^1H -MRS is performed at a static magnetic field which is relatively high, for example at least 4T, preferably at least 7T. Such high magnetic fields benefits in vivo ^1H -MRS detection of metabolites with substantial gains in signal-to-noise
20 ratio (SNR) and spectral resolution, enabling the detection of subtle changes in metabolite levels from small voxels and higher specificity than at 3T (Mekle et al. (2009)). That said, the ^1H -MRS may be performed at a static magnetic field which is lower, for example 3T.

The ^1H -MRS may advantageously be one-dimensional ^1H -MRS. As compared to two-dimensional ^1H -MRS which may provide good separation of the resonance associated
25 with 2-HG, one-dimensional ^1H -MRS has the advantage of significantly shorter acquisition times due to the use of less complicated MRS pulse sequences. This makes the method much more practical in a clinical setting.

As is conventional in MRS, the MRS apparatus 4 is controlled by applying a MRS pulse sequence, consisting of RF pulses and magnetic field gradient pulses. Many types of
30 MRS pulse sequence consisting of different sequences of pulses are known, and in general any known MRS pulse sequence may be applied.

Advantageously, the MRS pulse sequence may be one which provides semi-localization by adiabatic selective refocusing (semi-LASER) (for example as disclosed in van de Bank (2015)). This has the advantage of providing minimal chemical shift displacement

error and insensitivity to transmit field (B_1^+) inhomogeneities at ultra-high frequencies.

An example of a semi-LASER MRS pulse sequence optimised for the detection of the H3/H3' and H4/H4' at 7T is shown in Fig. 4, wherein B_1 labels the RF pulses, and G_x , G_y , and G_z label the magnetic field gradient in read, phase encoding, and slice selection

5 directions, respectively. Initially, there is applied a VAPOR water suppression pulse sequence and an outer volume saturation (OVS) for suppressing signals from outside the voxel 5, such as lipid signals from the subcutaneous tissue. Then, the semi-LASER MRS pulse sequence comprises RF pulses consisting of a 90° excitation pulse (6ms) followed by two couples of adiabatic refocusing pulses (each 6ms). Magnetic field gradient pulses are
10 applied simultaneously. The magnetic field gradient pulses define the voxel 5.

An example of a semi-LASER MRS pulse sequence optimised for the detection of the H3/H3' and H4/H4' multiplet of 2-HG amongst the overlapping resonances of Gln and Glu at 7T is shown in Fig. 5. The semi-LASER implementation is symmetric in the positioning of its two pairs of gradient selective refocusing pulses. The echo time (TE) is defined by the
15 duration of the 90° and 180° refocusing pulses t_{90} and t_{180} , and the inter-pulse durations τ such that $\tau_1 = 0.78$, $\tau_2 = 1.56$, $\tau_3 = 1.56$, $\tau_4 = (TE - 4t_{180} - 2\tau_3)/2$ and $\tau_5 = (TE - \tau_1 - \tau_2 - \tau_3 - \tau_4 - 4t_{180} - t_{90})/2$, in milliseconds. The manipulation of the echo time is therefore equivalent to varying the onset time of the final refocusing pulse (τ_4) and readout (τ_5). The excitation pulse in the semi-LASER implementation may be a hsinc 90° excitation (duration = 2.56 ms,
20 bandwidth = 3.4 kHz) and refocusing may be carried out using adiabatic pulses (HS4 R25, duration = 4.5 ms, bandwidth = 5.5 kHz).

Fig. 6 illustrates the optimisation process for the detection of 2-HG at 3T. Fig. 6A illustrates signal integral as a function of echo time (TE) for 2-HG, Glu and Gln. Spectral profiles may be derived at any echo time. For example, Fig. 6B shows spectral profiles
25 shown for four different echo times, the dotted lines illustrating where the spectral profile at 110ms is linked to Fig. 6B. Fig. 6C shows a close up of the 2-HG H3/H3' (1.9 ppm) resonance at 110 ms, with overlaps showing 15 Hz integration window. As can be seen in Fig. 6C, at 110 ms, the semi-LASER generates near zero overlap from Glu and Gln around 1.9 ppm.

30 In step S2, the ^1H -MRS performed by the MRS apparatus 4 is controlled to shape the magnetic resonance spectrum 8 to provide the resonance associated with 2-HG and to distinguish it from resonances associated with other biochemical molecules that overlap therewith. The ^1H -MRS is similarly controlled to shape the magnetic resonance spectrum 8 to provide the resonance associated with any other biochemical molecule used, for example

lactate. Some examples of such shaping are as follows.

The magnetic resonance spectrum 8 may be shaped to distinguish the resonance associated with 2-HG from said resonances associated with said other biochemical molecules by suppressing said resonances associated with said other biochemical molecules such as glutamate (Glu) and glutamine (Gln) relative to said resonance associated with 2-HG and/or by inverting the resonances associated with said other biochemical molecules with respect to said resonance associated with 2-HG. These effects may be achieved as follows.

The signal modulation due to J-coupling during the echo time (TE) results in amplitude and phase modulation of the resonance signals. Under the in vivo linewidth conditions, the complex effect of modulation on spectral pattern of Glu and Gln could be major signal attenuation. Thus, modulating resonance signal with sequence timings during TE can be used to uncover metabolites (2-HG) from the overlapped resonances in the human brain in vivo.

The effect of J modulation on resonances associated with (a) Glu, (b) Gln and (c) 2-HG at a relatively high static magnetic field of 7T are shown in Fig. 7 which illustrates in vivo ¹H-MRS spectra for (a) Glu, (b) Gln and (c) 2-HG, at echo times of 30ms and 110ms. The in vivo ¹H-MRS spectra of Fig. 7 at 110ms are plotted together in Fig. 8. As can be seen, changing the echo time from 30ms to 110ms, causes attenuation of the resonances associated with (a) Glu and (b) Gln around the resonance associated with (c) 2-HG at a chemical shift of 2.25ppm. In addition, changing the echo time from 30ms to 110ms causes a counterintuitive increase in the intensity of the resonance signal associated with (c) 2-HG, due to the fully absorptive negative (inverted) 2-HG signal at 2.25ppm with increasing TE. Consequently, this means that the resonances associated with (a) Glu and (b) Gln are suppressed relative to said resonance associated with (c) 2-HG.

As can also be seen, changing the echo time from 30ms to 110ms, causes inversion of the resonances associated with (b) Gln and (c) 2-HG, but not of the resonance associated with (a) Glu. Consequently, this means that the resonances associated with (a) Glu (but not (b) Gln is inverted with respect to said resonance associated with (c) 2-HG.

As can be seen in Fig. 8, both of these suppression and inversion effects assist in distinguishing the resonance associated with (c) 2-HG from the resonances associated with (a) Glu and (b) Gln at a static magnetic field of 7T by selection of the echo time of 110ms.

The effect of J modulation on resonances associated with (a) Glu, (b) Gln and (c) 2-HG at a relatively low static magnetic field of 3T are shown in Fig. 9 which illustrates in vivo ¹H-MRS spectra for (a) Glu, (b) Gln and (c) 2-HG, at echo times of 30ms and 110ms.

The in vivo ^1H -MRS spectra of Fig. 9 at 110ms are plotted together in Fig. 10.

As can be seen, changing the echo time from 30ms to 110ms, causes attenuation of the resonances associated with (a) Glu and (b) Gln around the resonance associated with (c) 2-HG at a chemical shift of 2.25ppm. For (a) Glu, the resonance above 2.25ppm reduces a little, but the resonance below 2.25ppm reduces significantly. For (b) Gln, the resonances above and below 2.25 ppm both reduce, and narrow. Changing the echo time from 30ms to 110ms does also cause attenuation in the intensity of the resonance signal associated with (c) 2-HG (in contrast to the increase apparent at a static magnetic field of 7T as shown in Fig. 7), but by a slightly lesser amount than the attenuation of the (a) Glu and (b) Gln. As a result, the resonances associated with (a) Glu and (b) Gln are suppressed relative to said resonance associated with (c) 2-HG, albeit to a lesser degree than at a static magnetic field of 7T as shown in Fig. 7.

As can also be seen, changing the echo time from 30ms to 110ms, causes inversion of one of the resonances associated with (b) Gln below 2.25ppm. The resonance associated with (c) 2-HG does not invert, meaning that the inverted resonance associated with (b) Gln is inverted with respect to said resonance associated with (c) 2-HG.

As can be seen in Fig. 10, both of these suppression and inversion effects assist in distinguishing the resonance associated with (c) 2-HG from the resonances associated with (a) Glu and (b) Gln at a static magnetic field of 3T by selection of the echo time of 110ms.

The ^1H -MRS may be controlled to shape the magnetic resonance spectrum 8 by applying a MRS pulse sequence selected to perform that shaping. In particular, the echo time of the MRS pulse sequence may be selected to perform the shaping.

In one example, the echo time of the MRS pulse sequence may be selected to have an echo time in a range from 90ms to 130ms, preferably from 100ms to 120ms. The benefits of this have been demonstrated as follows.

Density matrix simulations were conducted to establish the optimal inter-pulse delays of the semi-LASER MRS pulse sequence for detection of 2-HG. The results are shown in Fig. 11 which is a 3D plot of the level of the resonance associated with 2-HG at a chemical shift of 2.25ppm against the inter-pulse delays. By using a GAMMA/PyGAMMA simulation library of VESPA (Soher (2011)) to carry out the density matrix formalism, time delays between the RF pulses were tuned to optimum 2-HG detection. The simulations indicate that the 2-HG resonance (multiplets) at 2.25 ppm (H_4 , H_4') leads to a maximum absorptive negative (inverted) multiplet at a total echo time of 100–120 ms. A TE of 110 ms was chosen, since simulations showed a near fully absorptive negative 2-HG and lactate (Lac)

spectral pattern at 2.25 ppm and 1.35 ppm with timings $TE_1 = 11$ ms, $TE_2 = 65$ ms and $TE_3 = 34$ ms (total $TE = 110$ ms).

This was tested by performing 1H -MRS with this semi-LASER MRS pulse sequence on two phantoms, one of which contained 2-HG with glycine (Gly) and the other of which contained Lac with acetate (Ace). In this experiment, the repetition time (TR) was 5000 ms, the number of excitations was 128 and the voxel was a cube of side 2 cm. The two resulting magnetic resonance spectra are shown in Fig. 12. The LCModel (Provencher (2001)) fit of the simulated model spectra to the phantom spectra was performed over the spectral range from 0.5 and 4.2 ppm. Phantom spectra were line broadened to match line widths encountered in vivo. The spectral shape of the resonances associated with 2-HG and Lac obtained from these phantom experiments closely resembled the simulated 2-HG and Lac shape determined by LCModel fitting.

There will now be discussed the use of the classification made in step S3.

As the classification discriminates between, on one hand, wild type IDH and, on the other hand, IDH1 and IDH2, it provides similar benefits in assisting in a diagnosis of cancer to existing techniques for detecting 2-HG using 1H -MRS. As such, the method shown in Fig. 3 may be followed by a step performed by a medical practitioner of making a diagnosis of the presence or absence of cancer taking into account the classification of the tissue.

As the classification discriminates between IDH1 and IDH2, which are localized to the cytosol and mitochondria, respectively, this discrimination is also capable of indicating subcellular compartmentalization of 2-HG. As discussed above, this provides information that is useful in making a diagnosis of the presence or absence of cancer, and potentially allows the monitoring of treatment response.

Experiments

Experiments were performed using the following methods.

Experiment 1:

Experiment 1 was performed to study performance of the in vivo method at 7T.

Subject Inclusion

Fourteen glioma subjects (8 men, 45 ± 13 years old, mean $\pm$ SD) and 8 healthy volunteers (6 men, 42 ± 11 years old) participated in the study after giving written informed consent. One subject (P005) was excluded due to poor placement of dielectric pad resulting in high measurement noise and insufficient transmit field. The Oxfordshire B National Research Ethics Committee approved the study.

Immunohistochemistry for detection of the R132H mutation in IDH1

All tissues were routinely fixed and processed and sectioned at 4 μ m. Endogenous peroxidase activity was blocked (20 mins, 3% aqueous hydrogen peroxide) and epitopes were retrieved (autoclave, 121°C for 10 min in 10mM sodium citrate, pH 6.0). Sections were incubated with primary antibody (IDH1 R132H (clone H09, Dianova) 1:100, overnight at 4°C (Capper (2009))). Visualisation was achieved using Dako REAL EnVision/HRP, Rabbit/Mouse (ENV) kit (Dako).

IDH1 and IDH2 DNA sequencing

DNA was extracted from paraffin sections (5 sections, 10- μ m thickness) using QIAamp FFPE DNA mini kit (Qiagen). Regions of IDH1 and IDH2 containing codons 132 and 172 respectively were amplified by PCR (primers identical to (Horbinski et al. (2009))). Products were examined by agarose gel separation and purified (MinElute PCR Purification kit, Qiagen). Sequencing reactions were performed using BigDye Terminator chemistry and an ABI-3730 sequencer.

MR imaging and spectroscopy

Each subject participated in a 1 hour MR scan. MR experiments were performed using a 7T whole body MR system (Siemens, Erlangen) with a Nova Medical 32-channel receive array head-coil. Voxels were defined on each participant's anatomical scan (1mm isotropic resolution MPRAGE sequence: repetition time TR = 2.3s, inversion time TI = 1.05s, echo time TE = 2.8ms, total acquisition time = 3min). First- and second-order shims were first adjusted by GRESHIM (Shah (2009))). The second step involved only fine adjustment of first order shims using FASTMAP (Gruetter et al. (2000))). Barium titanate pads were used to increase the extent of the effective transmit field (B_1^+) (Teeuwisse et al. (2012))). Spectra were measured with a semi-LASER MRS pulse sequence (TE = 110ms, TR = 5-6s, number of transients NT = 128, spectral bandwidth = 6kHz, data points = 2048) with VAPOR (variable power and optimized relaxation delays) water suppression and outer volume suppression (OVS). For subjects, volumes of 8 ml (20 $\times$ 20 $\times$ 20 mm³) were acquired from tumor and, when feasible, contralateral healthy tissue regions. Tumor voxel positioning aimed to exclude very heterogeneous tissue and minimize inclusion of healthy-appearing tissue (except in one previously-operated subject (P002), a 2 ml (20 $\times$ 10 $\times$ 10 mm³) volume was measured). For healthy volunteers, an 8ml voxel was placed similarly to the subject tumor positions.

Results were obtained as follows.

Ten of 14 subjects studied with in vivo MRS were shown to have mutations of IDH in tumour tissue subsequently obtained at surgery

Fig. 13 shows images and sequences relating to three subjects representative of the cases of immunopositive (top row), immunonegative with rare mutation (middle row) and a wild type tumor subject (bottom row). Fig. 13(a) is images that are haematoxylin and eosin (H&E) stains and Fig. 13(b) is images of immunohistochemistry with anti-IDH1 R132H. All scale bars 100µm.

Tissue samples underwent IHC analysis for the common IDH1 R132H mutation. Cases that were IDH1 R132H immunonegative were subjected to DNA sequencing. Three immunonegative cases (P006, P012 and P014) harboured a less common IDH2 R172K mutation detectable in the sequencing electropherogram. Fig. 13(c) shows, for the two immunonegative cases, the PCR and direct sequencing of codon 132 of IDH1 (middle row) and R172 of IDH2 (bottom row).

The double localization accomplished by the semi-LASER MRS pulse sequence and OVS pulse sequence eliminated signals from outside the voxel 5, resulting in artefact-free spectra with a flat baseline in the spectral range [1.6, 4.2] ppm for all subjects.

Fig. 14 shows magnetic resonance images and magnetic resonance spectra for the same three representative subjects. Fig. 14(a) shows the magnetic resonance images, indicating contralateral healthy and tumor tissue voxel placement. Alongside each image, Fig. 14(b) shows the corresponding in vivo ¹H-MRS spectra obtained from contralateral all the voxels at 7T. In all cases, the residual water signal was smaller than the major metabolite resonances (total Choline (tCho) and N-acetylaspartate (NAA) for tumour and healthy tissue voxels, respectively).

Fig. 15 shows L2-normalized ¹H-MRS spectra from all subjects indicating the mean of the IDH1/2 +ve subjects, the healthy subjects and the IDH1/2 -ve subjects, with the ± standard deviation being shaded.

Given the successful phantom and in vivo measurements, to characterise the spectral pattern changes induced by the IDH mutations, particularly any visually discernible 2-HG signal, untargeted metabolomics analysis was performed for the spectral range restricted to [1.6, 3.1] ppm. As shown by the gray box in Fig. 16, the untargeted feature extraction of in vivo spectra from healthy and tumor voxels resulted in a spectral pattern deviation at 2.25, where the 2-HG resonance is located.

The feature identified was used for linear discriminant analysis (LDA) to classify subject data into IDH mutant and healthy. The projection space plot of the LDA classifier showed a distinct clustering with not only a complete separation between spectra from the IDH mutant tumours and the healthy tissue but also between IDH1 and IDH2 R172K

mutations at 7T. Fig. 16 shows the thus-derived linear discriminant analysis latent space for the discrimination of the spectra from tumor tissue voxel of IDH mutant glioma subjects and healthy tissue voxel.

Thus, these results, and results obtained in a similar manner in the future, may be used as the reference data 11 in the classification performed in step S3 above.

In order to characterize this difference in more detail, the detected level (concentration) of 2-HG and other metabolite concentrations using LCModel (Provencher (2001)) analysis to determine neurochemical profiles. This uses an *a priori* established basis set for a selected group of metabolites, that is: Glu, glutamate; GSH, glutathione; myo-Ins, myo-inositol; scyllo-Ins, scyllo-inositol; tNAA, total N-acetylaspartate; tCho, total choline; tCr, total creatine; Glc, glucose; Tau, taurine; and Lac, lactate. The results are shown in Fig. 17, wherein the error-bars indicate inter-subject standard deviation. Only metabolites quantified with Cramér-Rao lower bounds (CRLB) $\leq 30\%$ in at least half of the spectra from a brain region were included in the profiles.

As also shown in Fig. 17, high Lac, myo-inositol (*myo*-Ins), total choline (tCho) and glucose+taurine (Glc+Tau) concentrations were observed in tumor voxels, whereas glutamate and total NAA were decreased.

As can be seen, the high spectral quality enabled the quantification of a neurochemical profile consisting of 8 metabolites in both tumor and healthy tissue voxels. Fig. 18 shows levels (concentrations) of 2-HG (in $\mu\text{mol/g}$) detected for different subjects and a control case. Only healthy tissue voxels of two subjects resulted in 2-HG detection with CLRBs of 25 and 26%, respectively. As can be seen, 2-HG was only detected in subjects exhibiting IDH1 and IDH2 mutations. In agreement with a previous cell culture study (Ward (2013)), this demonstrates in vivo that mitochondrial IDH2 R172K mutations lead to higher levels of 2-HG than cytosolic IDH1 mutations (9.06 ± 0.87 and $2.53 \pm 0.75 \mu\text{mol/g}$, respectively).

Analysis of the CRLBs as a function of the number of signal averages clearly showed that the estimated quantification errors per number of transient were always less than for previously published data at 3T, due to the MRS having increased sensitivity and spectral resolution. Importantly, the experiments enabled quantification of 2-HG in the tumor voxel with a mean CRLB of 23% following only 2 transient averages (~ 10 s experimental duration).

There will now be described experiments which demonstrate the use of lactate, in combination with 2-HG, as a biomarker that assists in the discrimination of IDH1 and IDH2.

As illustrated schematically in Fig. 1, most tumor tissues predominantly produce energy through break down of glucose via anaerobic glycolysis followed by lactic acid production in the cytosol, rather than via oxidative metabolism of pyruvate in mitochondria. Such altered energy metabolism has resulted in multiple metabolite differences between not only IDH mutants and health tissues but also between IDH1 and IDH2 mutations.

Together with increased 2-HG level in IDH2s, increased lactate signal in IDH2s was another biomarker that separates IDH2s from IDH1s. Results of experiments in which the level (concentration) of 2-HG and lactate were detected from ^1H -MRS spectra of the type described above are shown in Fig. 19. This shows that the levels of 2-HG and lactate levels are separated the groups with no overlap.

Linear discriminant analysis (LDA) was used to classify the subject data into healthy, IDH1 and IDH2 classes. Fig. 20 shows the resultant projection space plot of the LDA classifier. This demonstrates a distinct clustering with separation between the three classes.

Thus, Experiment 1 demonstrates in vivo detection of 2-HG at 7T.

Experiment 2:

Experiment 2 was performed to test the sensitivity of the method

Method

A test phantom containing six 74 ml bottles of varying 2-HG concentrations (or R,S- α -hydroxyglutaric acid, Sigma-Aldrich) was constructed. 10mM of glycine (Gly) was included in each bottle to act as a reference peak, having only a singlet resonance at 3.55 ppm. Concentrations of 2-HG were 0.1mM, 0.5mM, 1mM, 2mM, 5mM and 8mM. Each bottle was buffered with PBS buffer and average pH was measured to be 7.5 ± 0.1 . The larger bottle was filled with NaCl solution. Phantom measurements were acquired on a 3T whole body MR Prisma system (Siemens, Erlangen) with 32-channel receive array head coil. Voxels of $(20 \times 20 \times 20) \text{ mm}^3$ volume were obtained and shimmed to second order.

Spectra were acquired using both a semi-LASER (TE = 110 ms) pulse sequence optimised as described above and also a PRESS pulse sequence (TE = 97 ms), as described in Gordon et al (1984) and Bottomley (1987). The spectra were acquired with VAPOR water suppression and outer volume saturation bands (30) at a TR of 3s. RF pulses and timings were equivalent to those in simulations and 128 transients were acquired for each bottle in the phantom together with an unsuppressed water reference used for eddy current correction.

Analysis

Basis sets are generated from the fully localised simulations to use in metabolite assignment with LCModel as described in Provencher (1993). The basis set includes 2HG,

Ala (alanine), Asc (ascorbate), Asp, GABA, Cr+PCr (total creatine: tCr), Gln, Glu, Gly (glycine), myo-Ins (myo-inositol), Lac (lactate), NAA+NAAG (total NAA: tNAA), PCho, PE, scyllo-Ins (scyllo-inositol), Tau (taurine), Glc (glucose), GSH (glutathione). Individual spectra were frequency-corrected and eddy current-corrected. For quantification of absolute metabolite concentrations, a scaling correction, based on the difference in transverse decay rate, T₂, in tumours, was applied to acquired water spectra using the average of recently published values in low and high grade tumours (150 ms) (Madan et al. (2014)) and was set to 83 ms for healthy brain (Ganji et al. (2012)).

Spectra were line-broadened (6 Hz) to match in vivo conditions. To assess the sensitivity of both methods to detect 2-HG across a range of concentrations in the phantom, spectral fitting with LCModel was performed.

Results

Fig. 21 shows, for each of the six phantoms, the Cramér–Rao lower bound (CRLB) of fitting the three acquired spectra with the simulated localised LCModel basis as a function of 2-HG concentration collected for 128 transients. As can be seen, the results reveal that an optimised semi-LASER MRS sequence offers high sensitivity and localisation for in vivo detection of 2-HG, characteristic of IDH mutated brain tumours, at both 3T and 7T.

In particular, both PRESS and semi-LASER maintain near identical CRLBs of 2-HG fitting for concentrations greater than 0.5mM, falling to 2% for 8mM of 2-HG (SNR: 57 semi-LASER; 64 PRESS). At 0.1mM the CRLB after 128 averages is 53% and 19% for PRESS and semi-LASER, respectively. Also, both methods maintain identical relationships with increasing number of spectral averages. Even with only 2 averages (SNR ~ 15) both methods fit 2-HG at 2mM with CRLBs of (15±7)% and (14±6)% for PRESS and semi-LASER, respectively.

Experiment 3:

Experiment 3 was performed to test performance of the in vivo method at 7T.

Methods

A total of 11 glioma patients (7 male; 4 female; age: 39±11 years) were scanned with the optimised semi-LASER (TE = 110 ms) sequence in addition to PRESS (TE = 97 ms). Of those, 6 patients were scanned with semi-LASER only owing to constraints during a time-sensitive MR-protocol. Written and informed consent was given and approved by the South Central - Oxford B National Research Ethics Committee. Scanning was carried out prior to resection and histological confirmation apart from one patient who underwent only a partial resection and whose residual tumour was scanned early post-operatively. To test both

methods, 5 healthy volunteers (2 male; 3 female; age: 28 ± 4 years) were also scanned with both sequences. Voxels of $(20 \times 20 \times 20)$ mm³ were acquired on 3T whole body MR Prisma/Trio systems and the protocol remained identical to the phantom investigation with 64-96 transients collected in patients and 96 in healthy volunteers. An unsuppressed water scan was also collected for absolute quantification.

The histology and immunohistochemistry methodology used was the same as that of Experiment 1.

Results

Fig. 22 shows an example of the in vivo spectral quality along with LCModel fits for an IDH1-mutant patient at 7T. This spectra are representative of the high spectral quality obtained with both sequences. In particular, the amplitude of the residual water peak is less than tNAA or tCr, and no significant lipid contamination was observed, owing to the use of outer volume suppression. This resulted in flat baselines over the analysis range of 0.5-4.2 ppm except in one patient for whom spectral analysis was restricted to 3.8 ppm.

The arrows indicate the 2-HG peak (2.25 and 1.9 ppm) visible with semi-LASER at 3T. The presence of the H3/H3' multiplet can be seen clearly in Fig. 22. The occurrence of this peak at 1.9 ppm, upfield of the NAA singlet at 2.01 ppm, gives in vivo 2-HG semi-LASER spectra a distinctive spectral profile and can be seen in the raw data even before LCModel fitting. Compared to PRESS, the optimised compartmental artefacts of the optimised semi-LASER are considerably reduced due to its reduced chemical shift displacement artefact (similar to that reported by Kaiser et al. (2008)), leading to the complete refocusing of a resonance at 1.9 ppm.

Fig. 22 also shows the LCmodel fits of 2- HG, glutamate, glutamine, GABA and Lac shown with CRLBs (%) below corresponding spectra. Glu and Gln are detectable with both semi-LASER and PRESS, however Glu with higher CRLBs using semi-LASER than PRESS. Additionally, at 110 ms, the lactate peak at 1.33 ppm is almost fully inverted for semi-LASER, which significantly aids in its fitting generating much lower average CLRBS across all scans of 13.1% compared to 46.3% with PRESS.

Fig. 23 shows the spectra and basis fitting with LCModel for a single IDH mutant tumour. As can be seen, excellent spectral quality for both field strengths achieved with semi-LASER at both 3T and 7T.

The arrows indicate 2-HG peak (2.25 and 1.9 ppm) visible with semi-LASER at 3T and 7T.

Fig. 23 also shows overlapping metabolites, 2-HG, Glu, Gln, GABA and important tumour metabolite lactate (Lac). CRLBs lower at 7T for 2-HG and Lac. Gln CRLB is higher at 7T but still present, whereas Glu not detected at 3T.

The results reveal that an optimised semi-LASER MRS sequence offers high sensitivity and localisation for in vivo detection of 2-HG, characteristic of IDH mutated brain tumours, at 3T. In addition to H4/H4' (2.25 ppm), these results show direct detection of the H3/H3' multiplet of 2-HG at 1.9 ppm and reduction of estimation error in the in vivo spectra. The results also show how the optimised semi-LASER pulse sequence generates reduced fitting correlations with overlapping glutamate and glutamine resonances and improved fitting of lactate compared to an existing long-TE PRESS pulse sequence.

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Claims

1. A method of in vivo analysis of tissue of a subject for discriminating between the presence of wild type isocitrate dehydrogenase, mutant isocitrate dehydrogenase 1 and
5 mutant isocitrate dehydrogenase 2, the method comprising:
performing ^1H -magnetic resonance spectroscopy in vivo on a voxel of the tissue and deriving a magnetic resonance spectrum;
determining the level of a resonance associated with 2-hydroxyglutarate in the magnetic resonance spectrum;
10 classifying the tissue as containing either (i) wild type isocitrate dehydrogenase, (ii) mutant isocitrate dehydrogenase 1, or (iii) mutant isocitrate dehydrogenase 2, on the basis of at least the determined level of the resonance associated with 2-hydroxyglutarate.
2. A method according to claim 1, wherein the ^1H -magnetic resonance spectroscopy is
15 controlled to shape the magnetic resonance spectrum to provide the resonance associated with 2-hydroxyglutarate and to distinguish it from resonances associated with other biochemical molecules that overlap therewith.
3. A method according to claim 2, wherein the ^1H -magnetic resonance spectroscopy is
20 controlled to shape the magnetic resonance spectrum to distinguish the resonance associated with 2-hydroxyglutarate from said resonances associated with said other biochemical molecules by suppressing said resonances associated with said other biochemical molecules relative to said resonance associated with 2-HG.
- 25 4. A method according to claim 2, wherein the ^1H -magnetic resonance spectroscopy is controlled to shape the magnetic resonance spectrum to distinguish the resonance associated with 2-hydroxyglutarate from said resonances associated with said other biochemical molecules by inverting said resonances associated with said other biochemical molecules with respect to said resonance associated with 2-hydroxyglutarate.
30
5. A method according to any one of claims 2 to 4, wherein said other biochemical molecules are glutamine or glutamate.
6. A method according to any one of claims 2 to 5, wherein the ^1H -magnetic resonance

spectroscopy is controlled by applying a magnetic resonance spectroscopy pulse sequence selected to perform said shaping of the magnetic resonance spectrum.

7. A method according to claim 6, wherein the magnetic resonance spectroscopy pulse
5 sequence has an echo time selected to perform said shaping of the magnetic resonance spectrum.

8. A method according to any one of the preceding claims, wherein the ^1H -magnetic
10 resonance spectroscopy is performed applying a magnetic resonance spectroscopy pulse sequence that provides semi-localisation by adiabatic selective refocussing.

9. A method according to any one of the preceding claims, wherein the ^1H -magnetic
resonance spectroscopy is performed at a static magnetic field of at least 4T.

15 10. A method according to any one of the preceding claims, wherein the ^1H -magnetic resonance spectroscopy is one-dimensional ^1H -magnetic resonance spectroscopy.

11. A method according to any one of the preceding claims, wherein said resonance
20 associated with 2-hydroxyglutarate is at a chemical shift of 2.25 parts per million.

12. A method according to any one of the preceding claims, wherein:
the method further comprises determining the level of a resonance associated with
lactate in the magnetic resonance spectrum; and
the step of classifying the tissue is performed on the basis of at least the determined
25 level of the resonance associated with lactate and the determined level of the resonance associated with 2-hydroxyglutarate.

13. A method according to claim 12, wherein the ^1H -magnetic resonance spectroscopy is
30 controlled to shape the magnetic resonance spectrum to provide the resonance associated with 2-hydroxyglutarate and the resonance associated with lactate.

14. A method according to any one of the preceding claims, wherein the ^1H -magnetic
resonance spectroscopy is controlled by applying a magnetic resonance spectroscopy pulse
sequence having an echo time in a range from 90ms to 130ms, preferably from 100ms to

120ms.

15. A method according to any one of the preceding claims, wherein the step of classifying the tissue is performed on the basis of comparison with reference data derived from magnetic resonance spectra of known samples containing wild type isocitrate dehydrogenase, mutant isocitrate dehydrogenase 1, and mutant isocitrate dehydrogenase 2.

16. A method according to any one of the preceding claims, further comprising making a diagnosis of the presence or absence of cancer taking into account the classification of the tissue.

17. A system for performing in vivo analysis of tissue of a subject for discriminating between the presence of wild type isocitrate dehydrogenase, mutant isocitrate dehydrogenase 1 and mutant isocitrate dehydrogenase 2, the system comprising:

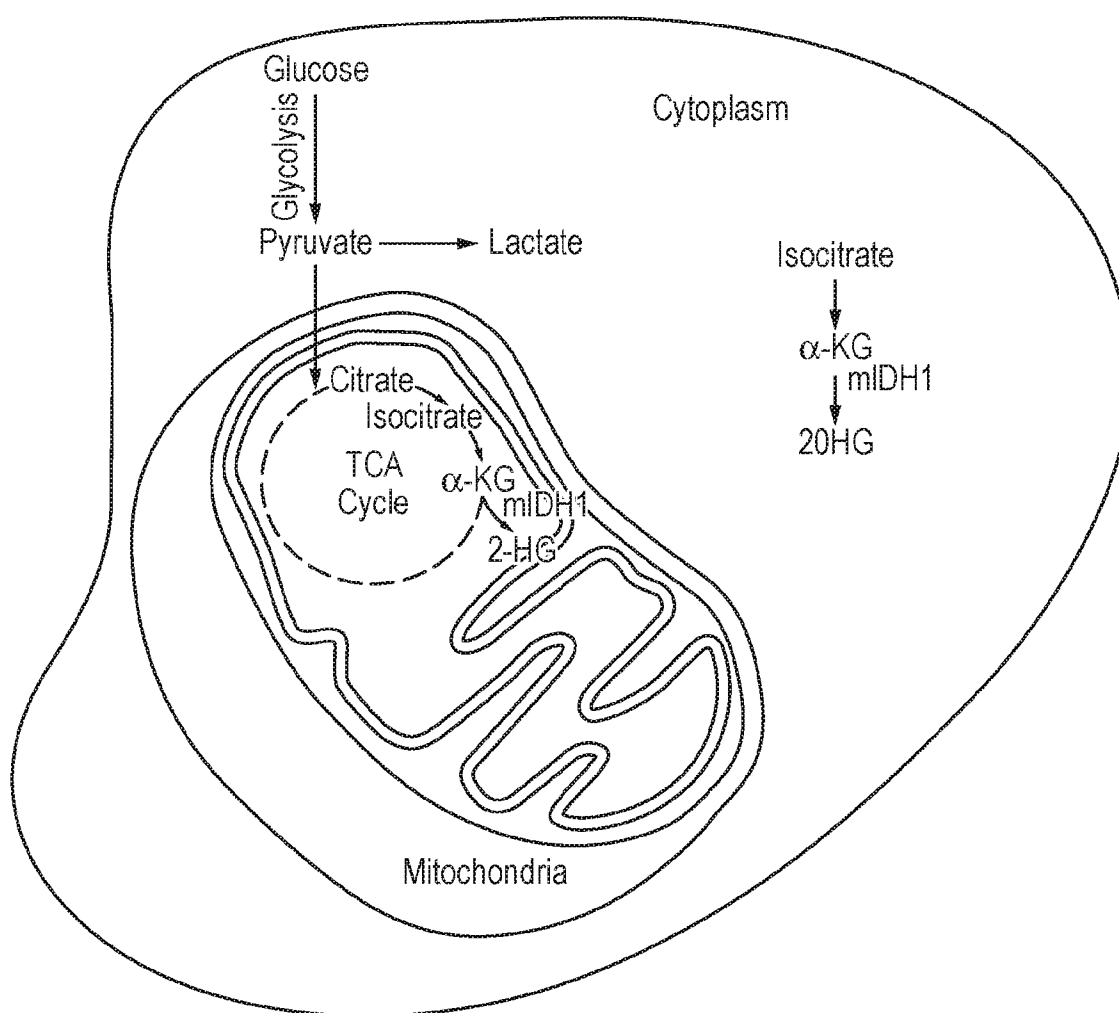
15 a magnetic resonance spectroscopy apparatus arranged to perform ^1H -magnetic resonance spectroscopy in vivo on a voxel of the tissue and to derive a magnetic resonance spectrum;

a signal processor arranged to determine the level of a resonance associated with 2-hydroxyglutarate in the magnetic resonance spectrum, and to classify the tissue as

20 containing either (i) wild type isocitrate dehydrogenase, (ii) mutant isocitrate dehydrogenase 1, or (iii) mutant isocitrate dehydrogenase 2, on the basis of at least the determined level of the resonance associated with 2-hydroxyglutarate.

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Fig. 1



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Fig. 2

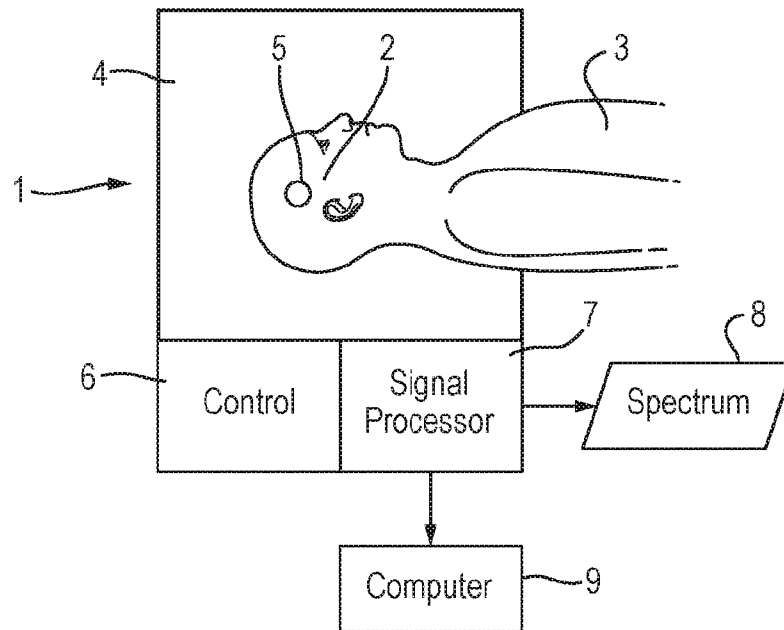
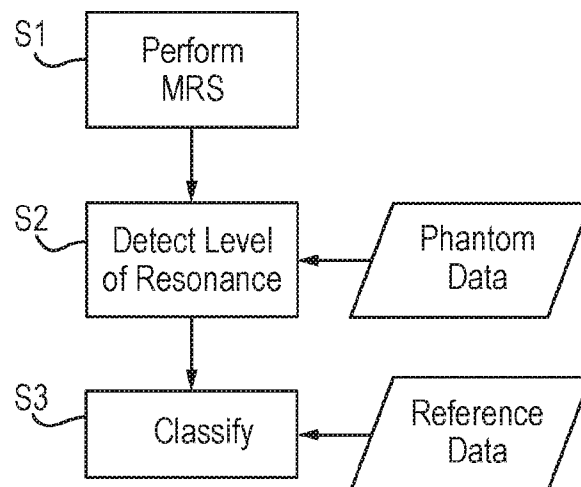


Fig. 3



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Fig. 4

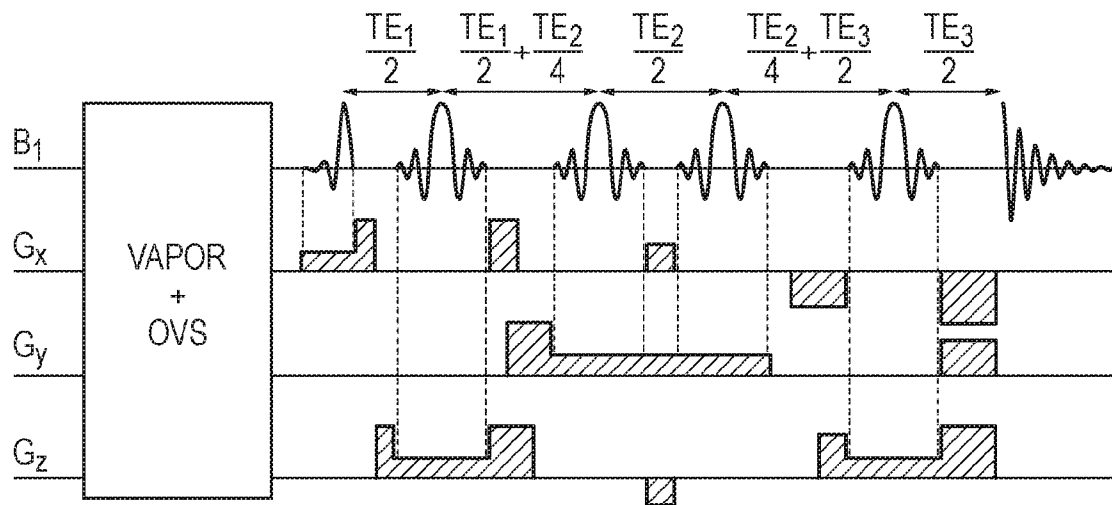


Fig. 5

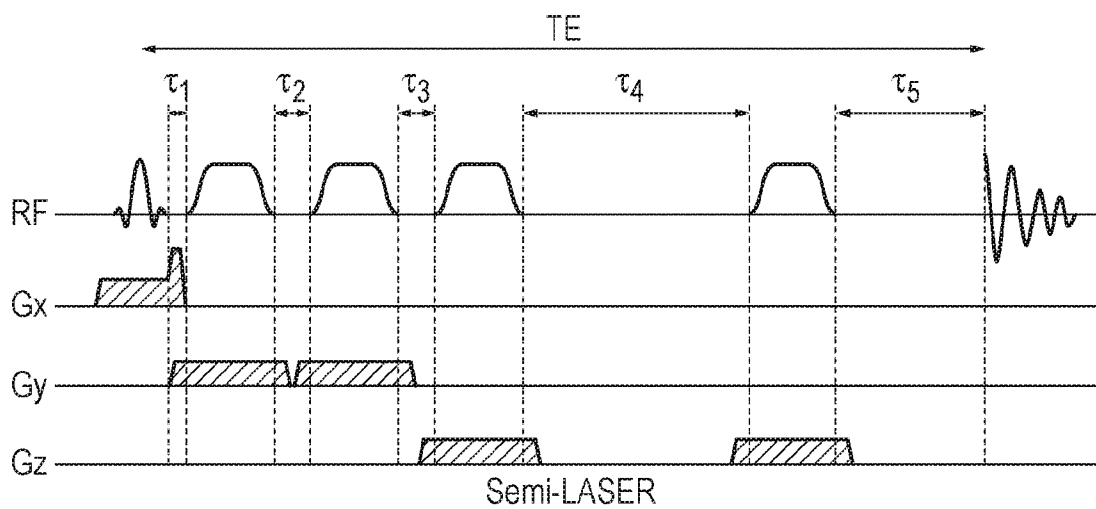


Fig. 6A

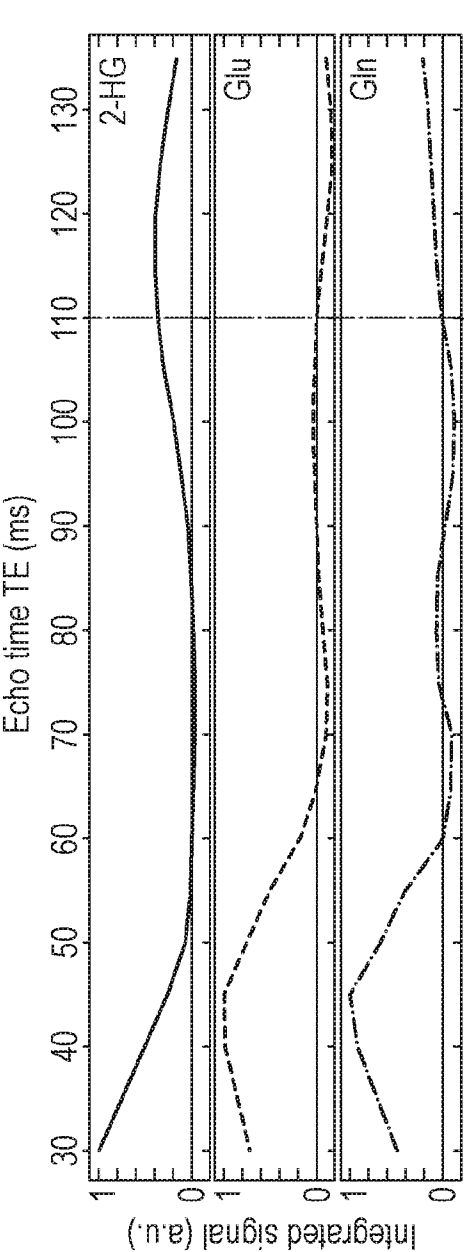
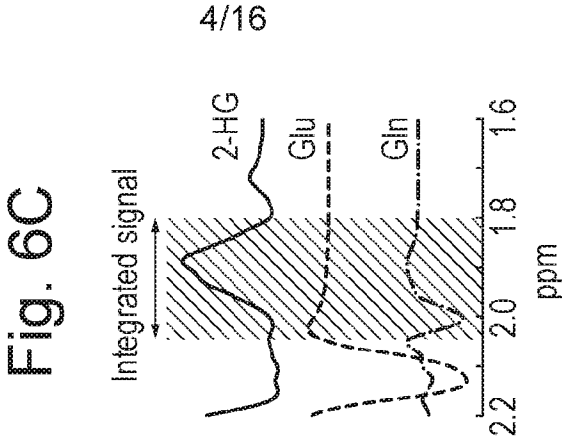
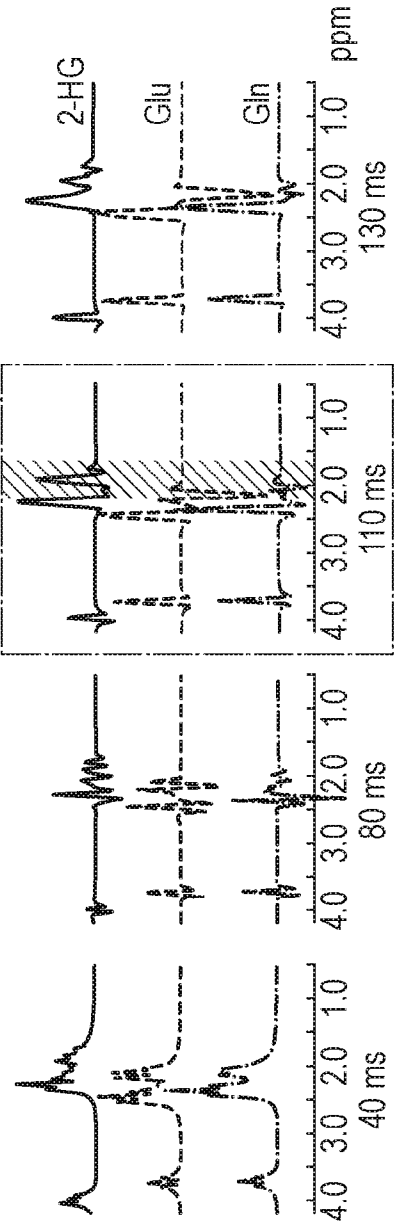
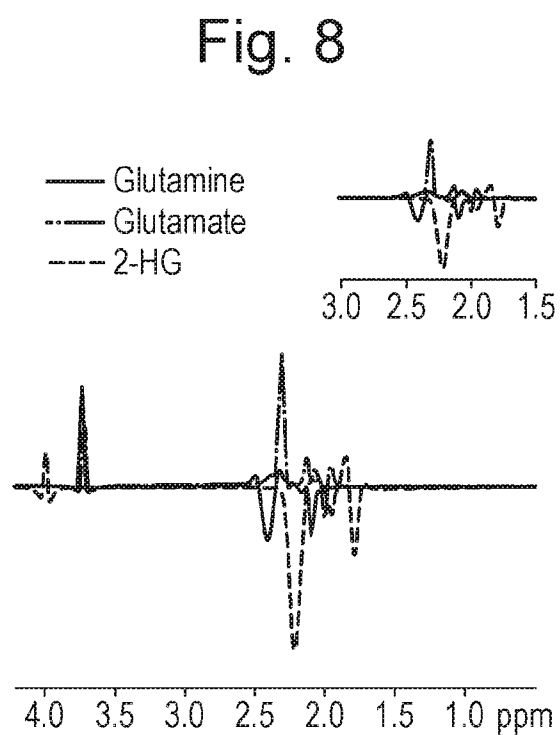
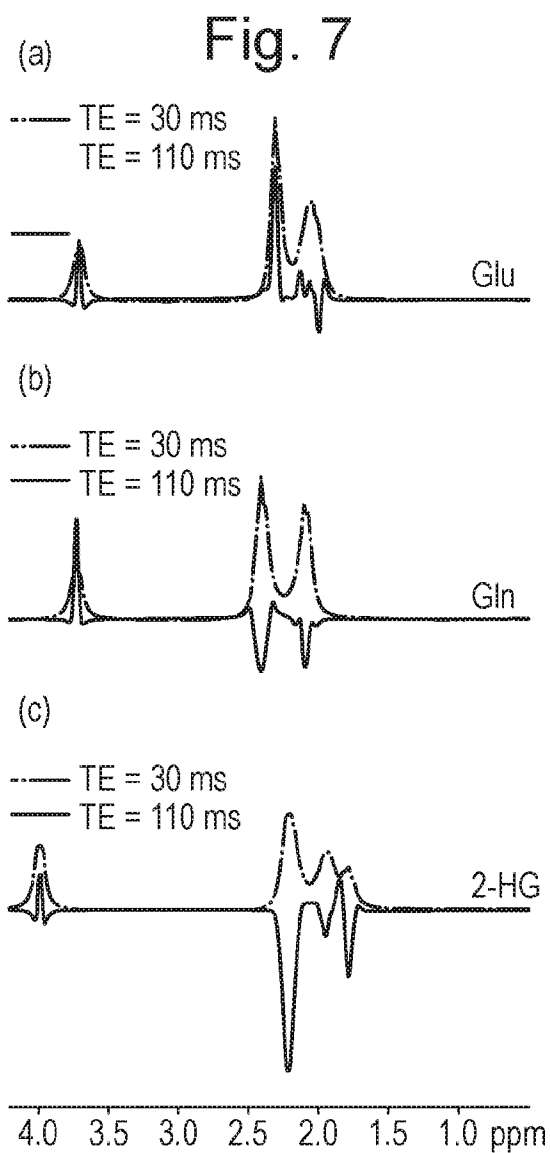


Fig. 6B



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Fig. 9A

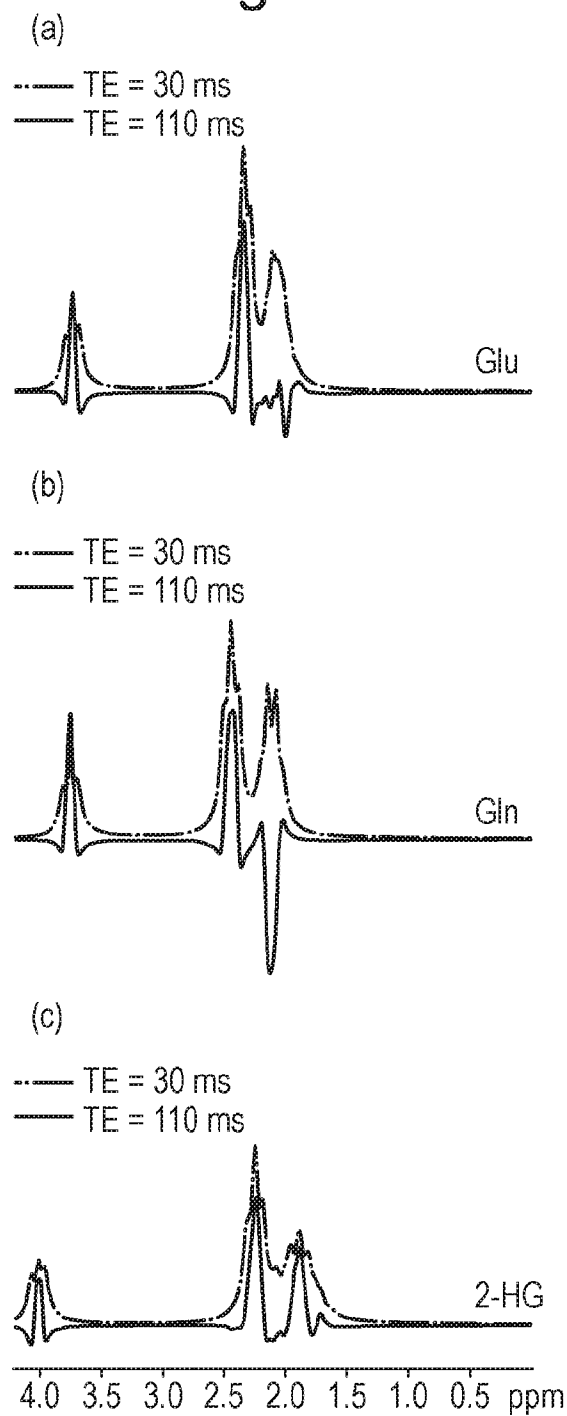
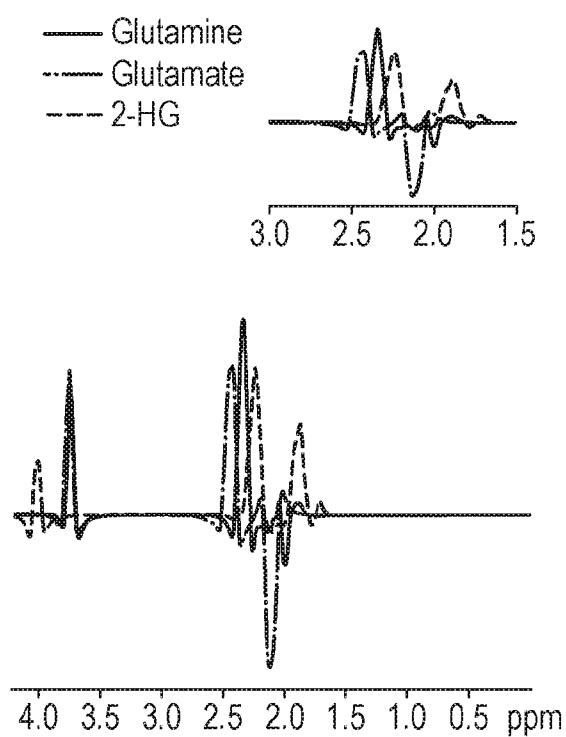
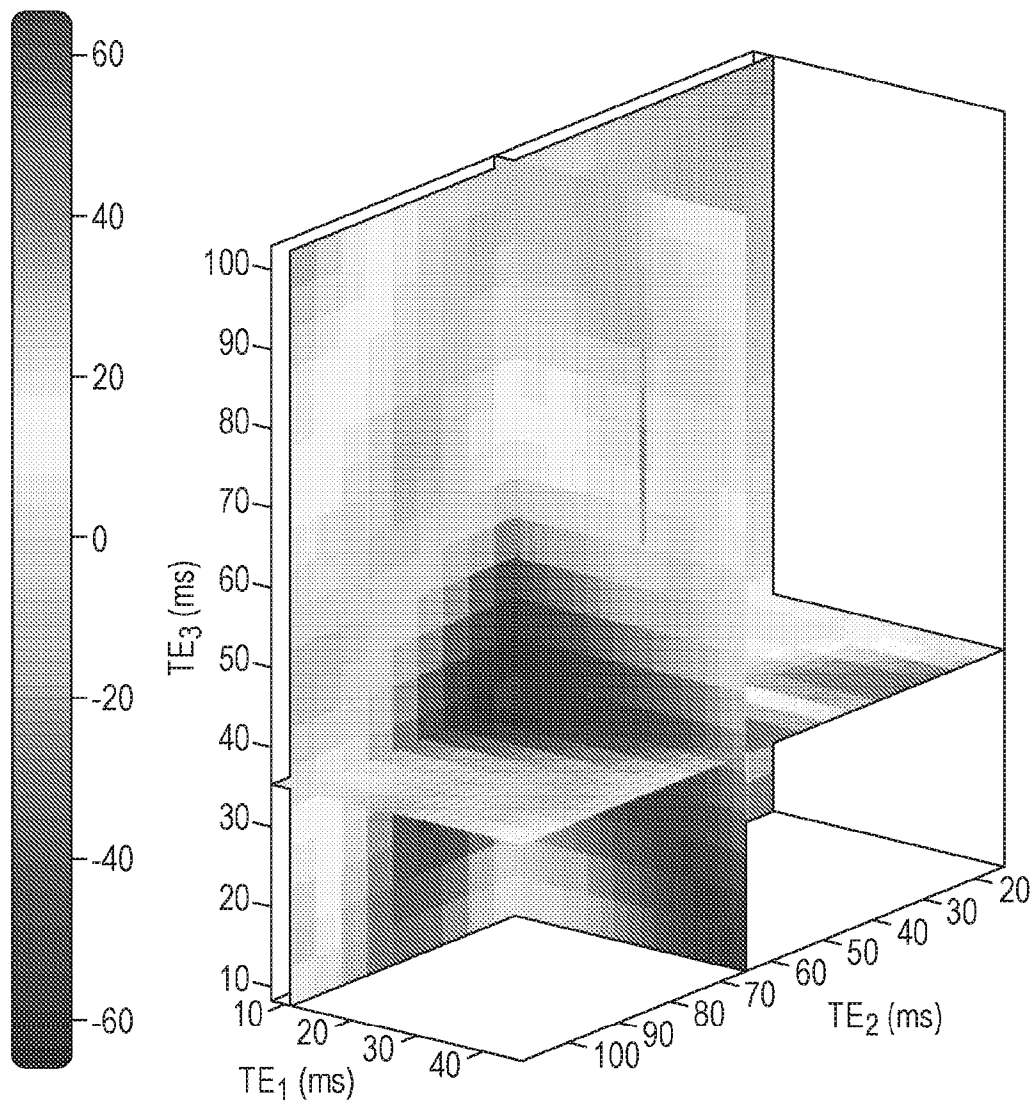


Fig. 10



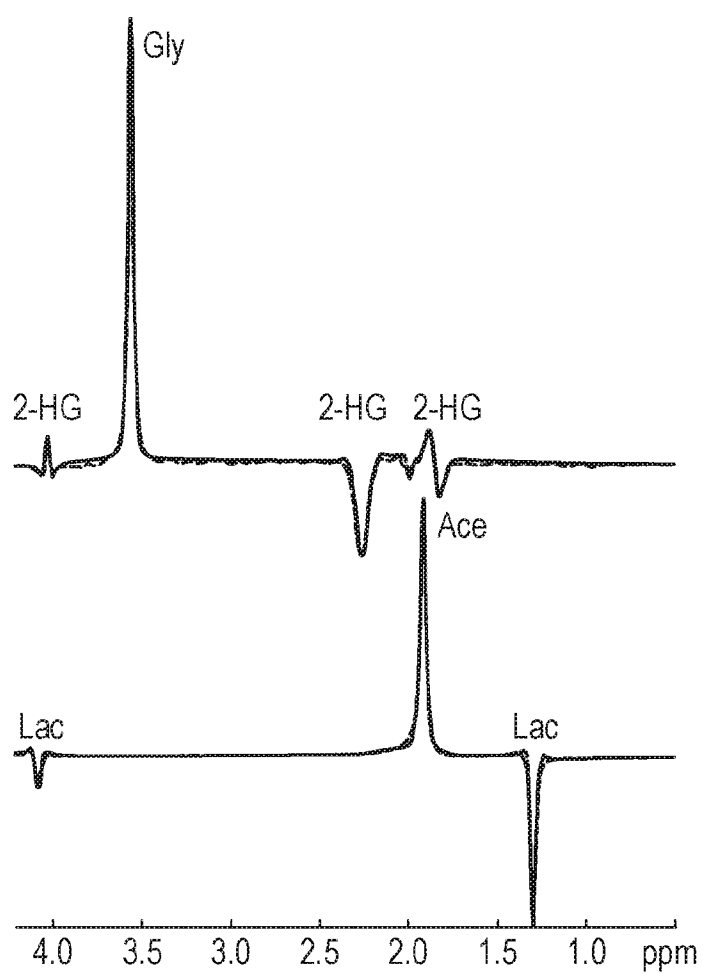
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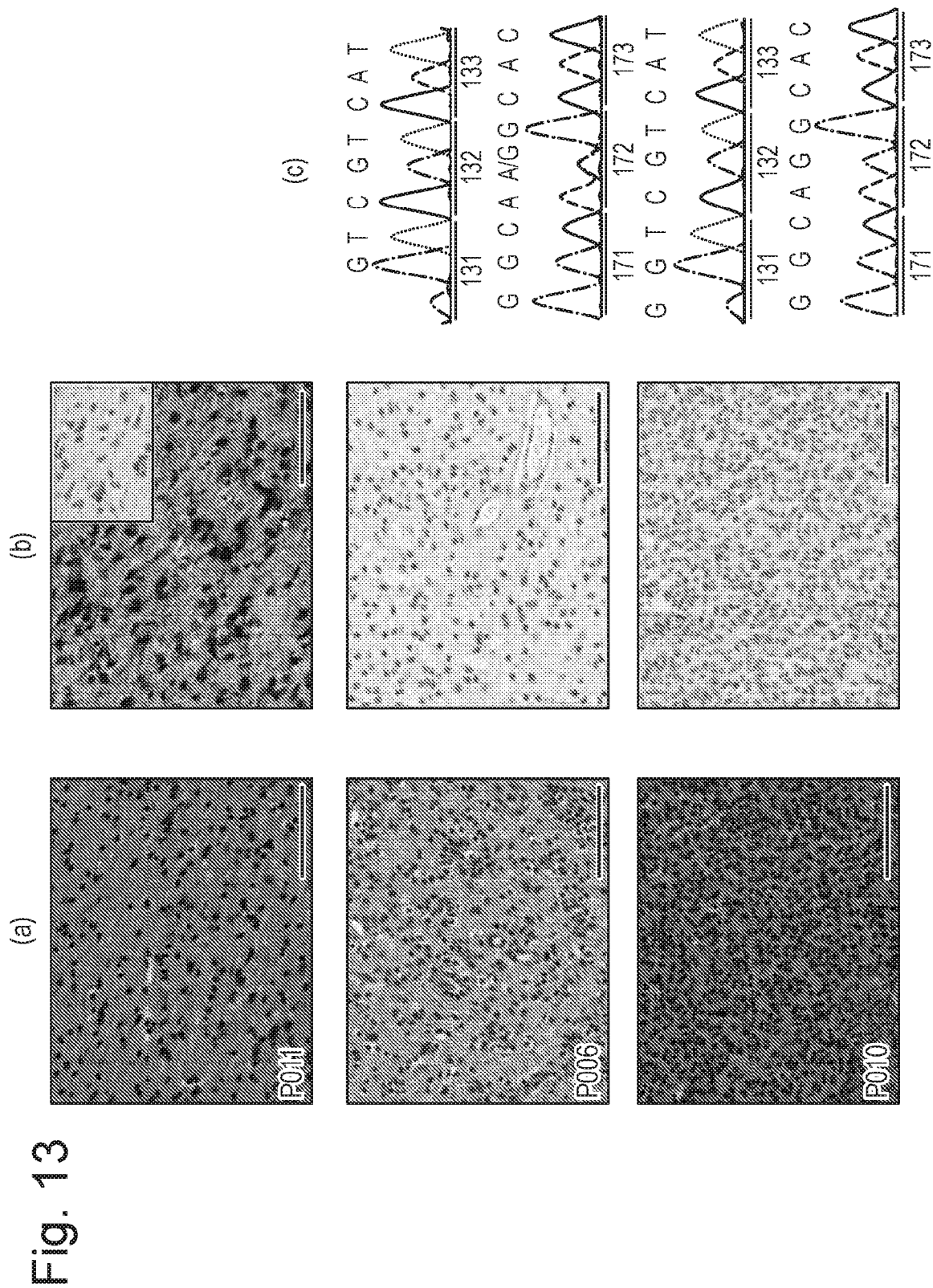
Fig. 11



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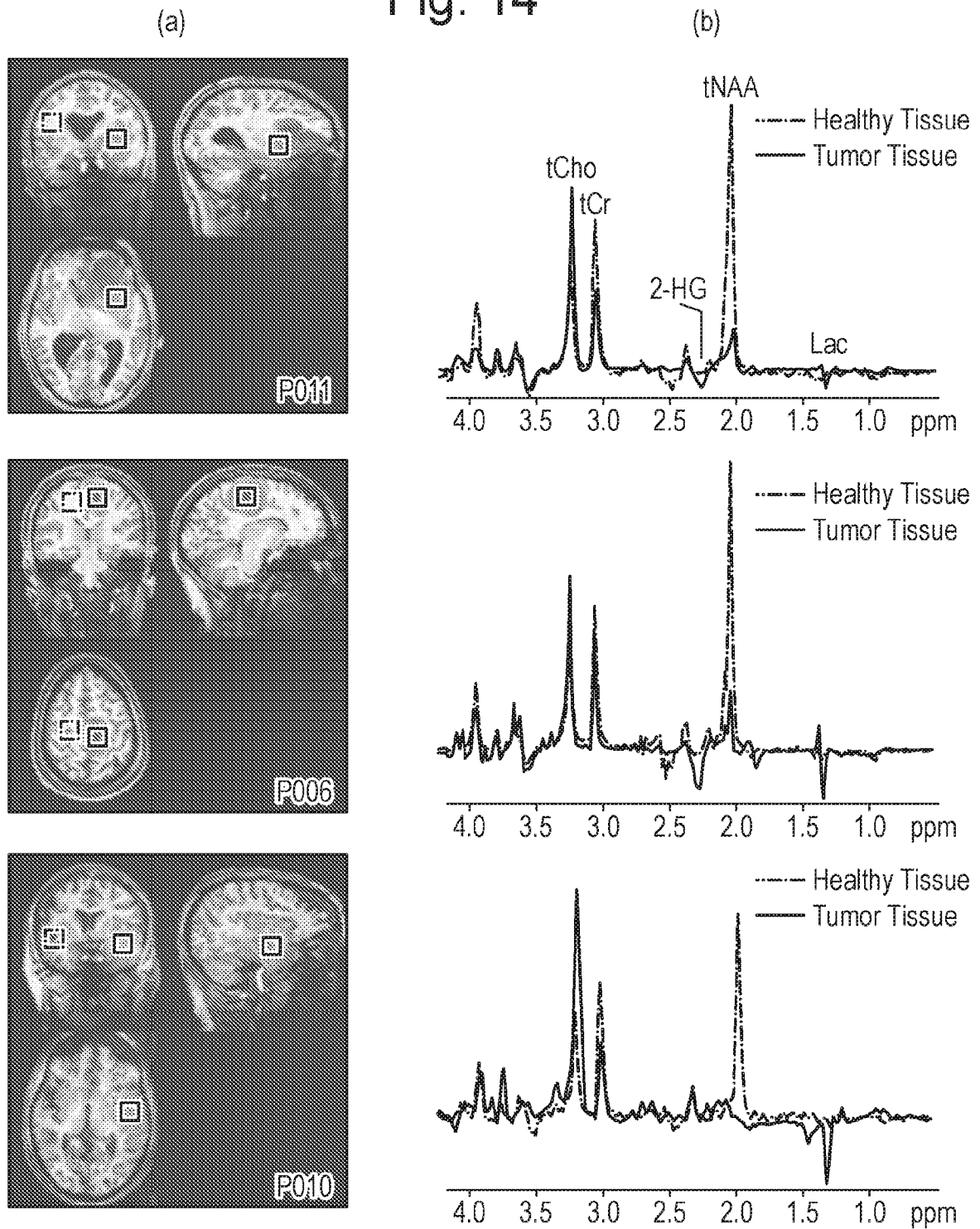
Fig. 12





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Fig. 14



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Fig. 15

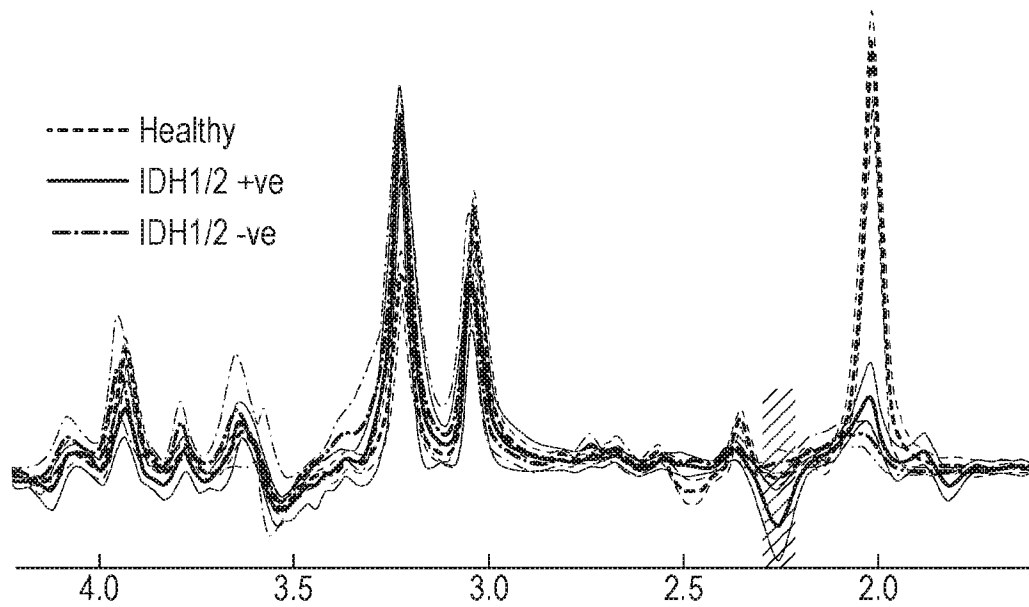
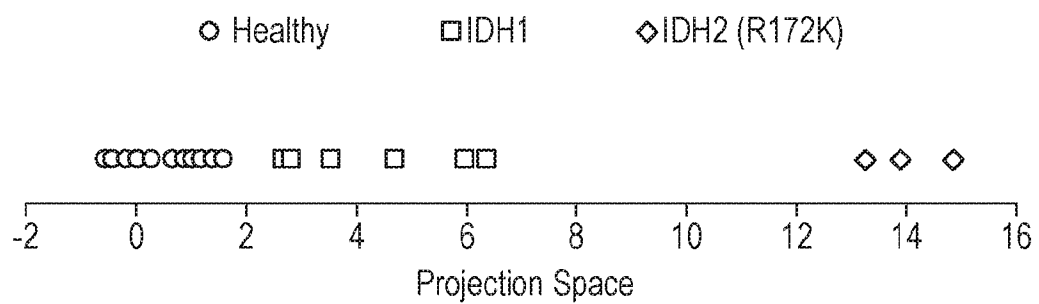


Fig. 16



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Fig. 17

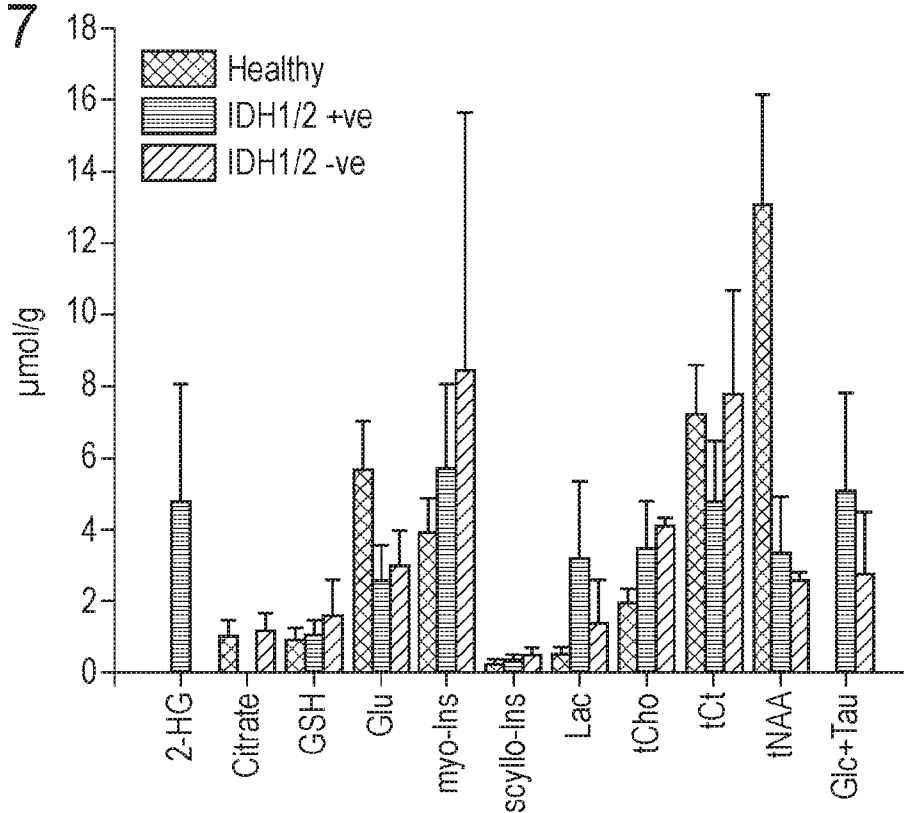
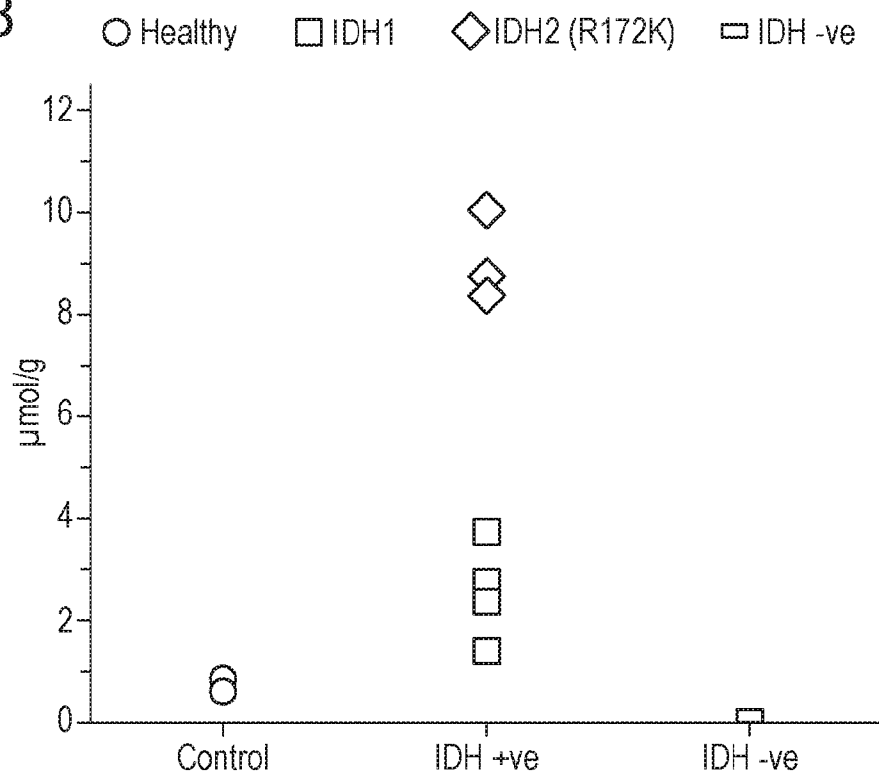


Fig. 18



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Fig. 19

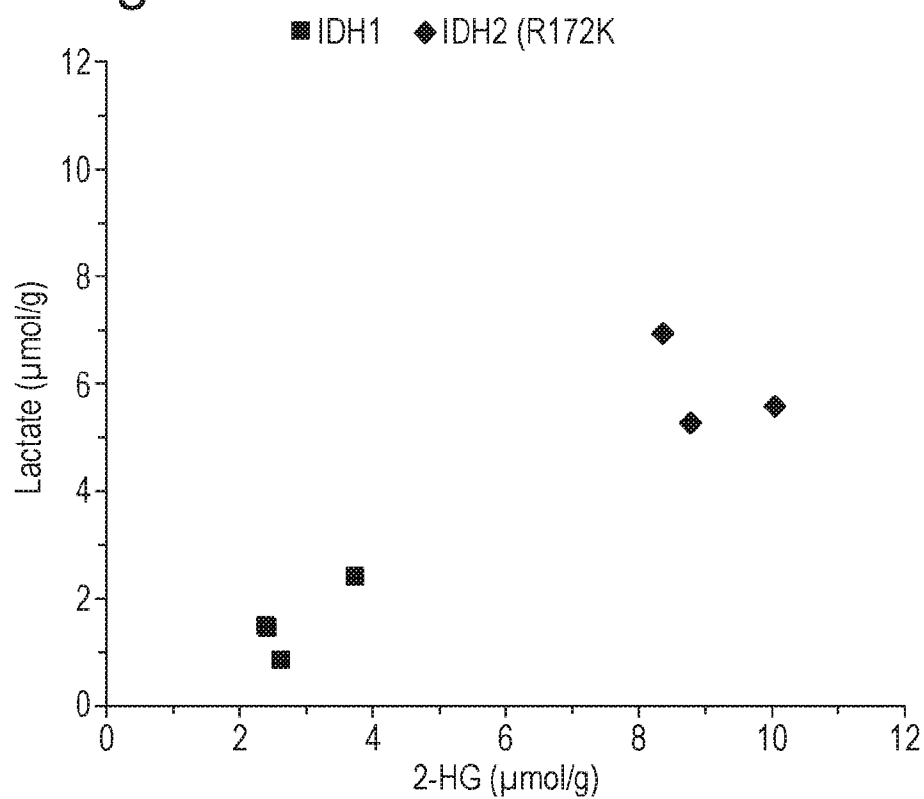


Fig. 20

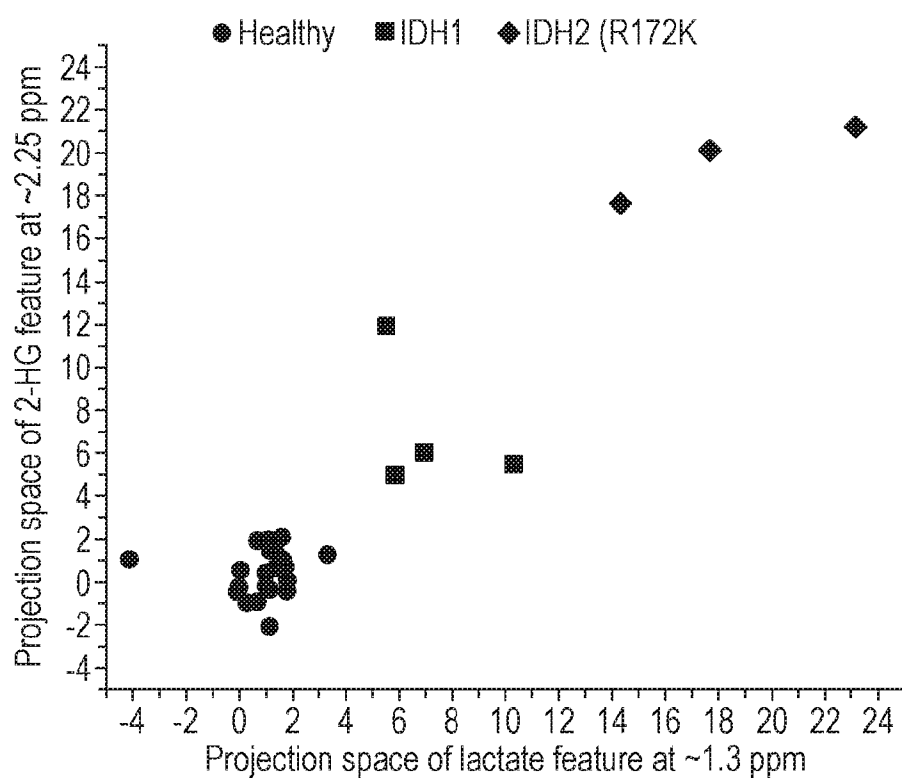
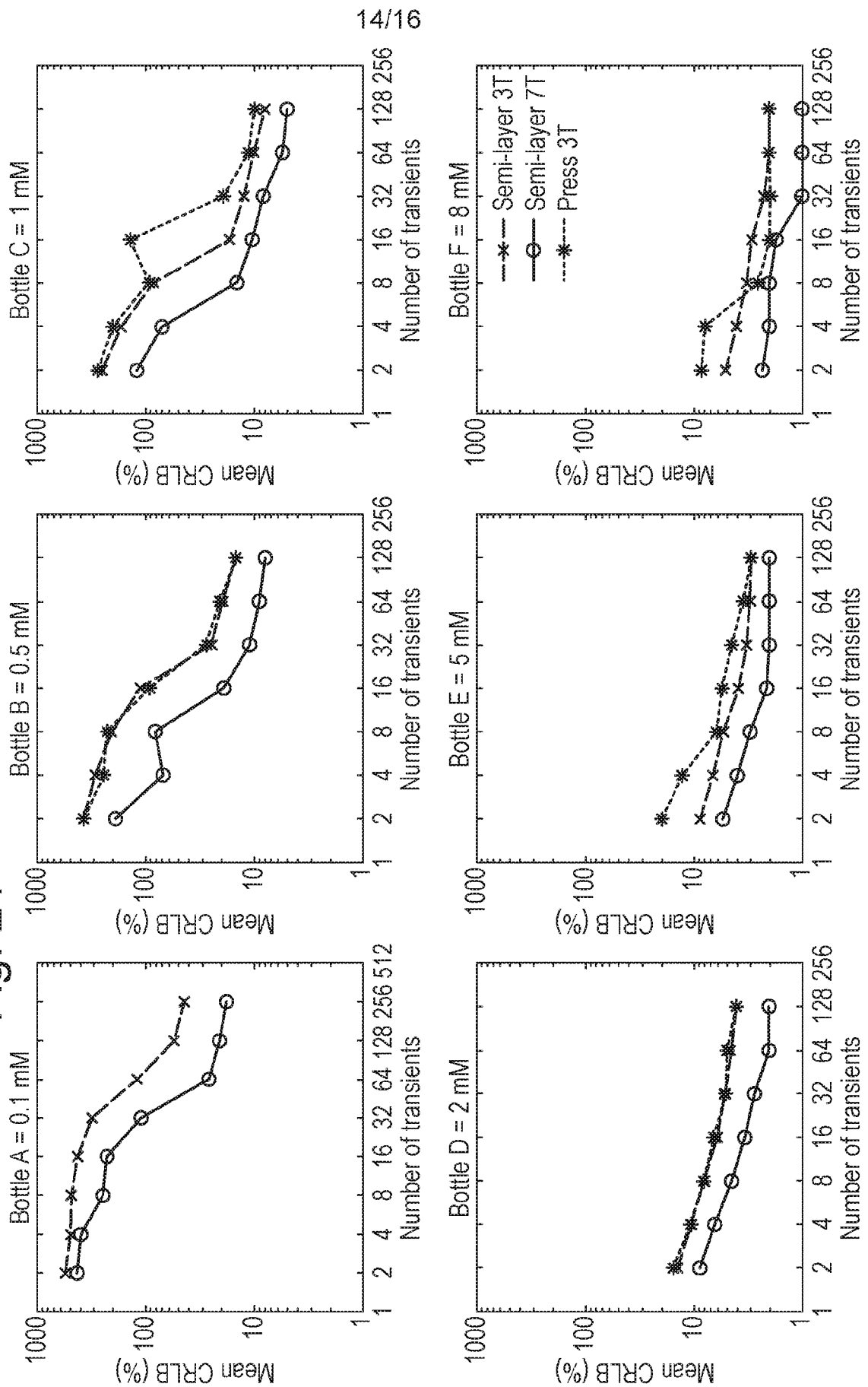
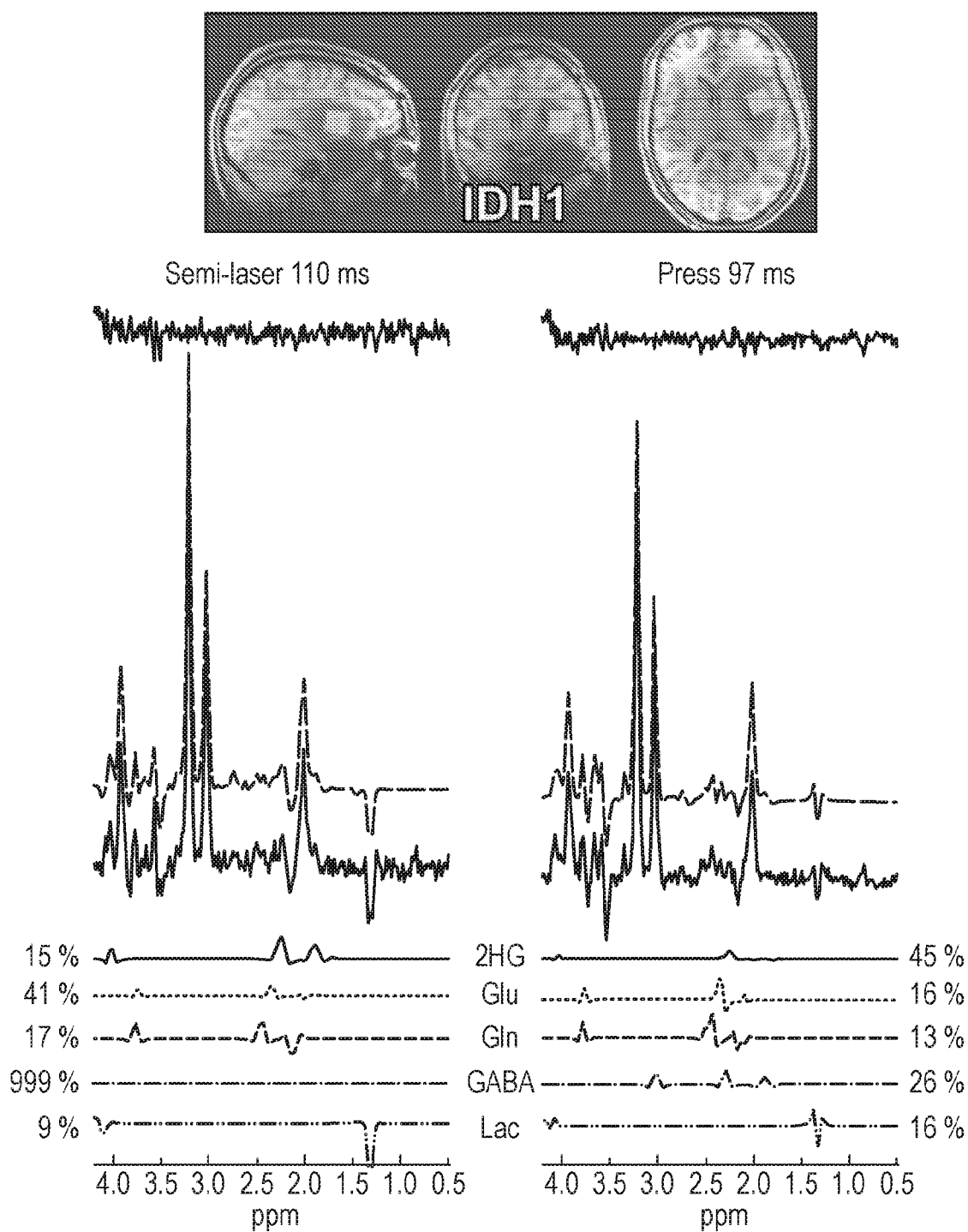


Fig. 21



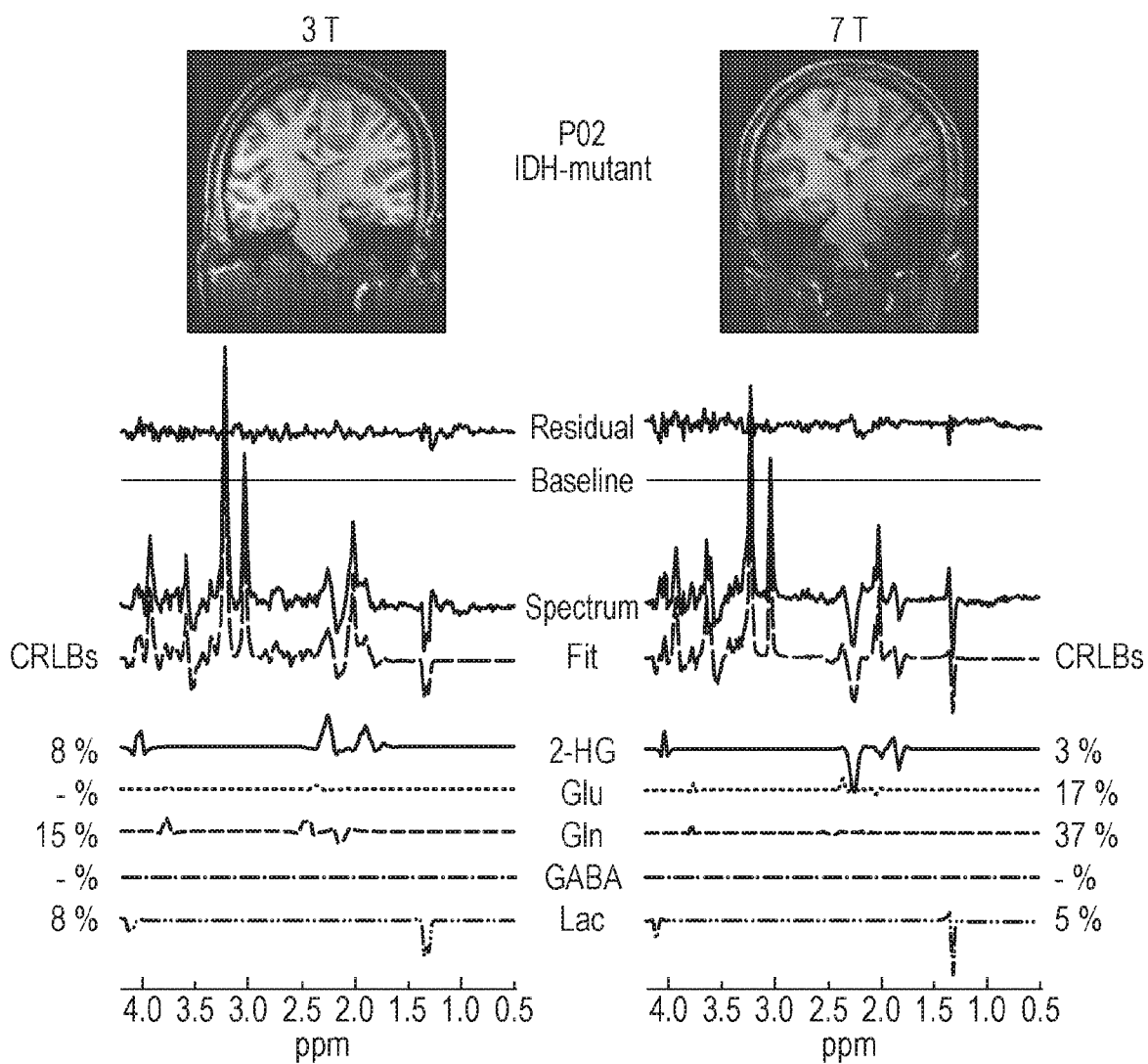
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Fig. 22



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Fig. 23



INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2016/051186

A. CLASSIFICATION OF SUBJECT MATTER INV. A61B5/055 C12Q1/32 G01R33/46 G01R33/465 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) G01R A61B C12Q		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Takako Kurimoto: "Feasibility of Adiabatic Refocusing Pulse for Detecting 2-Hydroxyglutarate using Proton Single-voxel Magnetic Resonance Spectroscopy at 7 Tesla", 1 January 2011 (2011-01-01), pages 1-2, XP055280830, Retrieved from the Internet: URL: http://archiv.ub.uni-heidelberg.de/vol1/textserver/14270/1/Kurimoto.pdf [retrieved on 2016-06-15] page 1 - page 2 -/-	1-17
☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search	Date of mailing of the international search report	
16 June 2016	28/06/2016	
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Lindberg, Pia	

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2016/051186

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	& Takako Kurimoto: "Feasibility of adiabatic refocusing pulse for detecting 2-hydroxyglutarate using proton single-voxel magnetic resonance spectroscopy at 7 Tesla - Deutsche Digitale Bibliothek", 1 January 2011 (2011-01-01), pages 1-1, XP055280832, Retrieved from the Internet: URL: https://www.deutsche-digitale-bibliothek.de/item/XJ3PWS6REAYCB02QITDHBK4WU522NYPB [retrieved on 2016-06-15] the whole document	
A	----- J.-A. LOSMAN ET AL: "What a difference a hydroxyl makes: mutant IDH, (R)-2-hydroxyglutarate, and cancer", GENES & DEVELOPMENT, vol. 27, no. 8, 15 April 2013 (2013-04-15), pages 836-852, XP055124908, ISSN: 0890-9369, DOI: 10.1101/gad.217406.113 abstract page 840, left-hand column, last paragraph.	1-17
X	----- CHANGHO CHOI ET AL: "2-hydroxyglutarate detection by magnetic resonance spectroscopy in IDH-mutated patients with gliomas", NATURE MEDICINE., vol. 18, no. 4, 26 January 2012 (2012-01-26), pages 624-629, XP055280972, US ISSN: 1078-8956, DOI: 10.1038/nm.2682	1-7, 10-17
Y	abstract, pages 624-626, the bridging paragraph on pages 627 and 628, figure 1a ----- -/--	8,9

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2016/051186

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JACOB PENNER ET AL: "Semi-LASER 1 H MR spectroscopy at 7 Tesla in human brain: Metabolite quantification incorporating subject-specific macromolecule removal", MAGNETIC RESONANCE IN MEDICINE., vol. 74, no. 1, 31 July 2014 (2014-07-31), pages 4-12, XP055280980, US ISSN: 0740-3194, DOI: 10.1002/mrm.25380 abstract, page 2, left-hand column, 1st full paragraph, page 3, left-hand column, 2nd full paragraph -----	8,9
Y	V.O. BOER ET AL: "7-T 1H MRS with adiabatic refocusing at short TE using radiofrequency focusing with a dual-channel volume transmit coil", NMR IN BIOMEDICINE, vol. 24, no. 9, 4 February 2011 (2011-02-04), pages 1038-1046, XP055023404, ISSN: 0952-3480, DOI: 10.1002/nbm.1641 abstract, page 1039, left-hand column, 1st full paragraph, pages 1043-1045 section "discussion". -----	8,9
X,P	U. E. EMIR ET AL: "Noninvasive Quantification of 2-Hydroxyglutarate in Human Gliomas with IDH1 and IDH2 Mutations", CANCER RESEARCH, vol. 76, no. 1, 15 December 2015 (2015-12-15), pages 43-49, XP055280987, US ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-15-0934 the whole document -----	1-17