Diagnosis/monitoring of glioblastoma and acute myeloid leukemia by (D)-2-hydroxyglutarate test (P-977)

Key facts
- Diagnosis and monitoring of IDH1/IDH2/IDH3 dependent diseases such as:
  - Glioblastomas, astrocytoma, oligodendrogliomas, oligoastrocytoma, acute myeloid leukemia (AML), chondrosarcoma, intrahepatic cholangiocarcinoma, angioimmunoblastic T cell lymphoma
- Simple and robust enzymatic assay; readout in 3 hours; suitable for 96-/384-well format
- Less expensive/time-consuming and high-throughput possible in opposite to established GC-MS test

Background
Isocitrate dehydrogenase (IDH) enzymes catalyze the oxidative decarboxylation of isocitrate to alpha ketoglutarate. In humans, three IDH isoforms are known, the homodimers IDH1 and IDH2 and the heterotetramer IDH3. Mutations in genes encoding IDH enzymes have been identified both in neurometabolic disorders and tumorous diseases. IDH1 have been found in secondary glioblastoma (83 %), diffused astrocytoma (71 %), oligodendrogliomas and oligoastrocytoma of WHO Grade II and III. In addition, the mitochondrial IDH2 have been also identified, although with less frequency (3 %). IDH mutations occur not only in diffuse gliomas, but also in acute myeloid leukemia (IDH1: 6.85 %; IDH2: 8.711 %), chondrosarcoma (56 %), intrahepatic cholangiocarcinoma (~25 %) and angioimmunoblastic T cell lymphoma (~45 %).

The diagnosis of low-grade gliomas is presently performed by immuno-histological analysis using the IDH1 R132H-specific antibody. However, other mutations in the IDH genes can at present only be detected by DNA sequencing of the corresponding exons.

In addition, detection of (D)-2-hydroxyglutarate (D2HG) in tumor tissue and in paraffin-embedded tissues of low-grade gliomas and in blood sera of AML patients can currently only be carried out by GC-MS, which is labor-intensive/expensive/time-consuming as well as not suitable for high-throughput analysis of samples. A robust D2HG test is therefore highly desired, but not yet available.

Technology
Researchers from DKFZ and University Hospital of Heidelberg developed a test for detecting (D)-hydroxyglutarate in a tumor sample by measuring the production of the reduced state of the dye. The technique can be used for diagnosis but in addition monitoring a (D)-2-hydroxy-glutarate-associated disease of a patient.

Advantages and Commercial Opportunity
Development of simple and robust enzymatic assays for the specific determination of the D- (or R-) enantiomer of 2-hydroxyglutarate, i.e. (D)-2-hydroxyglutarate (D2HG). The readout is available already in about 2 to 3 hours. Moreover, they are suitable for 96-well format and can be even further miniaturized to the 384-well format, thereby allowing for the parallel analysis (high-throughput) of numerous samples at the same time.

Development Stage
The test can be used for HTS and was successfully tested using on 96-well format.

Inventors
The invention was jointly conceived by researchers of DKFZ and University Hospital of Heidelberg: Andreas von Deimling, Jörg Balss, Stefan Pusch and Wolfgang Buckel from Max Planck Institute of Marburg.

Intellectual Property
Scientific Publications of inventors


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Figure 1: D2HG was diluted in water, blood serum, and urine to get a standard curve (0-375 pmol = 0-15 µM). Samples were prepared according to the protocol in Figure 2 and assayed with the diaphorase/resazurin read-out. In the graph, the relative fluorescence (RFU) is blotted against the D2HG concentration.

Figure 2: Measurement of sera of patients suffering from acute myeloid leukemia (AML). These patients carry an IDH1 mutation which results in the production of D2HG. Comparison between gas chromatography-mass spectrometry (GC-MS) (single determination) and enzyme assay of the invention using the diaphorase/resazurin read-out (in triplicate). In comparison, sera of healthy persons who did not carry an IDH1 mutation have been tested. These samples were D2HG negative.
Foreign Scientific Publications
supporting the findings and the commercial importance:


2) “Circulating oncometabolite 2-hydroxyglutarate is a potential surrogate biomarker in patients with isocitrate dehydrogenase-mutant intrahepatic cholangiocarcinoma.” In Clin Cancer Res. 2014 Apr 1;20(7):1884-90, by Borger et al.


6) “Mutant IDH1 promotes leukemogenesis in vivo and can be specifically targeted in human AML.” In Blood. 2013 Oct 17;122(16):2877-87, by Chaturvedi A. et al.

