

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 up to 1536 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 IDH1 and 2 enzymes have been identified in metabolic disorders, inborn tumor associated disease and numerous tumors. IDH mutations define secondary glioblastoma, diffused astrocytoma, and oligodendrogliomas (100%). IDH mutations occur not only in diffuse gliomas, but also in enchondroma (~80%), chondrosarcoma (~60 %), angioimmunoblastic T cell lymphoma (~45 %), intrahepatic cholangiocarcinoma (~25 %), acute myeloid leukemia (~20 %), , and other tumor entities (<5%) . All mutations lead to a nemorphic enzyme function, now producing (D)-2-hydroxyglutarate (D2HG), which can be used as surrogate marker for all mutations.

The diagnosis of IDH mutations is presently performed by immuno-histological analysis using the IDH1 R132H-specific antibody or sequencing.

As alternative, detection of D2HG in tumor tissue, in paraffin-embedded tissues and in blood/sera can be carried out by individual mass spectrometry analysis.

Technology

Researchers from DKFZ and University Hospital of Heidelberg developed a test for detecting D2HG in diverse samples by measuring the production of the reduced state of the dye. The technique can be used for diagnosis but in addition monitoring a D2HG-associated disease of a patient.

Advantages and Commercial Opportunity

Development and distribution of a simple and robust enzymatic assays for the specific determination of the D2HG. The readout is available already in about 2 to 3 hours. Moreover, it is suitable for 96-well format and can be even further miniaturized to the 1536-well format, thereby allowing for the parallel analysis (high-throughput) of numerous samples at the same time. Beside an already granted license for research use only, we now seek a licensee for the DIAGNOSTIC field.

Development Stage

The test can be used for HTS and was successfully tested using on 96 up to 1536 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

Priority patent application EP12157663.1 "Means and Methods for the Determination of (D)-2-Hydroxyglutarate (D2HG)" as well as a PCT patent application [WO 2013/127997](#).

Scientific Publications

"Detection of 2-hydroxyglutarate in formalin-fixed paraffin-embedded glioma specimens by gas chromatography/mass spectrometry." in [Brain Pathol. 2012 Jan;22\(1\):26-31](#) by Sahm F et al..

“Enzymatic assay for quantitative analysis of (D)-2-hydroxyglutarate.” in [Acta Neuropathol. 2012 Dec;124\(6\):883-91](#) by Balss J et al..

“Pan- mutant IDH1 inhibitor BAY 1436032 for effective treatment of IDH1 mutant astrocytoma in vivo.” In [Acta Neuropathol. 2017 Jan 25. PMID: 28124097](#) by Stefan Pusch et al..

“Pan-mutant-IDH1 inhibitor BAY1436032 is highly effective against human IDH1 mutant acute mye-

loid leukemia in vivo.” In *Leukemia*, Jan 2017; doi: 10.1038/leu.2017.46. by A Chaturvedi et al..

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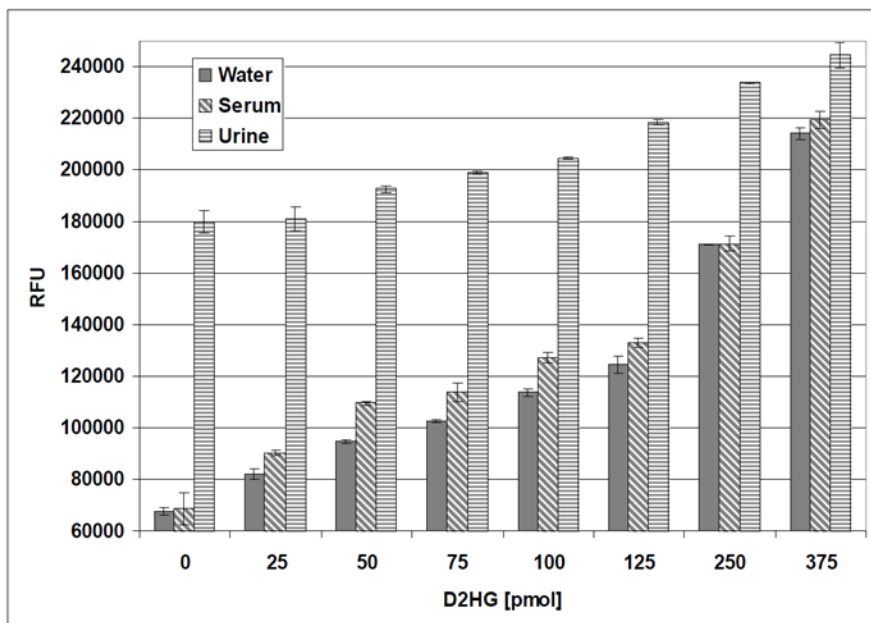


Figure 1: D2HG was diluted in water, blood serum, and urine to get a standard curve (0-375 pmol = 0-15 μ M). Samples were prepared according to the protocol in Figure 2 and assayed with the diaphorase/ resazurin read-out. In the graph, the relative fluorescence (RFU) is plotted against the D2HG concentration.

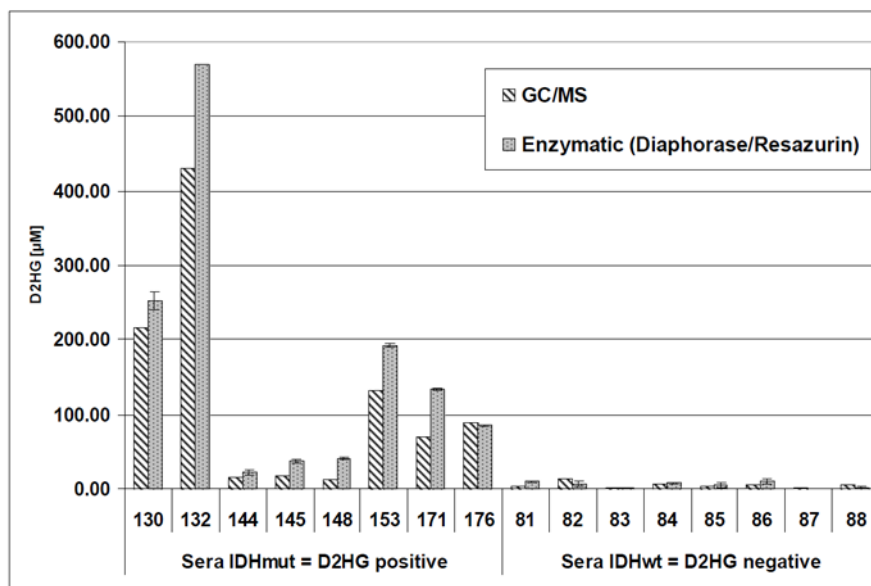


Figure 2: Measurement of sera of patients suffering from acute myeloid leukemia (AML). These patients carry an IDH1 mutation which results in the production of D2HG. Comparison between gas chromatography-mass spectrometry (GC-MS) (single determination) and enzyme assay of the invention using the diaphorase/ 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 for the D2-HG test:

- 1) „Isocitrate Dehydrogenase 1 (IDH1) Mutation in Breast Adenocarcinoma Is Associated With Elevated Levels of Serum and Urine 2-Hydroxyglutarate.“ [Oncologist. 2014 Jun;19\(6\):602-7](#), by Fathi et al.
- 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.
- 3) "Serum 2-hydroxyglutarate production in IDH1- and IDH2-mutated de novo acute myeloid leukemia: a study by the Acute Leukemia French Association group." In [J Clin Oncol. 2014 Feb 1;32\(4\):297-305](#). doi: 10.1200/JCO.2013.50.2047. Epub 2013 Dec 16, by Janin et al.
- 4) "MYC-driven accumulation of 2-hydroxyglutarate is associated with breast cancer prognosis." In [J Clin Invest. 2014 Jan 2;124\(1\):398-412](#), by Terunuma et al.
- 5) "Prognostic significance of 2-hydroxyglutarate levels in acute myeloid leukemia in China."in [Proc Natl Acad Sci U S A. 2013 Oct 15;110\(42\):17017-22](#), by Wang JH 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.
- 7) "Serum 2-hydroxyglutarate levels predict isocitrate dehydrogenase mutations and clinical outcome in acute myeloid leukemia." In [Blood. 2013 Jun 13;121\(24\):4917-24](#), by DiNardo CD et al.
- 8) "Prospective serial evaluation of 2-hydroxyglutarate, during treatment of newly diagnosed acute myeloid leukemia, to assess disease activity and therapeutic response." In [Blood. 2012 Nov 29;120\(23\):4649-52](#), by Fathi AT et al.
- 9) "2-Hydroxyglutarate in IDH mutant acute myeloid leukemia: predicting patient responses, minimal residual disease and correlations with methylcytosine and hydroxymethylcytosine levels." In [Leuk Lymphoma. 2013 Feb;54\(2\):408-10](#), by Pollyea DA et al.