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 D2HGin tumor tissue, in paraffin-embedded tissues and in blood/sera can be carried out by individual mass spectrometry analysis.

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

Scientific Publications

"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-

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.

**DKFZ Contact:**
Dr. Frieder Kern
Deutsches Krebsforschungszentrum
Technology Transfer Office T010
Email: F.Kern@dkfz.de
Tel.: +49-(0)6221-42-2952
Fax: +49-(0)6221-42-2956
Foreign Scientific Publications
supporting the findings and the commercial importance for the D2-HG test:


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.


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.