Genetic Study of anti-EGFR Antibody Therapy Response in Colorectal Carcinoma

For genetic diagnosis use.

PATIENT	
Name:	**N.A.**
Date of birth:	**N.A.**
Gender:	**N.A.**
Ethnicity:	**N.A.**
Consultancy referral number:	**GM042547**
Family history:	**N.A.**
Medical referral reason:	**Anti-EGFR therapy in metastatic colorectal carcinoma**
Genetic laboratory referral reason:	**N.A.**
Purpose:	**Pharmacogenetics**
Specimen type:	**FFPE colon adenocarcinoma**

HEALTHCARE PROVIDER	
Referring physician:	
Medical reference:	
Harvesting facility:	
Referring facility:	**HeartGenetics**
Requisition date:	
Fulfillment date:	

1. RESULTS

1.1. MOLECULAR TESTING

Predictive markers of treatment outcome with anti-EGFR antibody therapy (*KRAS*, *NRAS*):

Mutational status: **NEGATIVE**

No mutations were identified for these markers.

Negative prognostic markers (*BRAF*):

Mutational status: **NEGATIVE**

No mutations were identified for these markers.

Emerging markers (*ERBB2*, *EGFR*, *KRAS*, *NRAS*, *PIK3CA*):

Mutational status: **NEGATIVE**

No mutations were identified for these markers.

1.2. GUIDELINE RECOMMENDATIONS

According to the guidelines from the European Society for Medical Oncology (ESMO), in patients with metastatic colorectal carcinoma (mCRC) *RAS* mutations are negative predictive markers of anti-EGFR monoclonal antibody treatment outcome, whereas *BRAF* mutations are negative prognostic markers [1]. Hence, only patients with RAS wild- type mCRC should be under consideration for treatment with cetuximab and panitumumab [1]. The ESMO's Zurich treatment algorithm guides patient therapeutic management according to the *RAS* and *BRAF* mutational status [1]. Mutations in *PIK3CA*, exon 20, activating mutations in *ERBB2* and *EGFR* ectodomain mutations are all considered to be emerging biomarkers, and there is insufficient evidence to recommend their use for therapy selection outside of a clinical trial [1].

2. TECHNICAL INFORMATION

2.1. METHODOLOGY

1. A commercial kit was used to perform DNA extraction and purification from FFPE colon adenocarcinoma. DNA concentration and quality were evaluated with MultiskanGo spectrophotometer (Thermo Scientific).
2. The status of 171 mutations in 6 genes (OncoAlvo® Panel) was assessed using a high-throughput DNA Microchip platform, the iPLEX® system (Agena Bioscience, Inc). This array platform allows an optimal genetic analysis by combining the benefits of accurate primer extension chemistry with MALDI-TOF mass spectrometry. The different masses of each generated PCR product are then converted into mutational status information.
3. The iPLEX® system has an accuracy of 99%.

2.2. OncoAlvo PANEL

BRAF	**B-Raf proto-oncogene, serine/threonine kinase** | ENST00000288602	*KRAS*	**KRAS proto-oncogene, GTPase** | ENST00000311936
EGFR	**epidermal growth factor receptor** | ENST00000275493	*NRAS*	**neuroblastoma RAS viral oncogene homolog** | ENST00000369535
ERBB2	**erb-b2 receptor tyrosine kinase 2** | ENST00000269571	*PIK3CA*	**phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha** | NM_006218.1

2.3. RISKS AND LIMITATIONS

HeartGenetics applies a rigorous quality control which may not exclude the possibility of error that might influence the test results. The reliability of the results is always guaranteed as HeartGenetics, Genetics and Biotechnology SA standard quality recommendations have been followed for the execution of this genetic test. The results presented in this report are limited to the available scientific knowledge at the time this test was developed. The company guarantees the accuracy of the scientific knowledge presented in the report. It has been assumed as truthful all the above declarations about patient and medical identity, the purpose of the study, index case and nature of analysed biological products.

2.4. QUALITY ASSURANCE

HeartGenetics, Genetics and Biotechnology SA is an ISO NP 9001 and ISO 13485 certified company for Quality Management System and applies an External Quality Assessment program from UK NEQAS. The laboratory that performs this genetic test undertakes to, at all times, comply with the all applicable certifications and Law in its territory.

2.5. TERMS AND CONDITIONS

HeartGenetics, Genetics and Biotechnology SA will not be liable whether in contract, tort, warranty, or under any statute, or any other basis of special, incidental, indirect punitive, multiple, or consequential damages in connection with in arising from this document, or improper use of the product described herein or any use of such product outside the scope of the express written licenses or permissions granted by HeartGenetics, Genetics and Biotechnology SA, to the extent allowed by law.
The results presented in Section 3.1, Genetic Information, are the responsibility of the laboratory that performed the genetic test.

TECHNICAL DIRECTION
Cantanhede, 2017-07-12

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Associate Laboratory Director
(Operation responsibility)

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Laboratory Director
(Validation responsibility)

3. APPENDIX

3.1. GENETIC INFORMATION

Without genetic alterations.

3.2. EVIDENCES FOR MOLECULAR MARKERS

Without genetic alterations.

4. REFERENCES

[1] E. Van Cutsem, A. Cervantes, R. Adam, A. Sobrero, J. Van Krieken, D. Aderka, E. Aranda Aguilar, A. Bardelli, A. Benson, G. Bodoky, *et al.*, Annals of Oncology **27**, 1386 (2016).