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:	**GM042535**
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:	**Isabel Gaspar**
Medical reference:	**GM042535**
Harvesting facility:	**IPO Porto**
Referring facility:	**HeartGenetics**
Requisition date:	**2017-03-30**
Fulfillment date:	**2017-07-12**

1. RESULTS

1.1. MOLECULAR TESTING

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

Mutational status: **POSITIVE**

Analysed sample presents 1 mutation(s):
KRAS c.34G>T, p.Gly12Cys: This mutation is predicted to cause a lack of response to anti-EGFR antibody therapy.

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.

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The results presented in Section 3.1, Genetic Information, are the responsability of the laboratory that performed the genetic test.

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TECHNICAL DIRECTION
Cantanhede, 2017-07-12

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(Operation responsibility)

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Human Geneticist, Specialist; Molecular Biologist, PhD
Laboratory Director
(Validation responsibility)

3. APPENDIX

3.1. GENETIC INFORMATION

The results, described according to HGVS nomenclature (`http:www.hgvs.org`), are presented in the following table.
No other genetic variants from HeartGenetics panel were identified, than those shown in the table.

Gene	COSMIC reference	Nucleotidic change [1]	Aminoacidic change	Allelic frequency [2]
KRAS	COSM516	c.34G>T	p.Gly12Cys	57%

[1] The numeric identification associated with each variant is indexed to a reference sequence obtained from COSMIC database (`http://cancer.sanger.ac.uk/cosmic`).
[2] Allele frequency determined for analysed sample. This value does not necessarily reflect the allele frequency on the tumour itself.

3.2. EVIDENCES FOR MOLECULAR MARKERS

KRAS

KRAS is a proto-oncogene that encodes for a small GTPase protein, K-Ras, which relays signals from cell membrane EGFR, and other receptor tyrosine kinases, to the cell nucleus, controlling key processes such as cell proliferation, differentiation and survival [2]. Specific mutations in *KRAS* render the resulting protein constitutively activated, regardless of the EGFR status [2, 3]. For this reason, EGFR-targeted monoclonal antibodies cetuximab and panitumumab are ineffective against clones harbouring these *KRAS* activating mutations [4, 5, 6, 7, 8].

4. REFERENCES

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