3. Practical Course Detection of RAS Mutations in human metastastic Colorectal Cancer (mCRC)

Munich – Institute for Pathology of the University Munich – May 4th – 7th 2015

In the therapy of cancers personalized medicine became an important option. Thereby, personalized medicine is characterized by:
(1) The drug has a known TARGET (targeted therapy),
(2) There is a biomarker PREDICTing the action of the drug ahead of its application (predictive therapy).

Due to the biomarker it is possible to select subgroups of patients which will show response (positive biomarker) or will not response (negative biomarker). For metastastic colorectal cancer (mCRC) there are two classes of targeting drugs available in the moment:

(1) anti-EGFR (epidermal growth factor receptor) directed antibodies (cetuximab/ Erbitux® and panitumumab/ Vectibix®),
(2) anti-VEGF (vascular endothelial growth factor receptor) directed antibodies (bevacizumab/ Avastin® and aflibercept/ Eylea®).

Only for the anti-EGFR class of antibodies a biomarker was elaborated which predicts non-responsiveness thus being a negative biomarker. Together with the chemotherapeutic backbones FOLFIRI and FOLFOX three approval studies were conducted: CRYSTAL (FOLFIRI together with cetuximab), OPUS (FOLFOX together with cetuximab) and PRIME (FOLFOX together with panitumumab). They demonstrated an increase in PFS (progression free survival) and OS (overall survival) when patients with activating mutations in exon 2 of the KRAS oncogene (Kerstin Rat Sarcoma) were excluded from therapy (about 40% of patients). Both, PFS and OS were even more improved when patients with activating mutations in exons2, 3, and 4 of both the KRAS and NRAS (neuronal RAS) oncogenes were excluded (about 50% of patients).[1-6]

Mechanistically, this behaviour was explained by the RAS-Hypothesis. The EGFR like many other members of the receptor tyrosine kinase (RTK) family transmit their signal via the RAS/ RAF/ MAPK (RAS Associated Factor, Mitogen Activated Protein Kinase) pathway. In cases with mutated RAS molecules permanent activation of the signalling pathway takes place below the EGFR molecule resulting in a short-cut of the pathway making any anti-EGFR targeted therapy useless (Fig. 1).

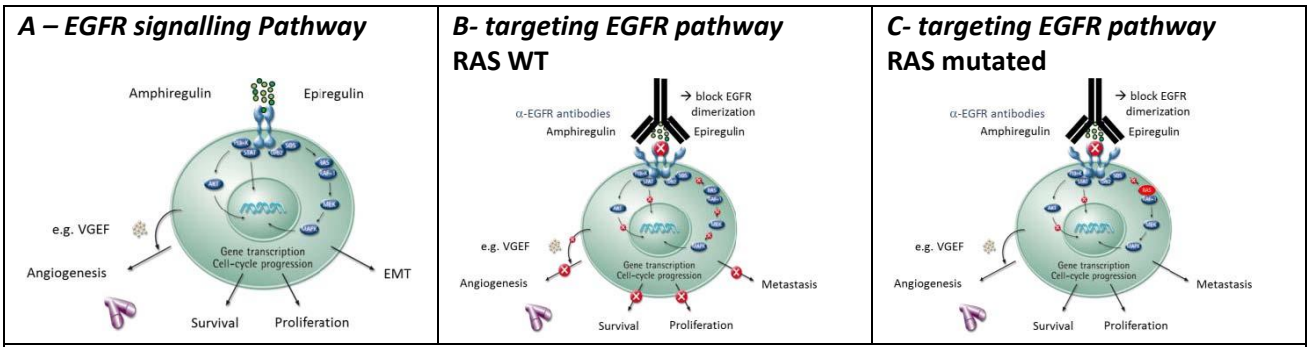

Figure 1: The EGFR signaling pathway and the mechanism of targeting using anti-EGFR directed monoclonal antibodies. (A) Binding of the EGFR receptors amphiregulin and epiregulin, which are the commonly used ligands of colorectal epithelial cells to activate the EGFR results in the dimerization of EGFR monomers and to an autophosphorylation of the intracellular region of the recpetors by transphosphorylation of the dimer. This results in the activation of SH (Src-homology) domain binidng domains enabeling the docking of adaptor molecules like GRB2, STAT and others. This results in the successive phosphorylation of downstream elements like the RAS components KRAS or NRAS and finally in the activation of transcription factors like ERK (extracellular receptor kinase) which translocate into the nucleus changing the expression profile of a cell. In case of transformed cells without proper control of this and other signaling pathways this results in the activation of proliferation, resistance to apoptosis (survival), angiogenesis, but also metastasis. **(B)** By blocking the binding of the ligands the EGFR initiated cascade is inhibited at the top of the signaling thus resulting in the shutdown of the signaling. **(C)** When RAS is mutated (marked in red) the signaling pathway is activated at this point in the signaling cascade thus reactivating the tumor driving characteristics again.

Moreover, it was shown that the usage of anti-EGFR targeted antibodies in the first-line therapy of mCRC in patients with RAS WT is more effective in terms of OS than the application of bevacizumab.[7,8] Therefore, the RAS status of each mCRC should be analysed ahead of therapy to give the patients the maximum

benefit of the therapy (statement of the German AIO – Working group intestinal oncology).

Taken together the testing of mCRC for the status of the biomarker RAS (KRAS- and NRAS oncogenes) is an essential step in the treatment of this disease. Due to the important role of the testing the European approval authority EMA (European Medicines Agengy) included in the approval of both of the anti-EGFR class antibodies that the testing for RAS mutation status should be done in an “experienced laboratory”. It is explained that “experienced” means that the laboratory doing the RAS mutation test should have been tested itself by an external quality assessment system (EQAS) which is done in the form of ring-trials.

Therefore, the Institute for Pathology of the Ludwig-Maximilians University (LMU) Munich founded the RAS^{LMU} Expert Laboratory which conducts in close cooperation with Merck-Serono (Dr. Karim Taha, Medical director) a comprehensive program for the quality assurance of RAS mutation testing (Fig. 2):

- ring-trials (RING – *ring trial*, IRIS - *intercontinental ring system* and valid.valued),
- mutation monitor,
- Training-course for the analysis of RAS mutations in mCRC.

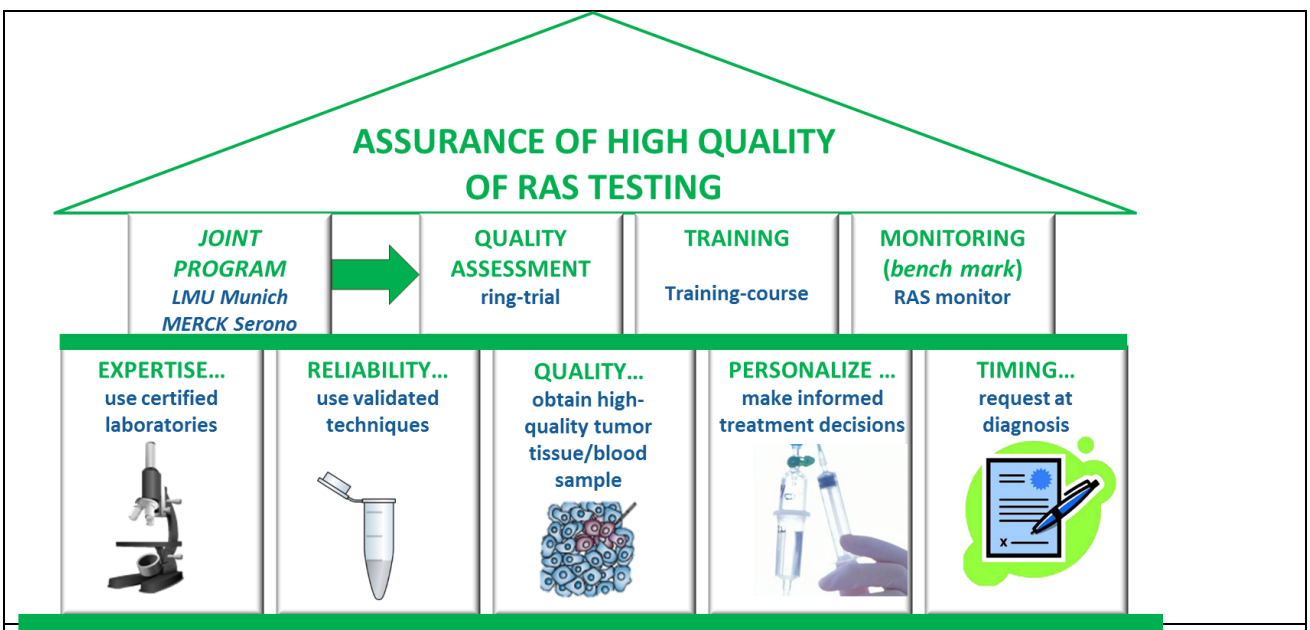

Figure2: Assurance of high quality of testing mutations in the RAS genes. Testing for mutations in predictive biomarkers requires a high standard of quality as the consequence of the result of the test directs the future therapy and thus the life span of patients. Quality is defined by a variety of factors like expertise, reliability, quality of tissue sampling, personalization, timing and others. To test this whole process in the lab three instruments were created by the RAS^{LMU} expert lab and Dr. Karim Taha (MERCK-SERONO, Intercontinental): 1. **Ring trials** (external quality assessment, EQA), 2. **Training-course** (hands-on training course for the detection of mutation in the RAS genes, 3. **RAS monitor** (monitoring the distribution of mutations, giving a benchmark of the frequency of mutations to be expected in the RAS genes KRAS and NRAS).

Between May 4th and 7th 2015 the *3. Practical course for the detection of mutations in the RAS genes* will be held in the Institute for Pathology of the University of Munich. It is a training-course which has its focus on the molecular-pathological detection of RAS genes mutation from tissue of human cancers. It has several goals:

(1) gain solid theoretical knowledge about the basis of the methodology,

(2) gain strategies of error-handling,

(3) learn in practical sessions how to perform the analysis (hands on, wet chemistry)

(4) mutational analyses,

(5) answer questions with existing analysis work roads.

The course takes four day and covers detection by pyrosequencing as well as Sanger-sequencing (Fig. 3).

Day time	time	Monday	Tuesday	Wednesday	Thursday	Friday
Forenoon (A.M.)	9.00		ISOLATION OF DNA	SEQUENCING HOW TO DO	PYROSEQUENCING ANALYSIS OF PYROGRAMS	
	10:00		PCR – HOW TO DO	SANGER-SEQUENCING PCR SETUP		
	11:00		PCR SETUP • PYROSEQUENCING • SANGER SEQUENCING	PYROSEQUENCING SETUP		
	12.00				EXAMINATION	
LUNCH	12.00 1.00					
Afternoon (P.M.)	1.00		PCR – THEORY • FFPE • Primer design • Creating Rules • Controlling Rules	SEQUENCING REACTION	SANGER—SEQUENCING ANALYSIS OF ELECTROPHEROGRAMS	
	2.00	WELCOME Introduction		SEQUENCING – THEORY		
	3.00	DNA PREPARATION				
	4:00		CONTROL OF PCR PRODUCTS • GELELECTROPHORESIS		BREAK	
	5:00				HANDING OVER CERTIFICATES DEPARTURE - FARE WELL	

Figure 3: Time Schedule of the 3. Practical Course for the detection of RAS mutations. Generally, the course is split into a practical and a theoretical part. In a first step the practical part is discussed and then transferred into a practical hands-on part. During incubation periods theoretical considerations and basics for the next practical part are discussed.

Travel and support of the course are organized by Merck-Serono. Please, get in contact with Dr. Karim Taha (Medical Director – Neurology and Immunology Intercontinental Region – Dubai, karim.taha@merckgroup.com) for more information.

References

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