Practical Course Detection of RAS Mutations in Human Colorectal Cancer

Munich – Institute for Pathology – March 17th – 20th 2014

In the therapy of metastatic colorectal cancer (mCRC) personalized medicine has conquered its place. There are two new aspects in personalized medicine:

(1) The drug has a well known TARGET.
(2) It is possible to PREDICT the action of the drug ahead of its application due to knowledge of a biomarker.

A good example is the class of anti-EGFR (epidermal growth factor receptor) targeted antibodies. These antibodies target the EGFR which is a driving force in the majority of mCRC. Thus, it is reasonable to block this signalling pathway thus affecting the tumor cells. This is done by the antibodies which compete with EGFR ligands for the binding to the EGFR-ligand binding site. Obviously, this blockade will work only in cases without other activating components downstream of the EGFR. Unfortunately, mCRC display frequently in one of the downstream elements KRAS or NRAS. The majority of cases (~40%) is characterized by oncogenic thus activating mutations in KRAS exon 2 and a minority (~10%) by mutations in KRAS exons3 or 4 or NRAS exons 2 to 4. Expectedly, mCRC with such activating mutations in the RAS genes should not respond to anti-EGFR targeted therapies what is known as the RAS hypothesis (Fig. 1).

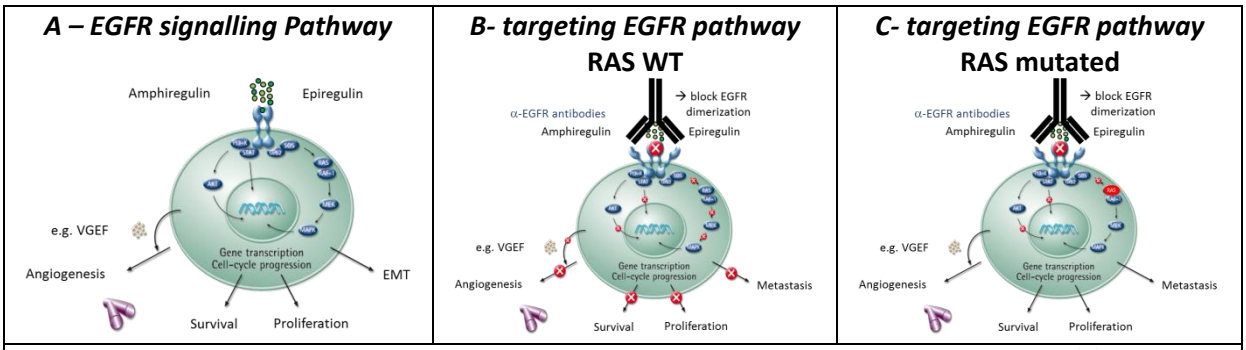

Figure 1: The EGFR signaling pathway and the mechanism of targeting using anti-EGFR directed monoclonal antibodies. (A) Binding of the EGFR receptors amphiregulin and epiregulin, which are the commonly used ligands of colorectal epithelial cells to activate the EGFR results in the dimerization of EGFR 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.

The RAS-Hypothesis was impressingly verified in a variety of clinical studies. Thus, mutations in RAS genes are a negative predictive biomarker for the action of anti-EGFR targeting antibodies. As a consequence the EMA (European Medicines Agency) approved anti-EGFR targeting antibodies as a therapeutical option only for mCRC which harbour WT RAS genes. Thus, it has become now become mandatory to check for the mutational status of the RAS proto-oncogenes ahead of therapy using tissue from the primary tumor.

The RAS^{LMU} expert laboratory of the Institute for Pathology of the Ludwig-Maximilians-Universität München offers in close cooperation with Merck-Serono Intercontinental Region a practical course therefore. This course aims to support laboratories which are in the beginning of the molecular-pathological detection of RAS genes mutation detection or encounter problems with this analysis to:

(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 (wet chemistry)
(4) and mutational analyses.

The course takes five day and covers detection by pyrosequencing as well as Sanger-sequencing (Fig. 2).

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

PATHOLOGISCHES INSTITUT DER LMU

DIREKTOR: PROF. DR. THOMAS KIRCHNER

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In close cooperation with: Merck Serono - Intercontinental Region

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 2: Schedule of the Practical Course

The course is organized byMerck-Serono - Intercontinental Region. For more information please, get in contact with Karim Taha - Deputy Regional Head of Medical Affairs Intercontinental Region - Dubai (karim. taha@merckgroup.com).