

Molecular Markers of tyrosine kinases signaling receptors pathways for clinical prognosis and prediction under TKI therapy in human gastrointestinal tumors *in vivo* and *in vitro*.

Lyros Orestis

Background

Recently, it has been recognized that tumor growth and the metastatic process are highly associated with the overexpression of protein kinases (PK), which trigger intracellular signal transduction by phosphorylating tyrosine, threonine and serine residues in key proteins, therefore regulating cell growth, apoptosis resistance and survival. Thus, excitement and optimism have been generated by the advent of new targeted therapies for treating of cancer. Undoubtedly, several small molecule inhibitors (Imatinib, Erlotinib, Sorafenib, and Sunitinib) of protein kinases have developed and are significantly contributing to the armamentarium of approaches to treat and –in the adjuvant setting- to possibly cure cancer. Signaling processes, which are critical to cancer cell division and survival, are targets of these agents designed with different biologic effects in mind, including anti-angiogenesis, anti-proliferation, and anti-invasion , to induce apoptosis or to re-establish the human immune system.

Gastrointestinal cancers with their most prevalent tumors of colorectal, pancreatic or gastric origin account for the most common cancers worldwide. Despite curative resections, many patients develop later recurrence or metachronic metastases. Many other patients already present with metastatic or surgically not resectable disease at time of diagnosis. Despite the recent improvements and approval of new chemotherapeutic agents like Irinotecan, Oxaliplatin, Bevacizumab and Cetuximab, there is an ongoing urgent need to improve the treatment options and establish new compounds with novel therapeutic profiles.

The identification of numerous molecular targets is a significant step forward, but it is just at the beginning of their clinical use. The present challenges are therefore:

- To understand how the different signaling pathways *interact in vivo* and *in vitro* in gastrointestinal cancers

- To define the roles of the different angiogenic phenotypes in gastrointestinal cancer as prognostic and predictive markers.

Aims

As new multi-target tyrosine kinase inhibitors are emerging and enriching the therapy options in various malignancies, we initiated a study of several novel multi-receptor tyrosine kinase inhibitors (SU11248, ZD6474, Sorafenib) against a variety of gastrointestinal tumor cell lines and animal models to investigate their activity and define their potentially antitumor effects but also to better understand the molecular pathways that intermediate in this type of cancer. Herein, combinations of extracellular antibodies (Cetuximab, Bevacizumab) with intracellular TKIs (such as ZD6474) have been very attractive for us.

Our translational research group recently published results revealing a high rate of receptor-tyrosine-kinases coexpression in gastric adenocarcinoma

The coexpression pattern of *VEGFR1-3*, *PDGFR α/β* and *EGFR1* was analyzed in 51 human gastric adenocarcinomas whereas the majority of samples revealed a *VEGFR1* (98%), *VEGFR2* (80%), *VEGFR3* (67%), *PDGFR α* (82%) and *PDGFR β* (82%) expression and only 62% exhibited an *EGFR1* expression. (Drescher D, Moehler M et al, World J Gastroenterol 2007)

Methods

Cell lines (AGS, MKN, NCI-N87, and OE33) originated from gastric or esophageal carcinoma (Barrett's metaplasia) known to express different RTKs like KDR, Flt-1, FLT3, c-KIT, PDGFR a+b, EGFR, were cultured in the presence of various concentrations of different new multi-tyrosine kinases inhibitors for 2 and 3 days. The effect of multi-RTKs Inhibitors on the proliferation was assessed by means of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. After the determination of the IC 50 the same method was followed for combination treatment with classical chemotherapeutic drugs, such as 5-FU, irinotecan, cisplatin, oxaliplatin,

cetuximab. Apoptotic cell death was examined with the Nicoletti method (Analysis of apoptosis by propidium iodide staining and flow cytometry).

Cellular assays were performed as well to detect the antiphosphorylation effect on RTKs and their downstream effectors (MAP Kinase, Akt/ ERK/ STAT). Cells were incubated with multi-tyrosine kinase inhibitors in indicated concentrations for limited time (hours) and then lysates were prepared for western blot analysis. Stimulation of the receptors was performed where needed.

Results

Our preliminary results revealed efficacy of multi-tyrosine kinases inhibitors such as SU11248 and ZD6474 in inhibiting cell proliferation and inducing apoptosis in gastric adenocarcinoma cell lines in a dose depended manner. The combination of several multi-tyrosine kinases inhibitors together led to synergistic effect, indicating the several molecular downstream pathways that intermediate in the tumor growth in gastric cancer. Furthermore, additive anti-proliferative effects were seen when these agents were combined with conventional chemotherapeutic compounds. In addition, the conventional immuno-blotting investigating Akt, Ras/Raf/MAPK-pathway depicted which exact pathways are being involved. The project is continuing and some results are about to be published. The ongoing cellular assays, as well as the investigations on xerograph models, will put further light on the molecular basis of gastric cancer.

These findings certainly encourage the application of multiple-target RTK-inhibitors in gastric adenocarcinoma as a step of a multi-drug therapy. In order to translate the results from the lab to a clinical setting, we currently initiate a phase II study analyzing the benefit of Sunitinib {Cabebe, 2006 #1364}, targeting the above analyzed RTKs in CPT-11 or Cisplatin refractory, disseminated gastric adenocarcinoma.

Personal Perspective

During my ESMO Fellowship apart from my research program, I performed clinical work as clinical investigator (Prüfarzt) at the University Outpatient clinic for gastrointestinal oncology, enrolling oncological patients in Phase II-III Studies initiated from the AIO (Arbeitsgemeinschaft Internistische Onkologie).

During this time, I continuously attended the interdisciplinary tumor board and I gained useful experience on handling tumor patients. Furthermore, having obtained written, informed consent, I created a collection of serum samples from patients with gastric adenocarcinoma (on day 1 of each cycle). Their characterization via ELISA to detect markers like VEGFR1-3 und their ligands VEGF A,/C/D, PDGFR α/β , cKIT, EGFR1, EGFR2 (Her2neu) and their ligands TGF α and EGF is the very next step to come.

During my ESMO fellowship I had the opportunity to attend several scientific meetings of ESMO, enriching my knowledge on oncology, and the honor to be awarded from the Hellenic Society of Gastrointestinal Oncology (HSGO) for the best poster presentation of Young Investigator. Furthermore I had the privilege to participate in the Translational Unit Visit in Vall d'Hebron Hospital, Barcelona, Spain, where I obtained additional experience; how translational research is being performed.

Acknowledgements

I would like to express my sincere gratitude to ESMO for providing the financial assistance that enabled me, in my very first steps as a clinician, to acquire important skills during the course of this work. I would like to thank my supervisor Dr. Markus Moehler for his continuous guidance and significant assistance as well as the whole team of biologists, technicians, doctors and nurses in the laboratory and clinic, who helped me to absolve this task. Additionally I want to express my gratitude to Prof. Meletios Dimopoulos as well as to the Dean of the Medical School of Athens, Greece Prof. G. Kreatsas for their support.