Molecular profiling allows us to better understand the regulatory pathways which drive tumor development, differentiation and growth. Recently, major players in these pathways have been identified as potential targets for anticancer therapy, including monoclonal antibodies against EGFR (epidermal growth factor receptor) and VEGF (vascular endothelial growth factor) or small molecules against tyrosine kinases, proteasome or cell-cycle dependent kinases. In this issue of Onkologie, Sutter et al. [1] are describing in detail these novel targets in critical pathways and the potential clinical implications for the treatment of gastrointestinal (GI) cancers. Cytotoxic therapy has shown only very modest efficacy but in some cases significant toxicity. Identification of novel targets in GI cancer will be critical for the development of more successful and less toxic treatment strategies. Molecular profiling is also critical to test for presence of the target in the tumor, and to validate whether the target is inhibited and functionally significant. In the future, molecular technologies will become an essential part of successful drug development in patients with GI cancers. Molecular signatures will help to select patients who will more likely benefit from chemotherapy and allow a better monitoring of efficacy of chemotherapy. We will be able to evaluate efficacy of chemotherapy within a couple of days and not weeks or months by measuring surrogate markers such as tumor DNA in serum or inhibition of the target in buccal mucosa.

The goal in administering chemotherapeutics is to develop the ability to predict the outcome of therapy in terms of response and toxicity. Technologies have been developed to allow tumor profiling with measurements of protein expression, gene expression levels and germline polymorphisms of genes involved in the pathways of the target as well as the metabolic pathways of drugs that may predict response and toxicity to particular chemotherapeutics. The chemotherapeutics for which particular markers have been shown to predict outcome include the fluoropyrimidines and platinum compounds [2–4]. However, ongoing prospective clinical trials will be critical to assess the benefits of profiling tumors and whether profiling will translate into an improvement in response and toxicity for patients with GI cancers.

When evaluating molecular correlates it is important to differentiate between prognostic markers and predictive markers. Prognostic markers are those that are applied to populations and not individual patients. These are usually reported as high/low or present/absent. Examples of such markers include 18q, p27, p53, and thymidylate synthase (TS). Predictive markers refer to predetermination of the likelihood of response to chemotherapy and toxicity and can be applied to an individual patient by evaluation of their tumor characteristics. The techniques employed to evaluate prognostic and predictive markers are usually measurement or assessment of protein expression, gene expression or genomic polymorphisms. These techniques have inherent technical limitations. For example protein expression, as evaluated by immunohistochemistry, is limited by the availability of antibodies and often reported subjectively, usually with an arbitrary grading system. Gene expression, assessed by polymerase chain reaction (PCR), was previously limited by its necessity for fresh tissue, however, this technology has recently been refined and techniques have emerged which allow pertinent data to be obtained from paraffin-embedded tissue [2]. The limitation in the utilization of this process may be access to this technology.

Determination of genomic polymorphisms is likely the most accessible technique, as assessment requires only a blood sample or a mouth wash from individual patients. In the future oncologists should utilize prognostic makers to decide when to treat, for example: is a patient with stage II gastric or colon cancer at high risk for tumor recurrence? In the US, the first cooperative trial is being initiated using 18q, p27, p53, and thymidylate synthase (TS). Predictive markers refer to predetermination of the likelihood of response to chemotherapy and toxicity and can be applied to an individual patient by evaluation of their tumor characteristics. The techniques employed to evaluate prognostic and predictive markers are usually measurement or assessment of protein expression, gene expression or genomic polymorphisms. These techniques have inherent technical limitations. For example protein expression, as evaluated by immunohistochemistry, is limited by the availability of antibodies and often reported subjectively, usually with an arbitrary grading system. Gene expression, assessed by polymerase chain reaction (PCR), was previously limited by its necessity for fresh tissue, however, this technology has recently been refined and techniques have emerged which allow pertinent data to be obtained from paraffin-embedded tissue [2]. The limitation in the utilization of this process may be access to this technology.

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may influence what treatment we may recommend, for example for local recurrence we may add radiation and for distant metastases we may recommend an inhibitor of angiogenesis. Predictive markers should help to decide what chemotherapeutic agents are more likely to benefit an individual patient. For the selection of the cytotoxic regimens, my laboratory has identified gene expression levels of TS, dihydropyrimidine dehydrogenase (DPD), ERCC-1 and others which can select patients who are more likely to benefit from 5-FU (5-fluorouracil-) or platinum-based chemotherapies. In fact, all patients at USC/Norris with GI cancers are profiled and treated based on their gene expression profile. These markers are being validated within a trial initiated by the Southwest Oncology Group (SWOG), the first prospective clinical trial to treat rectal cancer patients based on TS, DPD and ERCC-1 expression with either 5-FU/LV(leukovorin)/CPT-11(irinotecan), CPT-11/oxaliplatin or 5-FU/LV/oxaliplatin. Using gene expression profiles for treatment decisions is much easier for patients with metastatic gastric cancer because multiple combination chemotherapy regimens are being used which have only modest activity but differ significantly in toxicity. Using TS and DPD for 5-FU sensitivity, ERCC-1 for platinum sensitivity, beta-tubulin isoform III for taxane sensitivity and ribonucleotide reductase for gemcitabine sensitivity, it will become possible to tailor a combination chemotherapy which is more likely to be effective and avoid ineffective and toxic chemotherapy.

In the future we will face the challenge to understand how the novel targeted therapies will affect the sensitivity to cytotoxic agents. For example, if we understand how cetuximab is overcoming resistance to CPT-11, we will be able to use these drugs more effectively and possibly even develop more effective drugs. The future in oncology may be that oncologists will specialize in specific pathways such as EGFR or VEGF. Treatment may be only decided based on the molecular profile and not anymore on site and stage of the primary tumor.

Molecular signatures should also include germline polymorphisms. Studies by Wasserman et al. and our own laboratory have shown that germline polymorphisms can identify patients who are at risk of life-threatening toxicities [5, 6]. Patients with Gilbert’s disease have a high risk for neutropenic fevers. These patients have a germline polymorphism in the UGT1A1 gene, which can be easily tested in the blood prior to start of chemotherapy. At USC/Norris we routinely test for Gilbert’s disease prior to CPT-11-based chemotherapy to avoid life-threatening neutropenic fever. Interestingly, germline polymorphisms differ significantly in frequency between ethnic populations and may explain some of the differences in toxicity and efficacy of a drug tested in different ethnic populations. One cause of dose-limiting toxicity of oxaliplatin and cisplatin therapy is peripheral neurotoxicity. We have preliminary data on potential genes predicting neurotoxicity during oxaliplatin chemotherapy. These findings could not only lead to a more sophisticated dosing schedule but may allow the development of specific neuroprotectors.

In the future, oncologists will use predictive markers not only to select the most appropriate chemotherapy but also to use an optimal dosing. We are all aware that first-line chemotherapy is usually the most effective treatment and that about 1/3 of patients are not in the position to receive a second-line chemotherapy because of deterioration of their performance status. It is therefore important to select the most effective chemotherapy at time of diagnosis.

However, molecular profiling can be also used in patients with poor performance status to justify less aggressive therapy, for example patients who will likely benefit from 5-FU/LV/Avastin do not need to be exposed to CPT-11- or oxaliplatin-based treatment. Our own data suggest that molecular profiling may be also used in second-line chemotherapy, we identified that patients with metastatic colorectal cancer with high TS and ERCC-1 levels will unlikely benefit from 5-FU/oxaliplatin chemotherapy after they were treated with 5-FU/LV and CPT-11 [3]. In contrast, one may justify an aggressive chemotherapeutic regimen for patients who may be candidates for potential resection of their metastatic disease. However, these approaches need to be validated in prospective clinical trials. Being in the fortunate situation to have multiple targeted therapies available, we need to address the question of what combination therapy is the most effective. Evaluation of the target inhibition and its synergism with cytotoxic chemotherapy will be critical for the design of novel treatment strategies. For example, we identified that EGFR polymorphisms may be associated with success of oxaliplatin-based chemotherapy, making a combination of cetuximab and FOLFOX a promising combination [7].

One of the limitations, however, of these agents is the necessity to identify a particular receptor or target; for example, the suboptimal evaluation of the EGFR expression. More sensitive technologies could increase the number of patients who may benefit from cetuximab therapy by more accurately identifying those patients who express the receptor. This may be accomplished by assessing gene expression within the tumor that may lead to more accurate selection of patients. Ideally one would select patients for a particular chemotherapy or novel target based on their genetic makeup and the genetic characteristics of their tumor. This can help identify those patients that may have the best response to therapy. Future clinical trials with strong correlation markers will allow us to delineate some of these issues.

How molecular technologies will be integrated into clinical practice and can help to design better treatment regimens can be illustrated with EGFR. Expression of EGFR and its receptor has been found to correlate with prognosis in patients with gastric cancer [8, 9] and a significant number of gastric cancer cell lines express EGFR, which grow in response to EGFR/TGF-α. In fact, the presence of EGFR or TGF-α/EGFR positive gastric cancer shows a greater degree of gastric wall invasion and lymph node metastases, representing greater malignant potential [10]. Recently several studies have demon-

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strated that in vitro treatment of cancer cells with cetuximab (a monoclonal antibody, EGFR inhibitor) can downregulate the production of angiogenic factors, such as VEGF, interleukin-8 or basic fibroblast growth factor, and in vivo inhibition of EGFR results in growth inhibition and reduction in microvessel density [11]. These results suggest that the EGFR signaling pathways play a role in the regulation of angiogenesis. Understanding the pathway of EGFR and its downstream events is establishing EGFR as a promising target for the treatment of gastric cancer, and multiple trials have been initiated to use either monoclonal antibodies or tyrosine kinase inhibitors of EGFR in metastatic gastric as well as esophageal cancer. A better understanding of the limited efficacy of single-agent inhibitors of the EGFR led to the development of combination therapies with CPT-11 or oxaliplatin. More importantly, the lack of efficacy using single-target inhibitors also led to the development of multitargeted agents. For example, GW572016 is a dual tyrosine kinase inhibitor of both EGFR and HER2/erbB. Treatment with GW572016 in tumor xenografts that overexpressed both EGFR and HER2 resulted in reduced levels of phosphorylated tyrosine, which correlated with inhibition of tumor growth. Apoptosis and arrest of tumor cell growth has been demonstrated with this agent, even in the presence of saturating concentrations of EGF [12].

Given the evidence of expression of the EGFR and potentially HER2/erbB in patients with gastric cancer and preclinical evidence that blockade of these receptors may lead to inhibition of cell growth and apoptosis, this compound is now being studied in patients with advanced or metastatic gastric cancer. The success of targeted therapies will heavily depend on how important this particular pathway is for the development and progression of the cancer. Gleevec is a wonderful example, one of the reasons that it has been so successful in GIST and CML is that the c-kit pathway is the driving pathway in these cancers. Inhibition of this pathway results in dramatic growth inhibition and initiation of apoptosis. In tumors were multiple pathways are involved in growth, inhibition of one of them will unlikely be successful. Therefore multitargeted agents which inhibit VEGFR, EGFR and HER2, such as the Novartis compound AEE788, are more promising and are being tested in clinical trials. But again, these novel targets are most effective when used in combination with cytotoxic agents such as fluoropyrimidines, platinum, taxanes or irinotecan. Therefore, we need to identify the molecular profile which predicts sensitivity to these cytotoxic therapies and test these predictive markers in prospective clinical trials. The activity of these novel targeted agents may be multi-faceted in that they not only have anti-neoplastic effects, but also stabilize qualities that may allow the chemotherapy to be more effective. This may explain the enhanced efficacy of these agents when combined with chemotherapy.

Molecular markers will become an integrated part of our daily clinical practice. Over the next couple of years, we need prospective clinical trials to validate the predictive markers as well as to utilize molecular technologies to validate the novel targeted therapies and to identify patients who will benefit from these treatments. In some areas we need better technologies, for example evaluation of EGFR status. We need to incorporate molecular correlates into our clinical trials. We missed the opportunity to collect tissues in the Iressa lung trial as well as in the Avastin colon trials, which may have helped to understand why the lung trial was negative and who in the colon trials benefited from the combination therapy. We need better in vitro and in vivo models to understand the mechanism of action of the novel agents and interaction with cytotoxic therapeutics before we design large clinical trials. The future will bring us more tools to decide when to treat, with what to treat and how much to treat. Our patients will live longer and better when treated based on their molecular profile.

References