A second important challenge is the strategic questions on the best combination, on the best sequence and on the most optimal use of the different cytotoxic agents in combination with the biologicals in CRC. Also questions on whether 2 biologicals have to be combined with 2 cytotoxics in the first line treatment or whether biologicals have to be given only to selected patients. An important challenge is the understanding of the mechanism why tumors that initially respond to a combination of cytotoxics and biologicals may become resistant to this combination.
In conclusion: the biologicals have clearly increased the therapeutic armamentarium of patients with metastatic colorectal cancer and offer also prospects for an increased chance of a longer survival. The major challenge is now to implement strategies in which patients can be selected, based on molecular characteristics and/or pharmacogenomic profiles so that the new drugs and the resources can be used optimally for our patients with metastatic colorectal cancer.

92 INVITED

What should be the duration of treatment for metastatic colorectal cancer?

H.J. Schmoll. *Germany*

Abstract not received.

Symposium (Tue, 25 Sep, 14:45−16:45)
Pharmacogenomics: an ideal tool for optimising treatment in lung cancer

93 INVITED

Tumour and serum predictive markers of response to bevacizumab

M. Taron, J.L. Ramirez, F. Salazar, M. Berdiel, R. Rosell. *Hospital Universitari Germans Trias i Pujol, Medical Oncology Service-ICO, Badalona (Barcelona), Spain*

New blood vessel formation (angiogenesis) is a key process for tumor growth and metastasis. The vascular endothelial growth factor (VEGF or VEGF-A) pathway is known as one of the most important regulators of angiogenesis in normal and malignant tissue. The effects on generation and preservation of tumor vasculature include induction of endothelial cell division and migration, promotion of endothelial cell survival through protection from apoptosis, and reversal of endothelial cell senescence. VEGF exerts its effect by interacting with tyrosine-kinase receptors located in the cell membrane (VGFR-1/flt-1; VGFR-2/flk-1; VGFR-3/flt-4). VGFR-2 appears to be the main receptor responsible for mediating the pro-angiogenic effect of VEGF.
Bevacizumab (Avastin) is a recombinant human monoclonal antibody against VEGF. Bevacizumab binds and neutralizes all biologically active forms of VEGF with a high binding affinity. This monoclonal antibody has shown activity and outcome improvement in different solid tumor types, such as metastatic colorectal cancer, metastatic breast cancer, renal cell cancer and non-small cell lung cancer. Despite this clinical benefit, to date no biological markers have been found to correlate with bevacizumab activity. Given that bevacizumab targets VEGF it seemed logical that VEGF expression might predict benefit. However, in retrospective analyses of tumors, VEGF expression levels did not predict benefit from the addition of bevacizumab. Similarly, the expression of both pro-angiogenic (VEGF) and anti-angiogenic (thrombospondin-II) factors by the tumor stroma did not predict benefit. Other molecular factors have been analyzed without success. For instance, evidence from the clinic so far indicates that p53, k-Ras and Braf status and VEGFR-2 activation/phosphorylation are not relevant to the clinic efficacy of bevacizumab. Multiple studies are now focused on serum/plasma proteomics, since it has been observed that concentration of different soluble proteins (such as VEGF, PLGF-a VEGFR1 ligand) or circulating endothelial (CECs) and circulating endothelial progenitors (CEPs) change after bevacizumab treatment. Other markers are under analysis, such as thioredoxin plasma levels, carbonic anhydrase IX expression levels in tumor cells /stroma, or down-regulation of semaphorin-3F (a VEGF antagonist, with negative effect on cell attachment, spreading and migration) by gene promoter hypermethylation.

94 INVITED

Invidualised chemotherapy based on methylation of serum or plasma DNA

F. Salazar, J.L. Ramirez, M. Berdiel, M. Taron. *ICO Hospital Universitari Germans Trias i Pujol, Department of Oncology, Badalona (Barcelona), Spain*

Non-invasive tests for customizing chemotherapy could be performed based on the analysis of extracellular DNA circulating (cirDNA) in the blood. Numerous studies have demonstrated tumor-specific alterations, such as aberrant promoter hypermethylation, in cirDNA recovered from serum or plasma of non-small-cell lung cancer (NSCLC) patients and the absence of methylated DNA in healthy subjects (Ramirez et al. Cancer Lett 2003). For translational research studies in NSCLC, cirDNA is an abundant source of material that could be examined by methylation-specific PCR (MSP). Several layers of evidence indicate that several methylated genes in cirDNA could be potential predictive markers. Methylation of the mitotic checkpoint gene CHFR could indicate sensitivity to microtubule inhibitors, and we have shown that methylation of DNA repair genes, such as O6-methyl-guanine-DNA methyltransferase, in cirDNA indicates sensitivity to 1,3-bis(2-chloroethyl)-1-nitrosourea (Balaña et al. Clin Cancer Res 2003). In addition, 14-3-3σ, FANCF and BRCA1 methylation indicates sensitivity to cisplatin. Other genes, such as Werner, belonging to the RecQ family of helicases can indicate sensitivity to irinotecan. However, in NSCLC, BRCA1 methylation is not commonly seen, FANCF1 methylation is low, and Werner methylation has not been confirmed in our experience. We have also examined other crucial mitotic spindle checkpoint genes, such as BubR1, which is not methylated.
We have concentrated our translational research in CHFR and 14-3-3σ, since both are methylated in cirDNA in more than 30% of NSCLCs. The CHFR gene molecularly defines the existence of a checkpoint that regulates entry into metaphase. The CHFR protein contains a central ring finger domain that has ubiquitin ligase activity. CHFR directly ubiquitinates PlK1, Aurora-A and possibly other substrates, since it contains residues homologous to those of c-Cbl. We have observed that unmethylated CHFR in cirDNA confers greater sensitivity to second-line EGFR tyrosine kinase inhibitors (TKIs) in NSCLCs, both with and without EGFR mutations. The 14−3-3 proteins regulate cell survival and programmed cell death. We have found that in stage IV NSCLC patients treated with gemcitabine/cisplatin, median survival was longer in the cirDNA 14-3-3σ methylation-positive group (15 vs 10 months; P = 0.004) (Ramirez et al. J Clin Oncol 2005). A customized trial is planned for stage IV NSCLC patients, in which those with methylated 14-3-3σ in cirDNA will receive gemcitabine/cisplatin, and those with unmethylated 14-3-3σ will receive vinorelbine/cisplatin. One hundred and forty patients per arm are required to test that there are no differences in progression-free survival.

95 INVITED

Tailored chemotherapy in lung cancer

A. Adjei. *Roswell Park Cancer Institute, Department of Medicine, Buffalo, USA*

The variability in response to and toxicity from chemotherapeutic agents have been known for decades. However, understanding of the determinants for such variability has been rudimentary. It is clear that for an agent to cause tumor shrinkage or other changes in the tumor, it needs to be delivered to the tumor site. Recent advances in molecular biology have elucidated a number of tumoral factors that may influence the efficacy of chemotherapy drugs for lung cancer (such as irinotecan, taxanes, platinum analogues and the antimetabolites gemcitabine and pemetrexed). These include:

a. Expression and/or variations in target genes. For example, expression of thymydylate synthase in tumors have been correlated with TS inhibitors such as 5-fluorouracil and its pro-drugs.
b. Expression and variations in DNA damage repair genes such as ERCCI, XPD have been correlated with efficacy of platinum compounds.

With the focus on tumor-related factors, host related factors have been somewhat overlooked. Before a drug gets to the target, it needs to be absorbed, transported (sometimes activated) and can be inactivated. Thus the pharmacology of the drug, which deals with “what the body does to the drug” is important. In recent years, the role of genetic polymorphisms of drug metabolizing enzymes, drug transporters and drug targets in the response and/or toxicity to cancer agents is becoming increasingly important. Thus allelic variations in drug transporters such as mdr1 and Pgp have been correlated with disease outcome Polymorphisms in drug metabolizing enzymes such as UGT1A1, DPD and TPMT have been correlated with toxicity (and some times efficacy).
An example is the biotransformation of the chemotherapy prodrug irinotecan to form the active metabolite SN-38, an inhibitor of DNA topoisomerase I. SN-38 is primarily metabolized to the inactive SN-38