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

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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

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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

glucuronide by UGT1A1, the isoform catalyzing bilirubin glucuronidation. There is large interpatient variability in response to irinotecan, as well as severe side effects such as diarrhea and neutropenia, which might be explained in part by genetic variation in metabolic enzymes and transporters. Well-known variants are the promoter polymorphic repeat in UGT1A1 (UGT1A1*28) and the 1236C>T polymorphism in ABCB1. UGT1A1*28 genotype has been associated with toxicity and efficacy. The roles of ABCB1 variants and of variants the carboxylesterases continue to be elucidated. These polymorphisms and a systems biology approach evaluating variations in drug pharmacology as well as drug targets and host factors will be discussed.

96 INVITED

Predictive biomarkers for EGFR inhibitors in lung cancer

R. Dziadziuszko. *USA*

Abstract not received.

Symposium (Tue, 25 Sep, 14:45−16:45)
What is new in cervical and endometrial cancer?

97 INVITED

Update on pathology and terminology of uterine cancers

W. McCluggage. *Queen's University of Belfast, Department of Pathology, Belfast, United Kingdom*

A dualistic model of endometrial carcinogenesis is well established whereby the more common type 1 cancer (mostly endometrioid) develops from atypical hyperplasia under the influence of oestrogens while type 2 cancers (mostly serous) develop from an atrophic endometrium. In recent years, a presumed precursor lesion of type 2 cancer has been described, termed serous intraepithelial carcinoma (serous EIC). However, serous EIC may give rise to extrauterine disease, even in the absence of endometrial stromal or myometrial infiltration and, as such, may not represent a precursor lesion. In such cases, the term minimal uterine serous carcinoma may be more apt. Minimal uterine serous carcinoma has a marked propensity to arise in or involve endometrial polyps. Molecular alterations in both type 1 (PTEN, k-ras, β-catenin mutations and microsatellite instability) and type 2 (p53, p16, E-cadherin, Her2-neu mutations) cancers are now well described. It is also clear that mixed type 1 and type 2 cancers are not uncommon whereby the type 2 component develops from a type 1 cancer via a process of dedifferentiation secondary to p53 mutation. It is now clear that endometrial cancers are common in women with hereditary non-polyposis colorectal cancer (HNPCC) syndrome; the morphological types in women with HNPCC are not clear but surprisingly type 2 cancers may be more common than in the general population. The histogenesis of uterine carcinosarcomas has now been settled; most are, in reality, carcinomas with sarcomatous differentiation or metaplastic carcinomas, although a small number are true collision tumours. There has been a change in the terminology of malignant mesenchymal lesions derived from the endometrium with the terms high grade and low grade endometrial stromal sarcoma no longer used. Instead there is now a category of an indolent low grade malignant neoplasm composed of bland cells (endometrial stromal sarcoma) and an aggressive high grade malignant neoplasm composed of anaplastic cells (undifferentiated endometrial sarcoma).
In the cervix, adenocarcinomas appear to be increasing in incidence, especially in Western populations. Early invasive adenocarcinoma is now being diagnosed more commonly with refinement of the criteria for making this diagnosis. Neuroendocrine carcinomas in the cervix are also being diagnosed more frequently, especially large cell neuroendocrine carcinoma (LCNEC). This is in large due to the recognition that some undifferentiated carcinomas and poorly differentiated non small cell carcinomas in the cervix represent LCNEC.

98 INVITED

New molecular pathways in uterine cancers and their implications for new treatments

A. Oza. *Canada*

Abstract not received.

99 INVITED

New drugs in cervix cancer treatment

J.B. Vermorken. *University Hospital Antwerp, Department of Oncology, Edegem, Belgium*

The role of systemic treatment in cervical cancer has been changing over time. In the past chemotherapy has been considered appropriate only for patients with recurrent, metastatic or persistent disease for whom treatment with potentially curative intent is no longer amenable. Nowadays, chemotherapy is also often used in the treatment of primary disease for those who are at high risk for relapse. Cisplatin is considered to be the most active compound in all circumstances; it is mostly given alone when combined with radiotherapy, and given alone or combined with other agents when used for induction in the primary disease setting or applied for palliation. More and more patients in that latter setting will be offered chemotherapy after they have received cisplatin as part of primary chemoradiotherapy, and this will moderate the expected benefits. New drugs are more than ever needed. Taxanes, topo-I-inhibitors, vinca-alkaloids and gemcitabine have all been tested and suggested to be of benefit when used alone or in combination. Of these only paclitaxel, topotecan and irinotecan have been studied in combination with cisplatin in large randomized trials versus cisplatin alone. The greatest improvements in response, progression-free survival and median survival outcomes have been detected with the use of cisplatin plus topotecan. Targeted therapies alone, in combination with cisplatin or combined with (chemo)radiotherapy are under study. Recently, one of the prophylactic vaccines against HPV 6, 11, 16 and 18 (Gardasil) has been approved on the basis of several trials showing that CIN grades 2 and 3 and cervical adenocarcinoma in situ lesions were highly reduced by the use of this vaccine. Time will tell whether this will be the beginning of the end of cervical cancer.

100 INVITED

Progress with cervix cancer vaccines. The end in sight?

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Background: HPV infection in the genital tract is common in young, sexually active individuals, the majority of whom clear the infection without clinical disease. In a minority of subjects the immune system fails to control the persistent virus and malignancies develop. The natural HPV specific E6 and E7 T cell immune response is found to be different in patients compared to healthy individuals. Antibody responses to the major virus capsid protein L1 accompany the induction of successful cell mediated immunity and these responses are protective against subsequent viral. Results from phase III trials of HPV VLP vaccines show efficacy against HPV 6, 11, 16 and 18 of which the latter two are high risk types for invasive lesions of the female genital tract.
Therapeutic vaccination, in cases with persistent HPV infection or (pre) invasive lesions, has the aim to enhance the HPV type specific cellular immune response. Several modalities have been tested in animal studies and are currently being tested in humans.
Methods: Previously, we demonstrated that long overlapping HPV16 E6 and E7 long peptide vaccine in Montanide ISA 51 was safe and able to elicit strong HPV16 specific T-cell response in end-stage cervical cancer patients. A phase II study is currently in progress in 20 patients with histologically proven HPV16+ vulvar intraepithelial neoplasia (VIN) grade III, who are vaccinated 4 times at a 3-week interval.
Results: IFN-gamma-ELISPOT analysis reveal that almost all patients mount a T-cell response to multiple regions of HPV16 E6 and 75% of the patients against HPV16 E7, already after 2 vaccinations. In proliferation assays we see that this T-cell reactivity is associated with the production of IFNä and IL-5 similar to the cytokine profile of the HPV16-specific memory T-cell responses observed in healthy individuals. Analysis of the local immune response demonstrates that after vaccination HPV16-specific Th1/Th2 cells infiltrate both the vaccination site and/or the VIN lesion. At this moment clinical efficacy at 3 month follow-up can be measured in a subgroup of the patients.
Conclusion: Prophylactic as well as therapeutic vaccination against HPV related disease in the female genital tract is showing potential efficacy.