from the tumour. Proliferation (as measured by the nuclear antigen, Ki67) is substantially reduced in ER+ primary tumours by SERMs such as tamoxifen and aromatase inhibitors. With aromatase inhibitors at least 90% of patients show a reduction in Ki67 indicating that the large majority of ER+ breast carcinomas have some dependence on oestrogen although the withdrawal of this may lead to an insufficient change to elicit response. No increase in apoptosis has been measured, indeed significant decreases occur with aromatase inhibitors probably as a result of the close association between proliferation and cell death. Changes in Ki67 are only modestly predictive of clinical response but in the IMPACT trial they were predictive of the improved RFS seen with anastrozole over tamoxifen and the combination of tamoxifen and anastrozole. In keeping with this, Ki67 levels after 2 weeks treatment with endocrine therapy is more closely associated with recurrence free survival than pretreatment levels suggesting that the treatment induced change contributes to prediction of long-term outcome. The potential for this to be used for improved prediction of outcome in individual patients will be tested in the PeriOperative Endocrine Therapy for Indiividualising Care (POETIC) trial. Expression array analysis of biopsies before and after treatment with aromatase inhibitors reveals the profound changes in transcription that result from estrogen deprivation. The changes can be summarised as a Global Index of Dependence on Estrogen (GIDE) that may provide an additional index of benefit from the therapy. It is higher in patients with high ER+ tumours and lower in patients with HER2 positive disease. The study of the expression of other such markers or activity of biological pathways with ?Ki67 and/or the GIDE as indices of response should shed further light on the importance of the respective markers/pathways. Detailed analysis of the changes in gene expression after estrogen deprivation may identify pathways other than proliferation that determine the progression of estrogen independent disease and its response to endocrine therapy.

Symposium (Tue, 25 Sep, 14:45−16:45)
Treatment of advanced colorectal cancer in the era of biologics

89 INVITED

Molecular markers and patient selection in colorectal cancer

P. Johnston. *UK*

Abstract not received.

90 INVITED

Signal transduction pathways in colorectal cancer (CRC)

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An emerging understanding of the molecular pathways that characterize cell growth, cell cycle, apoptosis, angiogenesis and invasion has provided novel targets in cancer therapy. Numerous proteins have been implicated as having a crucial role in CRC. There are different targets according to their cellular localization like: (a) membrane receptor targets (epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), insulin-like growth factor receptor (IGFR), platelet-derived growth factor receptor (PDGFR), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)); (b) intracellular signaling targets (Ras/Raf/MEK/MAPK pathway, phosphoinositol-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR), src kinase and the hdm2/p53 complex, among others); and (c) other protein kinases that regulate cell division (Aurora and Polo kinases and cyclin dependent kinases (CDK)). In this session we aim to review the current knowledge of some of these signaling pathways as well as the potential targets for innovative drugging in CRC.
The PI3K/AkT/mTOR pathway controls many cellular processes that are important for the formation and progression of cancer, including apoptosis, transcription, translation, metabolism, angiogenesis and cell cycle progression. The PI3K signaling pathway is upregulated in many CRC, and this upregulation positively correlates with increased tumorigenic potential of colon adenocarcinoma cell lines. Mutations in PI3CA (which encodes the P110α catalytic subunit) have been identified in up to 40% of CRC. A correlation between PI3K mutations and advanced stage of tumorigenesis, just before cell invasion, has been observed. Increased expression and activation of Akt has been noted in CRC. mTOR downstream and upstream effectors have been shown to be activated in around 1/3 of CRC.
C-src is a non-receptor tyrosine kinase protein overexpressed and activated in many human cancers, including CRC and is associated with advanced-stage and distant metastases. C-src is also of particular interest in colon cancer because it is overexpressed and/or activated in a wide range of tumors that also overexpress several receptor tyrosine-kinases, indicating the potential role for cross-talk interactions in promoting tumorigenesis.
Emerging data from the clinical development of new drugs directed to these targets is providing novel opportunities in the treatment of patients with CRC that will probably translate in efficacy advantage in the next years.

91 INVITED

Optimal strategy for integration of biologics in treatment of metastatic colorectal cancers

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The management of patients with metastatic colorectal cancer (CRC) has changed dramatically over the last years, with increasing chances of prolonged survival. The development of new cytotoxic and targeted agents as well as the multidisciplinary management of patients with resectable and initially non-resectable metastases contribute to the progress. The development of the cytotoxic agents irinotecan, oxaliplatin and capecitabine and of the biological agents bevacizumab, cetuximab and panitumumab has clearly increased the therapeutic options for patients with metastatic CRC.
It has been shown in randomized phase III trials that bevacizumab, when combined with irinotecan plus bolus 5-FU/LV (IFL) in the first-line treatment of metastatic CRC and with FOLFOX in second-line treatment leads to an increased median survival, progression-free survival (PFS) and response rate (RR) compared to the cytotoxic chemotherapy alone. Moreover, it has been demonstrated in a few randomized phase II studies and in a combined analysis of these phase II studies that bevacizumab increases the activity of 5-FU/LV in the first-line setting. The recent randomized phase III study of FOLFOX compared to capecitabine plus oxaliplatin $\pm$ bevacizumab in the first-line treatment shows that capecitabine is as effective as IV 5-FU/LV when combined with oxaliplatin and that bevacizumab increases the PFS of the fluoropyrimidine/oxaliplatin combination. The data from phase 2 studies with irinotecan and capecitabine (without bevacizumab) show also a high activity, although more uncertainty remains on the optimal dose of this combination in view of some reports of higher toxicity of the combination capecitabine plus irinotecan.
Cetuximab is active in epidermal growth factor receptor (EGFR)-expressing irinotecan refractory metastatic CRC. The combination of cetuximab with irinotecan is more active in this setting than cetuximab alone. The combination of cetuximab plus irinotecan leads to an increased RR and TTP compared to cetuximab alone in irinotecan-refractory CRC. It has been shown also that panitumumab, a human monoclonal antibody against the EGFR is active in irinotecan- and oxaliplatin-refractory metastatic CRC. The RR of the anti-EGFR antibodies cetuximab and panitumumab as single agent in EGFR expressing chemorefractory CRC is consistently around 10%. In a large phase III trial it was shown that panitumumab increased significantly the PFS compared to best supportive care in EGFR expressing metastatic CRC refractory to oxaliplatin and irinotecan. In another large randomized trial of cetuximab versus best supportive care, cetuximab prolonged the PFS as well as the survival. The PFS of the combination FOLFIRI/cetuximab was significant longer than that of FOLFIRI alone in a phase 3 trial in the first line treatment of CRC.
With this information in mind, bevacizumab is often used in clinical practice in combination with an active cytotoxic regimen in the first-line treatment of metastatic CRC (FOLFIRI or FOLFOX) and cetuximab plus irinotecan in chemorefractory CRC, at least if patients are fit and if there are no contraindications for these therapeutic options.
Many open questions and challenges remain in relation to the use of the anti-VEGF and anti-EGFR antibodies in metastatic CRC. Answers are needed to optimize the outcome for patients and the more optimal use of the resources. A crucial challenge is to demonstrate which patients are more likely to respond to bevacizumab-containing regimens and to the anti-EGFR antibodies cetuximab and panitumumab. Predictive molecular markers for a benefit on angiogenesis inhibitors are not yet available. Despite intensive research, large studies validating predictive molecular markers for response to anti-EGFR antibodies are not yet available in metastatic CRC. The clinical studies evaluating the activity of cetuximab and panitumumab have been carried out in EGFR-expressing tumors, as determined by immunohistochemistry (IHC). The intensity of EGFR immunostaining is not related to antitumor activity, and a clinical benefit has also be noted in patients whose tumors had no EGFR immunostaining. EGFR gene mutations have not been demonstrated to play a role in the response prediction in CRC. Although it has been reported in a small study that EGFR gene copy number, as assessed by fluorescence in-situ hybridization (FISH), correlates with the propensity of CRC to respond to EGFR-directed antibodies, this finding is at the moment very controversial. In a few other retrospectieve studies K-ras mutations were associated with low activity to cetuximab.

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

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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