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BERLIN 2005 (Part I)

Highlights in Gastrointestinal Oncology

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Abstracts of Invited Lectures
Poster Abstracts

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GASTRO-CONFERENCE BERLIN 2005 (Part I)

HIGHLIGHTS IN GASTROINTESTINAL ONCOLOGY

Berlin (Germany)
October 1 - 2, 2005

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Of mice and men: Mouse models for colon carcinogenesis

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Because of its abundant genetic and genomic information, laboratory mouse (*Mus musculus*) has become one of the best model animal species in biomedical research today. Genetically engineered mice, i.e., gene knockout and transgenic mice have become essential tools in both mechanistic studies and drug development. In this lecture, I will review currently available mouse models for colon cancer and polyposis; adenomas, hamartomas and adenocarcinomas. Then I would like to discuss recent progress from our own laboratory, including the mouse models for chromosomal instability and inflammation.

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Session I

Esophageal cancer
Etiology, screening and treatment
Molecular mechanisms of esophageal cancer

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The esophageal mucosa comprises a stratified squamous epithelium that is shared across many sites such as the skin, oral cavity, pharynx, larynx, and anogenital tract. This epithelium comprises several compartments: proliferating basal cell layer, differentiating suprabasal cell layer and terminally differentiated superficial squamous cell layer. Cells migrate in an outward direction from the basal layer to the superficial squamous layer, and eventually undergo desquamation due to senescence and apoptosis. This process is continuously renewed, and at the same time, it can be appreciated why this epithelium is subject so readily to injury by acid, bile, infectious agents (viral, fungal), and chemoradiation therapy.

We have previously helped to define the molecular basis for the switch from the proliferative state to the differentiated state as involving in part plasticity in the expression and function of cytokeratins within the basal and suprabasal cell layers. In order to model esophageal carcinogenesis, which is the ultimate perturbation of the normal proliferation-differentiation gradient, we have also employed the Epstein-Barr virus ED-L2 promoter to target genes (e.g. cyclin D1) specifically to the oral-esophageal epithelium, resulting in squamous dysplasia. When the L2-cyclin D1 mice are bred into a p53 deficient background, oral-esophageal squamous cancer emerges with lymph node involvement. This robust model has been utilized in translational systems, namely provoking the mice with zinc deficient diets (a common nutritional deficiency in many parts of the world), and also, applying a chemopreventive agent, Sulindac.

Our current work is motivated by three interrelated directions: (1) Identification and characterization of stem cells as a basis to understand lineage specification in the normal esophageal squamous epithelium and transdifferentiation into the intestinal metaplastic phenotype that defines Barrett's esophagus; (2) Elucidation of the role of EGFR and signaling mechanisms in proliferation and transformation; and (3) Modeling transformation in organotypic culture, a three-dimensional cell culture system, as a platform for investigating epithelial-stromal interactions in the tumor microenvironment.
Protection and chemoprevention of Barrett's esophagus

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The rapid increase in Barrett's esophagus related cancers has prompted efforts in several different countries to develop strategies for the management of this pre-malignant condition. Development of esophageal adenocarcinoma is associated with a survival of less than 20% in Western Societies. However, in our current understanding, the vast majority of patients with Barrett's esophagus will not develop cancer. The primary problem is trying to identify those at highest risk of malignancy. Risk factors that have been identified to date include length of Barrett's esophagus, presence of dysplasia, extent of dysplasia, development of genetic abnormalities such as inactivation of p16 and p53, increase in proliferating cells, and abnormalities in DNA content. All of these risk factors except dysplasia are not widely accepted and have not been proven to be successful across multiple centers. Unfortunately, it is well recognized that dysplasia is not diagnosed in a standardized fashion by even experienced pathologists that further complicates this problem.

Strategies which have been studied in terms of chemoprevention include cyclo-oxygenase inhibition, acid suppression, ornithine decarboxylase inhibition, and photodynamic therapy. Of these, only photodynamic therapy has been found to actually decrease cancer risk. Complicating this situation is the advanced age of most of these patients that decreases their candidacy for surgical resection but opens the possibility for the use of strategies that are not curative, but may delay onset of cancer. Current management of these patients is still dependent on careful surveillance biopsies, endoscopic mucosal resection techniques to be certain areas of cancer can be detected, and careful selection of treatments based upon consideration of individual patient factors and cancer risks.
Surgical strategies in esophageal cancer

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Surgical strategies in esophageal cancer are based on exact preoperative staging and risk analysis of the patient. **In preoperative diagnostics** it is important first of all to differentiate between squamous cell cancer (SCC) and Barrett-Adeno cancer (B-ca) (by endoscopic biopsies). The second most important aspect is the exclusion of distant metastases - this can be done with a single PET-imaging (distant metastases means palliative treatment). Regarding preoperative neoadjuvant treatment a further stratification in early/localised tumours (T1/T2) and locally advanced tumours (T3/T4) this is most effectively done by EUS. Risk analysis in every patient is mandatory.

The therapeutical concepts are different in SCC and B-ca.

- **SCC:** Neoadjuvant treatment has the aim of local down-sizing of the tumour for R0-resection later on (problem of neighbourhood of the tracheal-bronchial tree as a consequence of the frequent location of SCC at the level or above the tracheal bifurcation). For this RTX is necessary. (The CTX is more active at cytosensitizer and applicated as combined RCTX). The problem is the immunosuppression following this RCX which makes modification of surgery sometimes necessary (so called "safety-surgery", i.e. two-step reconstruction)

- **B-ca:** As the consequence of the frequent location below the tracheal-bronchial-bifurcation. The local problems of R0-resectability are few. Therefore, CTX is indicated. With the first aim of systemic effects, CTX has no negative consequences for surgery.

In both entities PET for early response evaluation is desirable. In this way, identification of non-responders is possible two weeks after the beginning of the therapy (palliative treatment?).

The surgical strategies are also different in both tumour entities. B-ca will be treated with abdominal thoracic esophagectomy and interthoracic esophagogastostomy. This procedure is well proven in PCT (prospective controlled trials) and has a very low operative mortality (below 2%) and morbidity and is followed by a high quality of life. **SCC** - transthoracic total esophagectomy is necessary in the majority of patients. Reconstruction is done by gastric tube interposition with a cervical anastomosis. Morbidity and mortality are slightly higher but in experienced centres below 5%. As a consequence of the cervical anastomosis swallowing discomfort occasionally happens.

The prognosis of esophageal cancer has improved over the last decades. The survival rate is better in Barrett cancer (45% 5-year survival rate) than in squamous cell cancer (30% 5-year survival rate). The best prognosis have so called "responders" under induction therapy in both tumour entities. The best prognosis of all have patients with Barrett cancer who are responders (5-year survival of 55%).
Neoadjuvant treatment of oesophageal cancer

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Squamous cell carcinoma of the upper third of the oesophagus can be resected only when laryngectomy is performed simultaneously. In order to avoid consecutive disability to speak, chemoradiotherapy alone should be delivered. 10-year survival rates with chemoradiation alone are as high as with surgery alone. Salvage resection may be considered for patients with relapses after chemoradiotherapy.

There is no consensus on treatment strategy for squamous cell cancer of the intermediate and lower third of the oesophagus. Patients with locally advanced, not resectable cancer should be treated with chemoradiation, in case of partial or complete remission subsequent surgery may be an option. For patients with locally advanced, resectable squamous cell cancer neoadjuvant chemoradiotherapy followed by resection is the standard of care in most of the cancer centers (F. Fiorica et al. 2004). Although, surgery alone or chemoradiation alone are performed in and outside of clinical trials in some units. Neoadjuvant chemoradiotherapy increased postoperative mortality and morbidity in most of the studies.

Data from the MAGIC trial (D. Cunningham et al.) and other investigations indicate that patients with adenocarcinoma of the gastro-oesophageal junction benefit from neoadjuvant chemotherapy. ECF (Epirubicin/Cisplatin/5-FU) for adenocarcinoma and Cisplatin/5-FU for squamous cell carcinoma are cytotoxic combinations used in most of the trials.
Session II

Gastric cancer
Standard and novel therapies
Helicobacter pylori and risk of gastric cancer and lymphoma

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It is well established that persistent infection with Helicobacter pylori (Hp) is associated with an increased risk for gastric malignancies. Within the last years, new insights into the pathogenetic role of Hp in gastric carcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma became evident. Also, the preventive value of Hp eradication as well as its therapeutic potential in gastric malignancies can now be estimated more precisely.

In 1983, the gastric bacterium Hp, at that time still named Campylobacter pylori, was rediscovered by Warren and Marshall. An unbelievable story of success followed since then especially with the focus on peptic ulcer disease. In 1994, the WHO classified Hp as a class I carcinogen. The association of Hp and gastric cancer mainly came from large-scale epidemiological studies. According to them and to available metaanalyses, Hp infection increases the risk of gastric cancer two- to threefold. This association might have been underestimated because of possible clearance of the infection in the course of disease development. In a recent study from Germany, this hypothesis was addressed in a case-control study with serologic assessment of Hp infection in which various exclusion criteria were used to minimize potential bias from this source. By doing this, the odds ratio of noncardia gastric cancer increased to 18% for any Hp infection and to 28% for CagA-positive Hp Infection. There are several other arguments for a pathogenetic role of Hp in the development of gastric cancer:

- Hp causes gastric mucosal atrophy and intestinal metaplasia both known to increase the cancer risk
- Hp infection decreases gastric ascorbin acid concentration and eradication of the bacterium is followed by its normalization
- Hp induces hyperproliferation which is regularly found in the early steps of cancer development
- animal experimental data
- interventional studies

A first population-based randomized placebo controlled intervention study recently showed that eradication of Hp can prevent gastric cancer in a subset of patients. The significant reduction in cancer incidence was found in those subjects not revealing precancerous changes such as atrophy, intestinal metaplasia or dysplasia at the beginning of the 7.5-year follow-up. The missing preventive effect of Hp eradication in subjects with pre-existing precancerous changes can be explained by the fact that treatment of the infection was too late. It seems possible that atrophy, metaplasia and dysplasia represent a point of no return beyond that Hp eradication is no more protective. These theoretical considerations and the findings of the study advocate an early eradication treatment.
Up to now, a family history of gastric cancer, the presence of a risk (dominant corpus) gastritis, and the patients’ desire were accepted arguments for Hp eradication therapy under cancer preventive considerations. There is some evidence now that cytokine genetic polymorphisms may play an important role in the development of gastric cancer. This may offer more specific interventions in the future.

There is clear evidence from epidemiological, histomorphological, molecular biological and experimental data that Hp plays a decisive role in the development and progression of gastric MALT lymphoma. Hp eradication therapy has become the widely accepted initial treatment of stage I gastric MALT lymphoma leading to remission rates of some 70%. However, the curative potential of this strategy was still under debate until very recently. A large prospective series now confirmed an excellent long-term outcome of patients after exclusive eradication therapy. However, some 20-30% of gastric MALT lymphoma cases do not respond to Hp eradication. Detection of API2-MALT1 fusion may discriminate responders from non-responders as found recently. A very important aspect concerns patients revealing histologically persisting lymphoma infiltrates after successful Hp eradication despite normalization of the endoscopic findings. Patients with this condition are classified as having minimal residual disease. As treatment failures they were referred to radiation, surgery, or chemotherapy up to now. However, this obviously represents an unnecessary overtreatment. There is good evidence from a large international series that most patients with minimal residual disease have a favourable course of disease and that a watch-and-wait strategy with regular endoscopic-bioptic controls should become the approach of choice in this condition.
The adjuvant treatment of gastric cancer

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Gastric cancer accounts for approximately 10% of all cancers and is the second cause of cancer related mortality worldwide. Surgical resection of the primary tumour and regional lymph nodes was the only curative option for operable gastric cancer. Most experts advice to perform at least a D1 resection and advice to recover and to examine at least 15 lymph nodes (1-3). However, relapses are frequent even after adequate surgery. Several strategies have been explored to reduce the risk of recurrence and to improve the overall survival.

1. Adjuvant chemotherapy:
Most of the individual trials studying the effect of postoperative adjuvant chemotherapy do not show a survival advantage compared to surgery alone. These studies randomized often a low number of patients and are clearly underpowered. The trials studied also predominantly older chemotherapy regimens. Further, the patient populations studied were heterogeneous, including patient populations with both high and low risk of recurrence.

Five meta-analyses (or combined analyses) of adjuvant chemotherapy have been published (4-7). Most of the analyses show a small benefit in survival for patients treated with postoperative adjuvant chemotherapy. Because of the nature of the data, adjuvant chemotherapy is not generally advised to patients who undergo a complete surgical resection of gastric cancer.

2. Post-operative chemoradiotherapy:
A major change in the management of this disease is based on the results of the US GI- Intergroup study, which randomised 556 patients with resected adenocarcinoma of the stomach or gastro-oesophageal junction to surgery plus post-operative chemoradiotherapy or surgery alone (8). The adjuvant treatment consisted of 425 mg/m² 5-FU (bolus infusion) per day plus 20 mg/m² of Leucovorin (LV) for five days, followed by 45 Gy in 25 fractions of 1.8 Gy over 5 weeks with bolus 5-FU and LV during the first and last week of the radiotherapy. This was followed 4 weeks later by 2 cycles of bolus 5-FU/LV. The median overall survival in the surgery only arm was 27 months, compared to 36 months in the chemoradiotherapy group (p < 0.05). The survival at 3 years was 50 versus 40% in favour of patients treated with post-operative chemoradiotherapy. After a median follow-up of 5 years, compared to surgery alone, 5 year overall survival was improved by 11.6% (40% versus 28.4% respectively; p < 0.001) and the relapse-free survival was increased from 25 to 31%. Patients treated with postoperative chemoradiotherapy had significant less locoregional recurrences. Three patients died from toxic effects of chemoradiotherapy. Grade 3 toxic effects occurred in 41% and grade 4 in 32% of the patients treated with chemoradiotherapy (8).
Most patients did not undergo an extensive surgical resection although the protocol recommended a D2 resection. Fifty four percent of the patients did not even have a D1 resection (8). So it is possible that the adjuvant therapy was simply making up for inadequate surgery. The chemotherapy used in this study was never considered to be a highly effective combination for stomach cancer. Therefore better chemotherapeutic options should be investigated in this setting: actually infused regimens of 5-FU are recommended in this combination regimen. The addition of new cytotoxic agents is under investigation.

3. Perioperative chemotherapy

The Magic trial compared a strategy of surgery alone with the administration of 3 cycles of pre-operative chemotherapy followed by surgery followed by 3 cycles of post-operative chemotherapy (perioperative chemotherapy) in 504 patients with gastric and GE junction adenocarcinoma. The ECF regimen was selected as chemotherapy regimen: epirubicin, cisplatin and protracted 5-FU every 3 weeks. The patients treated with perioperative chemotherapy had a significantly better survival: 50 vs 41% were alive at 2 years and 36 vs 23% at 5 years compared to patients who were treated with surgery only. The median survival was significantly longer for patients treated with peri-operative chemotherapy: 24 vs 20 months (p = 0.009). The progression survival was also significantly improved for patients treated with peri-operative chemotherapy: HR 0.66 (95% CI 0.53 - 0.81; p = 0.0001) (9).

Conclusions: Relapses after gastric cancer are frequent. Recent data on the (neo-)adjuvant therapy have changed clinical practise in patients with gastric at risk of recurrence after complete gastric cancer resection. There is actually level 2 evidence for the strategy of post-operative chemoradiotherapy and for the strategy of perioperative chemotherapy and level 3 evidence for post-operative chemotherapy. The strategy of post-operative chemoradiotherapy and of peri-operative decreases the risk of recurrence and improves the outcome for patients fit to undergo these treatment options.

References:


Chemotherapy and new treatments for gastric cancer

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Adenocarcinoma of the stomach remains one of the leading causes of cancer death worldwide. Complete resection is the only curative therapy for gastric cancer, however, approximately two-thirds of patients have locally advanced or metastatic disease at diagnosis.

Cisplatin-based chemotherapy (e.g., ECF [epirubicin, cisplatin, 5-fluorouracil]-regimen, modified AIO [weekly infusional 5-fluorouracil, leucovorin, cisplatin]-regimen) has been widely accepted as treatment option for patients with advanced gastric cancer.

Recently, the final results of a randomized trial comparing the DCF (docetaxel, cisplatin, 5-fluorouracil [5-FU]) chemotherapy with FUP (infusional 5-FU/cisplatin) have been reported. The DCF-regimen showed significant superiority in terms of remission rate, time to progression and overall survival. However, the overall survival of the DCF-regimen only reached 9.2 months (Table 1). Severe neutropenia was the main toxicity of DCF (Moiseyenko et al., J. Clin. Oncol. 23, Suppl. 16S, abstr. 4002, 2005).

In a second randomized phase III study a modified AIO-regimen (weekly infusional 5-FU/leucovorin/irinotecan, IFL) was tested against FUP. In this trial 337 patients with advanced gastric cancer were included. The remission rate (31.8% vs 25.8%) and time to progression (5.0 months vs 4.2 months, p = 0.088) of IFL were superior compared to FUP, but the overall survival showed no significant differences (9.0 months vs 8.7 months) (Dank et al., J. Clin. Oncol. 23, Suppl. 16S, abstr. 4003, 2005). However, the toxicity profile was in favour of the IFL-regimen (neutropenia, neutropenic fever, mucositis). Furthermore, the results of the randomized REAL-2 study (> 900 patients, U.K.) are of significant clinical importance. In the REAL-2 study the standard ECF-regimen [epirubicin, cisplatin, infusional 5-FU] is compared with the substitution of cisplatin with oxaliplatin (EOF or EOX) and 5-FU with capecitabine (ECX or EOX) by a two-by-two design. The results of this study are awaited to be presented next year.

Future strategies may also implicate molecular targets like the Epidermal Growth Factor (EGF) or Vascular Endothelial Growth Factor (VEGF) receptor. The EGF receptor plays a major role in cancer cell proliferation, angiogenesis, metastatic spread and apoptosis inhibition and is expressed in 60% of gastric cancer. Preliminary data on the humanized anti-EGFR mAb matuzumab showed efficacy in combination with cisplatin-based chemotherapy in patients with advanced gastric cancer (ECX/matuzumab [Rao et al., J. Clin. Oncol. 23, Suppl. 16S, abstr.. 4028, 2005], PFL/matuzumab [Trarbach et al., J. Clin. Oncol. 23, Suppl. 16S, abstr. 3156, 2005]).
The role of cytotoxic agents and new molecular strategies in the treatment of patients with advanced gastric cancer will be discussed.

**Table 1: Advanced gastric cancer. Randomized Phase III Studies**

<table>
<thead>
<tr>
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<th>ECF vs MCF (N = 580)</th>
<th>DCF vs FUP (N = 457)</th>
<th>IF vs FUP (N = 337)</th>
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<tbody>
<tr>
<td>Ross et al. 1999</td>
<td>Remission rate (%)</td>
<td>36.0 vs 33.0</td>
<td>36.7 vs 25.4 (s)</td>
</tr>
<tr>
<td>Moiseyenko et al., 2005</td>
<td>Overall survival (months)</td>
<td>9.4 vs 8.8</td>
<td>9.2 vs 8.6 (s)</td>
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<td>Dank et al., 2005</td>
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Colorectal tumors develop as a consequence of genetic alterations in various tumor suppressor genes and oncogenes. The tumor suppressor APC (adenomatous polyposis coli) is mutated at an early stage of colorectal tumorigenesis and has been proposed to act as a gatekeeper in this process. Mutations of APC lead to aberrant activation of the Wnt signaling pathway by stabilization of the cytoplasmic component $\beta$-catenin and formation $\beta$-catenin/TCF transcription factor complexes. Thus, constitutive activation of $\beta$-catenin/TCF target genes including c-myc is a key event in the development of colorectal carcinomas and also various other tumors, such as hepatocellular carcinomas. Wnt signaling is controlled by the negative regulator conductin (also termed axin2 or axil) or the related protein axin, which induce degradation of $\beta$-catenin by functional interaction with APC and the serine/threonine kinase GSK3$\beta$. We show that conductin but not axin is a target of the Wnt signaling pathway and is upregulated in human colorectal and liver tumors as well as in the APC-deficient intestinal tumors of Min mice. Upregulation of conductin may constitute a negative feedback loop that controls Wnt signaling activity. Furthermore, we present evidence that conductin is involved in mitotic checkpoint control and generation of chromosomal instability linking aberrant Wnt signaling to alterations of genomic integrity of cancer cells.
Session III

Colorectal cancer
The controversy of polyp development - Beyond APC?
Cooperative signalling by TGFβ and Wnt pathways

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The TGFβ superfamily of growth and differentiation factors are important regulators of many developmental processes and interference with their activity is implicated in a variety of human diseases. TGFβ superfamily ligands act by binding to heteromeric complexes of ser/thr kinase transmembrane receptors which then directly phosphorylate members of a family of intracellular mediators, known as Smads. Once activated, heteromeric Smad complexes translocate from the cytoplasm to the nucleus. Mutations in TGFβ pathway components have been identified in a number of human tumors including those of colorectal and pancreatic origins. While TGFβ superfamily members can function independently to control developmental events, cooperative interactions between the TGFβ and Wnt/wingless pathways have also been described. The Wnt/wingless pathway is distinct from that of TGFβ and signals are mediated through the Frizzled family of receptors to β-catenin, a co-activator of the DNA binding HMG box transcription factors, LEF1/TCFs. As for the TGFβ pathway, mutations in Wnt pathway components have been detected in human tumors, most prominently in colorectal cancer.

We have been investigating the molecular mechanisms that mediate the cooperative activity of these two pathways and previously demonstrated that TGFβ and Wnt pathways synergistically activate transcription of the homeobox gene, twin during early Xenopus development through direct physical interactions between Smads and LEF/TCFs. To investigate cooperative signaling by TGFβ and Wnt in mammals, we have produced soluble, active Wnt ligands and are using cDNA microarrays to identify synergistically-regulated target genes in epithelial cells. Our results have revealed a complex pattern of independently-, antagonistically- and cooperatively-regulated targets. Of note, increased expression of a subset cooperatively-regulated genes are increased in tumor samples obtained from mouse models of colorectal and mammary cancer and in FAP patients. Thus, these pathways appear to control a specific transcriptional program that may be involved in promoting tumorigenesis.
Beyond the adenomatous polyposis coli (APC) era: Alternative pathways to colorectal cancer

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Based on the model provided by the autosomal dominant condition familial adenomatous polyposis it has been assumed that the majority of colorectal cancers (CRCs) originate within tubular adenomas that are in turn initiated through inactivation of the tumour suppressor gene APC. Subsequent steps in the transition from adenoma to carcinoma are mutation of KRAS and TP53. It is likely, however, that only 60% of CRCs have mutation of APC and that only a fraction of these will have mutation of both KRAS and TP53. It is also apparent that up to 95% of tubular adenomas will remain as benign lesions. These observations point to the existence of alternative pathways of colorectal tumorigenesis that must explain more than a small minority of CRCs. Lesions that have been relatively neglected as potential precursors of CRC include the rare types of adenoma (villous, serrated and flat) and recently recognized subtypes of hyperplastic polyp described as sessile serrated adenoma (or sessile serrated polyp). Some of the alternative precursor lesions, notably the serrated polyps, have been linked to the class of CRC that shows extensive DNA methylation, DNA microsatellite instability, and mutation of the oncogene BRAF. These CRCs are also characterized by distinct clinical features (proximal location and female predilection) and pathological features (mucinous and/or poor differentiation). A single linear model cannot explain the evolution of all CRCs. CRC represents a set of different diseases, each with its own natural history and clinical behaviour. Based on the understanding that CRC is not a homogeneous entity, it should be possible to devise novel and more targeted strategies for the prevention, early detection and treatment of this disease.
Tumor progression driven by the tumor environment in colorectal cancer

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Most human colorectal adeno-carcinomas (CRC) develop via an adenoma-carcinoma sequence. All steps in this progressive sequence are histologically well definable. On the molecular level this progression is paralleled by the accumulation of mutations in oncogenes, like K-ras as well as tumor suppressor genes, like APC (adenomatous polyposis coli), SMAD4/DPC-4 (deleted in pancreatic carcinomas) or p53. The connection between histology and genetic alterations cumulated in the multistep carcinogenesis model developed by Fearon and Vogelstein. In this model malignant progression is indicated by the gradual loss of differentiation of colorectal tumor cells, which is the prerequisite for invasion, migration and finally metastasis. But most metastases of human CRC present histologically the same or even a better grade of differentiation compared to their primary tumors. If dedifferentiation of tumor cells is a prerequisite for malignant transformation, how do metastases arise then? Interestingly, in most CRC the two compartments "central tumor area" and "invasion front" can be discriminated on the basis of the differentiation of the tumor cells. Tumor cells in central tumor areas are well differentiated, express E-cadherin together with $\beta$-catenin in the context of adherens junctions (zonula adhaerens). In contrast, tumor cells at the invasion front display a dedifferentiated phenotype which is reminiscent of mesenchymal cells. Accordingly, these cells express the mesenchymal markers vimentin as well as fibronectin, and have lost the membranous expression of E-cadherin and $\beta$-catenin. Moreover, $\beta$-catenin is expressed in the nuclei of most of the tumor cells in this compartment which was shown recently to induce EMT (epithelio-mesenchymal transition). Both compartments are found as well in the primary tumors as their corresponding metastases. Thus, the phenotypical change of colorectal tumor cells must be regulatable and the driving force seems to be contributed by the environment of the tumor cells. Taken together, the process of colorectal tumorigenesis is highly dynamic and not linear as suggested by the multistep carcinogenesis model.

Nuclear $\beta$-catenin has an important role in the process of colorectal carcinogenesis, as the Wnt-pathway controlling the stability of $\beta$-catenin, is functionally affected in more than 80% of all cases. Tumor cells at the invasion front displaying high levels of nuclear $\beta$-catenin display a strong expression of $\beta$-catenin target genes like the proteases uPA (urokinase plasminogen activator), MMP-7 (matrix metalloproteinase-7), MT-MMP1 (membrane type MMP1) which facilitate invasion and migration. Other $\beta$-catenin target genes are the extracellular matrix (ECM) component tenascin-C a well known inductor of EMT, invasion and migration as well as laminin-5 $\gamma$2. Laminin-5 $\gamma$2 is degraded by MT-MMP1 and a short fragment generated by this proteolysis is a very potent inductor of invasion and migration. For a global and more detailed analysis RNA microarrays (Affymetrix) were done using a mixture of tumor and stroma cells from either the invasion front or central areas of
the tumors. After clustering the genes according to functional groups it turned out that genes affecting invasion and migration as well as genes indicative for inflammation were strongly up-regulated in tumor cells of the invasion front. Thus, nuclear β-catenin induces EMT and migration in colorectal tumor cells, which has prognostic value.

Moreover, β-catenin induces the expression of TERT (telomerase RT-component) and survivin. As TERT and surviving expression are found mainly in epithelial stem cell their expression in tumor cells might indicate tumor stem cells in CRC. In such a sense the tiny amount of nuclear β-catenin expressing tumor cells may resemble the biologically active component of human CRC which might have been underestimated in the past.
Session IV

Colorectal cancer
New screening strategies for the new millennium
Impact of the National Polyp Study

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The U.S. National Polyp Study (NPS) was a multicenter prospective randomized trial designed to evaluate follow-up surveillance strategies in patients who have had colonoscopic polypectomy. The study was sponsored by the 3 national U.S. GI Societies and funded by the NCI. Eligible patients were randomized to more frequent or less frequent follow-up which included colonoscopy, FOBT and DCBE of a referred cohort of 9112 patients, 1418 were eligible for randomization. When the NPS was initiated common practice in the U.S. was annual colonoscopy following polypectomy. The NPS demonstrated that the first follow-up colonoscopy could be deferred for 3 years, leading to this recommendation in U.S. guidelines. Colonoscopy revealed only 3% advanced adenomas whether the exam was 1 yrs. or 3 yrs. after polypectomy. Recent NPS data showed that patients could be stratified into lower and high risk subgroups for subsequent neoplasia which led to the risk stratification concept in updated U.S. Multisociety Task Force guidelines. The NPS provided evidence to support our belief in the adenoma-carcinoma sequence demonstrating a 76%-90% reduction to the expected incidence of colorectal cancer following polypectomy. This reduction required baseline clearing of the entire colon with high confidence. Miss rates of adenomas and cancers are directly related to the quality of the clearing colonoscopy. The NPS also demonstrated low (48%) sensitivity of the DCBE for adenomas > 1 cm in a blinded comparison, an increased risk of colorectal cancer in first degree relatives of probands with adenomas younger than age 60 and evidence for the long natural history of the adenoma carcinoma progression and its dynamic aspect with regression of adenomas. When published pathology criteria for flat adenomas were used, 27% of all baseline adenomas could be classified as flat. In the NPS flat adenomas were no more likely to have HGD than sessile (polypoid) or pedunculated adenomas and were not at greater risk for subsequent advanced adenomas. In conclusion, the NPS provided an opportunity to study many aspects of the adenoma-carcinoma sequence: its long natural history, the long interval that could be used in post-polypectomy surveillance, the insensitivity of DCBE, the risk attributable to close relatives, the importance of high quality clearing colonoscopy, regression of adenomas, and provided a model for chemoprevention studies and blinded comparison of colonoscopy with imaging modalities.
Colorectal cancer screening: Cost effectiveness and adverse events

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Colorectal cancer (CRC) remains a major cause of morbidity and mortality throughout the world. Population screening has been shown to be effective in reducing mortality from CRC. Every form of screening has limitations and potential adverse events which impact outcome, which are summarized in this abstract.

Fecal Occult Blood Testing (FOBT):
Issues: FOBT is a complex program with many layers. Failure of adherence at any level has an impact on test performance. First, the test must be accepted by patients, and must be repeated every 1-2 years to be effective. Adherence with repeat testing after a negative test is poor. Single, one-time testing will not be effective due to poor sensitivity of one-time testing. Second, the test is being used to identify patients with an increased risk of CRC, and positive results should be followed by colonoscopy. Failure at this level renders the test ineffective.

Cost effectiveness: Demonstrated in models

Flexible Sigmoidoscopy (FS)
Issues: If patients with distal adenomas at FS receive complete colonoscopy, FS is able to identify 70% of men with advanced neoplasia. A recent study found that the test is much less effective in women - only 35% with advanced neoplasia detected (NEJM 2005; 352: 2061-8). In addition, FS in men is less effective with increasing age, because lesions in the proximal colon are more prevalent.

Cost effectiveness: Demonstrated in models

Colonoscopy
Issues: Colonoscopy can be used as a primary screening test. However, it is the most invasive screening test, with the highest risk of adverse events. Serious events are rare - significant bleeding in 2/1000 and perforation in 1/1000. The miss rate for colonoscopy is a potential limitation - 4-12% of polyps ≥ 10 mm are missed.

Cost effectiveness: Demonstrated in models

CT Colonography
Issues: This is an evolving technology. Current data reflect this evolution, with significant differences in sensitivity and inter-observer variability. Other issues include the need for a bowel prep and radiation exposure.

Cost effectiveness: Not demonstrated. Factors which influence cost are 1) the threshold polyp size for recommending colonoscopy and 2) rate of extracolonic findings which require evaluation.
Fecal DNA test
Issues: The most recent data find sensitivity for cancer (51.6%) and advanced neoplasia (18.2%). Further refinements of the method may improve sensitivity, but this represents a significant limitation of the test.

Cost effectiveness: Not shown
Detecting dysplasias in ulcerative colitis

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Patients with ulcerative colitis (UC) have a significantly higher risk for the development of colitis-associated colorectal cancer. Risk factors include the duration of the disease, the degree and extent of inflammation, as well as the presence of primary sclerosing cholangitis. The growing pattern of dysplastic tissue is often multifocal and flat. Thus, significant lesions can be overlooked. Randomized biopsies are recommended but do not erase the fear of overlooked cancers with severe consequences for the patient. Chromoendoscopy with targeted biopsies may solve this problem. Another clinical problem in ulcerative colitis is that dysplastic changes can have two different causes. Colitis-associated dysplasias (DALM) are driven by inflammation whereas sporadic adenomas in UC patients follow the adenoma-carcinoma sequence, which leads to different clinical managements. Local resection by polypectomy is sufficient for sporadic adenoma, but multifocal DALMs require colectomy. In ulcerative colitis, inflammation and dysplasia are first limited to the mucosa. Thus, methylene blue as an absorptive stain helps ideally to characterize the surface architecture due to the stable staining pattern in comparison to indigo carmine. In 2003, the first randomized, controlled trial was published to test whether chromo- and magnifying endoscopy might facilitate early detection of intraepithelial neoplasia in patients with ulcerative colitis by using magnifying chromoendoscopy. In the chromoendoscopy group, more targeted biopsies were possible, and significantly more intraepithelial neoplasias were detected in the chromoendoscopy group. Using the modified pit pattern classification (Pit-Pattern I-II: endoscopic prediction non-neoplastic; Pit Pattern III-V: endoscopic prediction neoplastic), both the sensitivity and specificity for differentiation between non-neoplastic and neoplastic lesions were 93%. The ability of the dye technique to identify neoplastic from non-neoplastic lesions to enhance detection of more dysplastic lesions in flat mucosa is a potential major advance in dysplasia surveillance.

This new technique may help target biopsies so that they are less random and ultimately some form of a proven dye technique may help the endoscopist who has little enthusiasm to persist through the tedium of 30 to 40 biopsies. Perhaps considerably fewer biopsies will be necessary.

Recently, Rutter et al. found no dysplastic tissue in 2904 non-targeted biopsies. Taken together, these data suggest that targeted biopsies after intravitral staining will replace random biopsies in the future, although prospective studies fully addressing this point are required. Although panchromoendoscopy requires additional time during surveillance colonoscopy (about 8-9 minutes) the use of targeted rather than random biopsies will save time. Therefore, the total time required for panchromoendoscopy with targeted biopsies is similar compared with colonoscopy with random biopsies in experienced hands but chromoendoscopy has a higher efficacy for detection of intraepithelial neoplasias.
In conclusion, magnifying chromoendoscopy is a new valid tool for improving endoscopic detection of intraepithelial neoplasia in patients with long-standing ulcerative colitis. Chromoendoscopy increased the diagnostic yield of intraepithelial neoplasia as compared with conventional colonoscopy and biopsy techniques 3- to 4.5-fold, which further suggests that more patients with ulcerative colitis could be considered as candidates for colectomy. Differentiation of non-neoplastic from neoplastic lesions is possible with a high overall sensitivity and specificity. Thus, the old days when endoscopists were groaning about too much random biopsies seem to be over and a new era where few smart biopsies are sufficient for surveillance colonoscopy in patients with long-standing ulcerative colitis has started to come.

References:


Virtual colonoscopy

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CT colonography (CTC) or MR colonography is a rapidly evolving technique which has induced a lot of controversy. With increasing emphasis on the early diagnosis of colorectal cancer the potential of this specialized examination to detect colonic polyps has captured increasing attention.

Method: CTC is the term used to describe thin-section helical CT of the prepared large bowel with the volumetric data set reviewed both as 2D and 3D images of the colonic surface. Meticulous bowel cleansing is one of the most crucial requirements as residual stool and large amounts of residual fluid impair the assessment of the colon. Bowel distension can be supported by intravenously given spasmolytic agents.

Indications: Failure to visualize the complete colon occurs in 5-15% of colonoscopies and is an adequate indication for CTC. Apart from its ability to demonstrate the entire large bowel in the majority of patients with distal occlusive carcinomas and its accuracy in depicting synchronous neoplasms, CTC before surgery provides additional benefits. CTC combines study of the colon with evaluation of target organs for metastases, in particular the liver.

People who are candidates for screening should be given adequate information on the risks and benefits of the various screening procedures.

Patient acceptance is a key feature of a diagnostic method used for screening. Recent publications have been presented containing substantial data on the good acceptance of CTC compared with conventional endoscopy. Other factors like the absence of sedation, the shorter procedure time, and the lower physical challenge of CTC play an equally important role.

The exposure to ionising radiation is a drawback of CTC that hampers implementation for colorectal cancer screening. Several efforts have been made to reduce radiation dose.

Summary
The advantages of CTC over conventional endoscopy include safety, its ability to demonstrate the entire colon in patients who had an incomplete colonoscopy, to accurately localize lesions, to examine the proximal bowel with minimal risk inpatients with obstructing lesions and in frail, debilitated patients, and to provide staging information in the preoperative evaluation of patients with cancer. The examination is performed without sedation in less time than conventional colonoscopy and involves little risk of complications. The disadvantages include the need for bowel cleansing and infusion of gas to expand the colon. Scanning hardware is expensive, and the interpretation of the images is relatively difficult and time consuming.
Session V

Colorectal cancer
Prevention
A statistical model for post-polypectomy surveillance - a virtual alternative to virtual colonoscopy?

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Introduction. The National Polyp Study (NPS) demonstrated that detection and removal of adenomatous polyps is associated with a 76% to 90% reduced risk of subsequent colorectal cancer. This incidence reduction was achieved with initial polypectomy and surveillance colonoscopy for patients with adenomas in a randomized controlled trial comparing surveillance intervals at 1, 3 and 6 years versus at 3 and 6 years post polypectomy. Of importance is parsing out whether the incidence reduction was due to the initial or surveillance colonoscopies, or to both, and how long the interventions had an effect. We cannot directly observe the separate effects of the initial and the surveillance colonoscopy because all adenoma patients received both initial and surveillance colonoscopy. However we can use micro-simulation modeling to assess the separate effects. We also use micro-simulation modeling to address the important question of whether all patients require 3-year surveillance or whether the surveillance colonoscopies could be lengthened for lower risk patients. An additional issue is whether virtual colonoscopy could be used for the surveillance for lower risk patients without diminishing the incidence reduction achieved.

Methods. We use micro-simulation modeling of the NPS adenoma cohort under three scenarios - 1.) no initial and no surveillance colonoscopies 2.) initial colonoscopy intervention but no surveillance and 3.) initial colonoscopy and surveillance colonoscopies to assess the separate effects of the initial and surveillance colonoscopies. We further assess the timing of the surveillance colonoscopies (3, 6, or 10 years post polypectomy) by the patient characteristics for the multiplicity (1 or 2 versus 3 or more) and the size ($\leq 0.5$ cm, 0.6-0.9 cm, or $\geq 1.0$ cm) of the adenomas at initial colonoscopy. We also consider whether virtual colonoscopy has a role in surveillance for the lower risk adenoma patients. We use the MISCAN-Colon micro-simulation model to simulate a population of adenoma patients of the same age, sex and baseline adenoma characteristics of the National Polyp Study.

Results. Over 95% of the impact of colonoscopic intervention is due to the initial colonoscopic polypectomy rather than to surveillance colonoscopies in the first 6 years of surveillance. By 10 years post polypectomy the impact of the initial colonoscopy has dropped to 75% with the surveillance colonoscopy accounting for 25%. However, the impact of surveillance colonoscopy is primarily in the 20% of the NPS patients with 3 or more adenomas at initial colonoscopy. Performing surveillance colonoscopy at 3 years for those with 3 or more adenomas at baseline and lengthening the surveillance to 6 or more years for those with 1 or 2 adenomas at baseline preserves the incidence reduction observed by the NPS with a marked reduction in the number of surveillance colonoscopies.
Conclusions.
The initial colonoscopy provides the largest impact on CRC incidence in the subsequent 10 years following initial polypectomy. For patients with only a single adenoma of size < 1.0 cm, the initial polypectomy could be considered to be sufficient impact to reduce subsequent risk. For those with 3 or more adenomas, surveillance colonoscopy at 3 years shows benefit. Tailoring subsequent surveillance recommendations to the baseline colonoscopy findings would achieve the benefits of colonoscopic polypectomy with a reduction in the number of surveillance colonoscopies required and associated risks.
Molecular targets for prevention of colorectal cancer

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It has been well established that chronic NSAID users have a 40-50% reduction in mortality from colorectal cancer. Both aspirin and non-aspirin NSAIDs were found to be protective. This occurs when "anti-inflammatory" or even lower doses of NSAIDs are given. Other reports indicate that mortality from other types of cancer may also be affected by COX-2 status and/or NSAID exposure. Clinical trials have shown a significant reduction in polyp number in patients receiving low dose aspirin over several months. Research efforts have been focused on understanding the molecular basis for the chemoprotective effects associated with use of aspirin and other NSAIDs. Nonselective NSAIDs inhibit both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) activity. Since COX-2 levels are increased in a number of solid tumors, this enzyme may serve as a molecular target for cancer prevention. Recent clinical studies indicate that the presence of COX-2 in human lung and colon cancers is associated with a negative clinical prognosis. Therefore, COX-2 inhibitors are currently being evaluated for prevention and/or treatment of cancer in humans. Recent clinical evidence indicates that use selective COX-2 inhibitors are also associated with significant thrombotic side effects. These serious side effects seem to vary with the particular COX-2 inhibitor (rofecoxib >> celecoxib) and with the dose of drug given. Interestingly, the cardiovascular side effects do not become apparent until after 18 months of daily drug use and "short-term" use does not appear to cause these problems.

COX-2 overexpression in cultured cells confers a resistance to apoptosis, an increased metastatic potential and increased release of pro-angiogenic factors. In some instances, prostaglandins can mimic the effects of COX-2 overexpression. Treatment with a selective COX-2 inhibitor inhibits tumor growth in several preclinical models. Recently we and others have found that combinations of these inhibitors with agents designed to block ErbB receptor signaling are even more effective than either agent alone. Furthermore, studies evaluating the effects of PGE2 demonstrate an activation of the ErbB signaling pathway and increase in polyp burden. Hence, there is important crosstalk between the prostaglandin and growth factor receptor signaling network which is being explored further. Use of agents which target certain pathways downstream of COX-2 deserve attention due to the recent problems associated with use of COX-2 selective inhibitors.

References


Opposing effects of tumor-promoting and tumor-inhibiting bile acids on Ras, p38 and Cox-2 in AOM tumorigenesis and colon cancer cells

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Environmental factors, including dietary fats, are implicated in colonic carcinogenesis. Dietary fats modulate bile acid concentrations in the colon, which are thought to contribute to the nutrition-related component of colon cancer risk. In the azoxymethane (AOM) model of experimental colon cancer in rodents, bile acids including cholic acid are known to promote tumorigenesis. In contrast, we previously showed that ursodeoxycholic acid (UDCA), a low abundance bile acid used to treat cholestatic liver diseases, inhibited AOM tumorigenesis. Dietary UDCA blocked the development of tumors with activated Ras and inhibited cyclooxygenase-2 (Cox-2) induction in AOM tumors. Furthermore, UDCA inhibited hyperproliferation and formation of aberrant crypt foci (ACF). ACF are the earliest identifiable premalignant precursors of colon cancer. To identify potentially causal changes induced by tumor-promoting cholic acid and tumor-inhibiting UDCA prior to ACF formation, we examined their effects on premalignant colonocytes five days after AOM treatment. Thirty six rats, in a balanced 2 x 3 factorial design, were treated once with AOM (20 mg/kg) or saline (vehicle) and then gavaged twice daily with cholic acid (10 mg/kg), UDCA (50 mg/kg) or DMSO (vehicle). On day 5, animals were sacrificed and colonocytes isolated. Cholic acid significantly enhanced, whereas UDCA inhibited AOM-induced Ras and p38 activations. Cholic acid also increased Cox-2 expression in premalignant colonocytes. In separate experiments, AOM tumors were induced in rats fed AIN-76A chow alone, or chow supplemented with 0.4% cholic acid or UDCA. As in premalignant colonocytes, cholic acid enhanced Cox-2 up-regulation in AOM tumors. In agreement with previous studies, UDCA inhibited Cox-2 induction in tumors. UDCA concomitantly and significantly decreased AOM-induced C/EBPβ, a positive regulator of Cox-2 gene expression. We next examined HCA-7 colon cancer cells to elucidate the mechanisms of bile acid-induced changes in Cox-2 expression. These cells have regulated wild type Ras and constitutively express low levels of Cox-2. Deoxycholic acid (DCA), the major metabolite of cholic acid in the colon, significantly increased Ras-GTP and phospho (active) p38 and induced C/EBPβ and Cox-2 in HCA-7 cells. UDCA inhibited basal Ras-GTP and DCA-induced p38 activation and Cox-2 induction. DCA also significantly activated the Cox-2 promoter and increased Cox-2 mRNA, while UDCA inhibited these changes. The p38 inhibitor, SB 203580 blocked p38 activation and completely abrogated Cox-2 induction by DCA. Similarly, UDCA inhibited DCA-induced p38 activation and Cox-2 induction. Taken together, these studies suggest that p38 mediates Cox-2 induction in colonic tumorigenesis and that UDCA inhibits Cox-2 induction by a p38-dependent mechanism. These studies have identified p38, and possibly C/EBPβ, as potential novel targets for colon cancer chemoprevention strategies.
Towards a vaccine to prevent familial forms of colon cancer

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About 15% of all human cancers arise as consequence of deficient DNA mismatch repair that results in microsatellite instability (MSI), i.e. rapid accumulation of mutations in short repetitive DNA stretches, termed microsatellites. MSI is particularly a hallmark of cancers associated with the hereditary non-polyposis colorectal cancer syndrome (HNPCC) but is also found in a variety of sporadic cancers, usually due to methylation of genes coding for components of the DNA-mismatch repair complex (Altonen et al., 1993, Yonov et al., 1993, Miyakura et al., 2001). MSI results in mutation of microsatellites that are located in intergenic or intron regions. However, they may also be located in coding sequence stretches (coding microsatellites, cMS). If such cMS are affected by MSI, the respective gene might be functionally inactivated. It is commonly accepted that the MSI-mediated inactivation of defined critical genes is the major driving mechanism of MSI-associated carcinogenesis (Duval et al. 2002, Woerner et al. 2001, Woerner et al. 2003).

MSI-associated mutations in cMS may result in translation of novel frameshift peptides at the carboxy terminus of the respective gene products. A growing number of such frameshift peptides were shown to be presented to the immune system as a novel class of tumor-associated antigens (Linnebacher et al. 2001; Saeterdal et al. 2001; Ripberger et al. 2003; Schwitalle et al., accepted for publication). These recent findings suggest that based on the major carcinogenic mechanism of MSI-H cancers a large number of frameshift induced neoepitopes is generated that may explain the pronounced immunogenic properties of MSI-H cancer cells, as indicated for example by the dense infiltration with cytotoxic T-cells (Dolcetti et al. 1999, Smyrk et al. 2001, Kloor et al., in preparation). Based on the frequency of mutations in specific genes encompassing coding microsatellites in MSI-associated cancers, we were able to predict a set of shared antigens in MSI-cancers that may represent interesting candidates for potential vaccines (either prophylactic or therapeutic) for MSI cancers and in particular for patients affected by the hereditary non-polyposis colorectal cancer syndrome (HNPCC). The detailed analysis of humoral and cellular immune responses against these antigens revealed that patients suffering from MSI-associated cancers frequently develop a pronounced immune response against these antigens. Moreover, since the penetrance of this hereditary cancer syndrome is only 80% or less, we speculated that individuals affected by predisposing germline mutations but who do not develop MSI-associated cancers may develop a sufficient immune response to prevent outgrowth of transformed cell clones based on the MSI-mediated carcinogenesis. The immunological analysis of those patients revealed an even stronger immune response against the predicted candidate antigens in healthy gene carriers compared to patients who developed MSI-cancers, whereas normal control individuals were not reactive against the predicted class of frameshift antigens.
This strongly suggests that immune responses against MSI-induced neo-antigens play an important role in the natural history of MSI-associated cancers. They may further be an interesting source to identify shared antigens that may form the platform for prophylactic or preventive vaccines against MSI-associated cancers and in particular in the HNPCC context.

In line with these observations, we found that antigen presentation pathways were also affected by frameshift mutations in coding microsatellite sequences and therefore pose an important immune escape mechanism for MSI-associated cancers.

References


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Gene expression profiles and molecular markers to predict recurrence of Dukes' B colon cancer

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Background: The 5 year survival rate of Dukes' B colon cancer patients is approximately 75%. Identification of the patients at high risk of recurrence in this group would allow better staging and more informed use of adjuvant chemotherapy. Although a number of molecular prognostic factors have been evaluated in their association with recurrence, clinical and pathological factors are preferably used to determine tumor stages in present medical practice for colon cancer. In this study, we used DNA chip technology to systematically identify new prognostic markers for tumor relapse in Dukes' B colon cancer patients. A prognostic gene signature was built and further validated by using independent patient samples.

Methods: Using Affymetrix U133a GeneChip containing approximately 22,000 transcripts from the human genome, RNA samples from 197 Dukes' B colon cancer patients were analyzed. Forty-four of these patients were selected because they developed tumor relapse in less than 3 years while 153 patients remained disease-free for more than 3 years after surgery. A multivariate Cox proportional hazards model was built to predict tumor recurrence. The model was then validated in independent patients from multiple institutions. Results: In a training set of 74 tumors we identified a 23-gene signature. This gene profile was highly informative in identifying patients who develop distant metastasis within 3 years (HR, 6.70, 95% CI, 2.30-19.5, p = 0.0001), even when corrected for traditional prognostic factors in multivariate analysis (HR, 5.78; 95% CI, 1.93-17.3, p = 0.002). Next, we tested this gene signature in an independent and more diverse population of Dukes' B patients. In this completely independent validation set of 123 patients, the 23-gene signature proved to be a prognostic factor in identifying patients who developed distant recurrence (hazard ratio, HR 2.95; 95% CI, 1.16-7.48, p = 0.017), even when corrected for traditional prognostic factors in multivariate analysis (HR, 3.50; 95% CI, 1.22-10.0, p = 0.02).

Conclusions: This study suggests that gene expression profiling provides a successful strategy to identify a combination of molecular markers that can be used to build a classification model of colon cancer recurrence. The clinical value of these markers is that the patients at a predicted high risk of relapse could be up-staged to receive adjuvant therapy, similar to Dukes' C patients. Our data highlights the feasibility of a prognostic assay that could help to focus more intensive treatment for localized colon cancer.
Session VI

Colorectal cancer
Predictive markers - Gene profiling
Chromosomal deletions as predictors for recurrence of early stage colorectal cancer

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The driving force behind the dynamic tumor genome is genetic instability, which, when coupled with environment selection, is responsible for the activation of oncogenes and the inactivation of tumor suppressor genes in tumor cells. The majority of colorectal cancers exhibit chromosome instability (CIN tumors), while a small percentage of them exhibit microsatellite repeat instability (MIN tumors). MIN phenotype is due to defects in a limited number of genes, all of which are involved in mismatch DNA repair. In addition, disease progression is usually favorable in MIN tumors with appropriate clinical treatment. Molecular mechanism for CIN phenotype is still under investigation but appears to be more complicated and may involve a large number of players. Molecular predictors for disease recurrence in early stage colorectal tumors have been actively pursued for the past decade. Results from initial loss of heterozygosity (LOH) studies using microsatellite repeat markers have been controversial partly due to inherent technical limitations of this method in analyzing archived paraffin-embedded tissue. Single-nucleotide polymorphisms (SNPs) are more suitable for this type of analysis because of their size uniformity, abundance and high throughput-analysis potential. Initial LOH analyses based on digital SNPs have provided promising results, demonstrating the potential power of SNP analysis. Future implementation of high-throughput SNP analysis using affymetrix oligonucleotide arrays may facilitate the development of an accurate prognostic test for early stage colorectal cancers based on chromosomal deletions.
Methylation profiling of gastrointestinal lesions?

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Patients with Barrett's esophagus (BE) are at increased risk of developing esophageal adenocarcinoma (EAC). Clinical neoplastic progression risk factors, such as age and the length of the esophageal BE segment, have been identified. However, improved molecular biomarkers predicting increased progression risk are needed for improved risk assessment and stratification. Using real-time quantitative methylation-specific PCR, we screened 10 genes (HPP1, RUNX3, RIZ1, CRBP1, 3-OST-2, APC, TIMP3, p16, MGMT, p14) for promoter hypermethylation in 77 EAC, 93 BE and 64 normal esophagus (NE) specimens. A subset of genes manifesting significant differences in methylation frequencies between BE and EAC was then analyzed in 20 dysplastic specimens. All 10 genes except p14 were frequently methylated in EACs, with RUNX3, HPP1, CRBP1, RIZ1, and OST-2 representing novel methylation targets in EAC and/or BE. p16, RUNX3, and HPP1 displayed increasing methylation frequencies in BE vs. EAC. Furthermore, these increases in methylation occurred early, at the interface between BE and low-grade dysplasia (LGD). To demonstrate the silencing effect of hypermethylation, we selected the esophageal adenocarcinoma cells BIC1, in which the HPP1 promoter is natively methylated, and subjected them to 5-aza-2′-deoxycytidine (AZA-C) treatment. Real-time RT-PCR analysis indicated increased HPP1 mRNA levels after 3 days of AZA-C treatment, as well as decreased levels of methylated HPP1 DNA. Hypermethylation of a subset of six genes (APC, TIMP3, CRBP1, p16, RUNX3 and HPP1) was then tested in a retrospective longitudinal study of 99 Barrett's metaplasia (BE) and 9 low-grade dysplasia (LGD) specimens obtained from 53 BE patients undergoing surveillance endoscopy. Only HGD or EAC were defined as progression endpoints. Two patient groups were compared: 8 progressors (P) and 45 nonprogressors (NP), using Cox proportional hazards models to determine the relative progression risks of age, BE segment length, and methylation events. Multivariate analyses revealed that only hypermethylation of p16 (OR 1.74, 95% CI 1.33-2.20), RUNX3 (OR 1.80, 95% CI 1.08-2.81), and HPP1 (OR 1.77, 95% CI 1.06-2.81) were independently associated with an increased risk of progression, whereas age, BE segment length, and hypermethylation of TIMP3, APC, or CRBP1 were not independent risk factors. In combined analyses, risk was detectable up to, but not earlier than, 2 years preceding neoplastic progression. Hypermethylation of p16, RUNX3 and HPP1 in BE or LGD may represent independent risk factors for the progression of BE to HGD or EAC. These findings have implications regarding risk stratification, early EAC detection, and the appropriate endoscopic surveillance interval for patients with BE.
Session VII

Colorectal cancer
Interdisciplinary treatment
Oxaliplatin/5-FU/LV in the adjuvant treatment of stage II and stage III colon cancer: Efficacy results for patients with 56.2 months median follow up

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MOSAIC trial was designed to demonstrate an increase in Disease Free Survival (DFS) at 3 years in stage II and III colon cancer. 2246 patients with completely resected stage II (40%) or III (60%) colon cancer were randomly assigned to receive LV5FU2 or FOLFOX4 for 12 cycles. Final results of the study have been reported for the overall population, with a median follow-up of 3 years (Andre et al., NEJM, 2004). With a follow-up of 56.2 months, a 23% reduction in the relative risk of relapse was observed with the FOLFOX4 combination in the overall population (p < 0.001). Among the 1247 patients with stage III disease, difference for DFS is 8.6% in favor of FOLFOX4, translating into a relative risk reduction of 25% in this subset of patients (HR 0.75 [0.62-0.89]). Among the 899 patients with stage II disease, difference for DFS is 3.5% in favor of FOLFOX4, translating into a relative risk reduction of 18% in this subset of patients (HR 0.82 [0.60-1.13]). An analysis of stage II patients with at least one adverse prognostic factor (T4 tumor, bowel obstruction or tumor perforation, poor differentiation, venous invasion or fewer than 10 lymph nodes examined) was reported recently. In this specific subpopulation (n = 576), difference for DFS is 5.4% in favor of FOLFOX4 translating into a relative risk reduction of 24% decrease in the risk of relapse (HR 0.76). These benefits were within the range of those observed in stage III patients and were associated with limited toxicity. In the overall population probability of surviving are 82.6% and 78.1%, respectively in the FOLFOX4 and LV5FU2 arms (HR 0.91 [0.75-1.11]). This translates into a reduction in mortality risk of 9% in favor of FOLFOX4. Patients are still followed for survival as number of events has not been reached so far for final analysis. After up to 4 years of follow up, 3.4% of patients presented either with persisting localized paresthesias of moderate intensity (2.7%) or with paresthesias that may interfere with functional activities (0.7%). Based on these results, FOLFOX4 is now the standard treatment for patients with stage III colon cancer.
Advances in chemotherapy for colorectal cancer

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Suddenly the pace at which our options for treating patients with colorectal cancer using both chemotherapy and biologic drugs has accelerated. This is true both in the adjuvant and metastatic disease settings and is a consequence of the common availability and usage of five active agents for managing colorectal cancer. These agents include fluorouracil with leucovorin (5-FU/LV) and its oral prodrugs, irinotecan, oxaliplatin, bevacizumab, and cetuximab. A host of other novel agents are in clinical testing and the expectations remain that the therapeutic armamentarium will continue to expand in the near future.

Presentations made at the last two annual meetings of the American Society of Clinical Oncology (ASCO) have led to a new consensus regarding managing stage III patients in the adjuvant setting. Two large, randomized trials, the MOSAIC study from Europe and the NSABP C-07 trial from the U.S., have provided us with consistent data that one of two oxaliplatin + 5-FU/LV regimens, FOLFOX (oxaliplatin plus infused 5-FU with LV) and FLOX (oxaliplatin plus bolus 5-FU with LV) lead to a 3 year disease free survival (DFS) advantage for patients over 5-FU/LV regimens. Three trials based on the use of irinotecan plus 5-FU/LV regimens in the adjuvant setting have failed to provide any evidence with respect to DFS to justify the use of irinotecan based regimens in the adjuvant setting. These negative trials include the CALGB study (Saltz et al.), the PETACC 3 trial (Van Cutsem et al.) and the Accord 02 trial (Ychou et al.).

In the past 25 years our expectations for the 5 year overall survival (OS) in Stage III patients has risen from less than 50% to approximately 70%. Considering the fact that over 1 million people are diagnosed with colorectal cancer annually worldwide this translates into many lives saved. Current endeavors are focusing on evaluating whether the addition of cetuximab and bevacizumab to oxaliplatin based chemotherapy can provide even a bigger advantage to patients in the adjuvant setting. The consensus for management of Stage II patients is that this population has a similar relative benefit from adjuvant therapy but because of the fact that a higher proportion are cured by surgery alone (70-80%) the absolute additive benefit for a course of chemotherapy in terms of lives saved is less striking. The use of single agent 5-FU/LV or capecitabine as adjuvant therapy should now be restricted to patients who are unwilling or unable to tolerate combination therapy.

The treatment of metastatic colorectal cancer has also changed for the better in the past decade with the appreciation for the activity and toxicity advantages of delivering 5-FU by infusion, the advent of the new chemotherapies irinotecan and oxaliplatin, and the integration of newly approved biologics for use alone and with chemotherapy. The approval of the oral agent capecitabine has also added to the options for both monotherapy and combination therapy.

Data from both individual and pooled analyses of randomized trials clearly indicates that combination chemotherapy leads to better outcomes than do single agents. (Grothey et al.) In phase III studies oxaliplatin based regimens coupled with infused 5-FU are advantageous with respect to response rate (RR), time to progression
(TTP), and OS, and the rates of grade 3,4, and 5 toxicities over the irinotecan and bolus 5-FU based regimen known as IFL. (Goldberg et al.) The data comparing regimens that are based on 5-FU infusion coupled with either irinotecan (FOLFIRI) or oxaliplatin (FOLFOX) is also available. (Tourignand et al.) In this trial that enrolled 220 patients there was no difference in outcomes between these two regimens noted. However, patients who were initially unresectable were more often able to undergo hepatic resection with FOLFOX than with FOLFIRI. Also FOLFIRI treated patients experienced more alopecia and diarrhea while FOLFOX treated patients were more commonly neutropenic (rarely associated with fever or sepsis) and developed sensory neuropathy. Proponents debate the advantages of FOLFOX versus FOLFIRI but both are commonly employed as first-line therapy in the metastatic setting. Trials seeking to refine the regimens and optimize them are ongoing worldwide. At the moment we have good evidence on which to base a choice for combination chemotherapy, coupling irinotecan or oxaliplatin to infused 5-FU, and can expect predictable toxicity profiles. The data supporting the substitution of capecitabine for infused 5-FU come largely from Phase II studies. While the current data are less robust than will be the case once Phase III trials have been completed the activity and toxicity appears to be similar to that observed with FOLFOX or FOLFIRI.

Two monoclonal antibodies, cetuximab and bevacizumab, were recently approved by the U.S. Food and Drug Administration for treatment of metastatic colorectal cancer. Bevacizumab is a humanized monoclonal antibody that targets the vascular endothelial growth factor receptor (VEGF) present on both normal and abnormal blood vessels. The trial by Hurwitz published recently in the New England Journal of Medicine enrolled over 900 patients. They were randomized to either IFL with or without bevacizumab or to 5-FU plus leucovorin with bevacizumab. There was a 4.6 month advantage in overall survival in the cohort of patients treated with the IFL + bevacizumab combination. Outcomes with IFL + bevacizumab were very similar to those observed with FOLFOX, and both IFL + bevacizumab and FU/LV + bevacizumab were better than IFL alone. Consequently the US FDA approved the use of bevacizumab as indicated for the treatment of previously untreated colorectal cancer patients with metastatic disease coupled with 5-FU alone, 5-FU + irinotecan or 5-FU plus oxaliplatin. The combination of FOLFOX + bevacizumab in the second line setting has also been shown in a large randomized Phase III study to provide an advantage with respect to RR, DFS, and OS over FOLFOX or bevacizumab alone in a population of patients with progressive disease after therapy with both irinotecan and 5-FU. (Giantonio et al.)

Cetuximab is a monoclonal antibody that targets the endothelial growth factor receptor that appears to be present on 80% of the tumor cell surfaces in advanced colorectal cancers. It has been tested in the second line setting both as a single agent and in combination with irinotecan in patients with prior irinotecan exposure. The response rates have been in the range of 10% as a single agent and 23% for the two drug combination. (Cunningham et al.) Phase III trials are in progress with this agent combined with irinotecan and oxaliplatin based regimens in the advanced disease and adjuvant settings. Recently data from a randomized Phase II study known as the BOND 2 trial were presented in which patients were treated with cetuximab plus bevacizumab with or without irinotecan after they had manifest progressive disease on an irinotecan
based regimen. In this study the response rates, TTP and overall survival rates were promising suggesting that there may be effective strategies in disease management that include biologic agents without the use of chemotherapy and its attendant side effects.

The options available to physicians and their patients with colorectal cancer have increasing at such a rapid pace that the field and what we can do for our patients is evolving rapidly. What do I currently recommend to my own patients with metastatic colorectal cancer? My current first line choice is to enroll willing patients on a clinical trial such as the jointly sponsored CALGB and SWOG U.S. Intergroup Trial. This study allows the physician to choose between FOLFOX or FOLFIRI and randomizes patients to cetuximab, bevacizumab, or both antibodies in addition to the multi-agent chemotherapy. In patients who are not eligible for the trial or choose not to enroll, I personally recommend FOLFOX + bevacizumab. I prefer FOLFOX over FOLFIRI because of the potential for rapid response, the potential for sufficient response to permit patients to undergo disease resection in some circumstances, and because of the toxicity profile.

In second line, my preference is again to enroll patients on a clinical trial when possible. When no such option exists, I suggest a cetuximab plus irinotecan based therapy. Third and even fourth line regimens are a common reality these days as well. Clearly we are doing better things for our patients in 2005 but still have a lot more research to do before we can realize the goal of curing advanced colorectal cancer for a majority rather than a small minority of patients.
Targeting the vascular endothelial growth factor receptor for the treatment of colorectal cancer

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Vascular endothelial growth factor (VEGF) is a key regulator of physiological and pathological angiogenesis. VEGF and has been shown to regulate all critical aspects of tumor angiogenesis, including endothelial cell proliferation, migration, gene expression, and survival. Targeting tumor angiogenesis through blockade of the VEGF axis is a recently validated strategy for the treatment of colorectal cancer. The most highly developed of these anti-VEGF approaches is bevacizumab, a monoclonal antibody to the VEGF ligand.

The value of adding bevacizumab to standard 1st line chemotherapy was first conclusively demonstrated in a phase III trial of 923 patients with previously untreated metastatic colorectal cancer. The treatment arms consisted of 1) weekly irinotecan, bolus 5-FU, and leucovorin as described by Saltz et al. (bolus IFL or Saltz regimen; 2) bolus IFL plus bevacizumab (5 mg/kg q 2 weeks), or 3) 5-FU/LV (Roswell Park regimen 5-FU 500 mg/m², LV 500 mg/m²) plus bevacizumab (5 mg/kg q 2 weeks). The third arm was included since bolus IFL had just become the standard 1st line regimen in the United States, and there was not yet safety data on the IFL/bevacizumab combination. Compared to the IFL/placebo arm, the IFL/bevacizumab arm demonstrated an improved overall survival (median 15.6 vs. 20.3 months, hazard ratio of death 0.66, p < 0.001), progression free survival (median 6.2 vs. 10.6 months, hazard ratio of progression 0.54, p > 0.001), and response rate (34.8% vs. 44.8%, p = 0.004). Treatment benefit was also seen in all prespecified clinical subgroups, including age, gender, race, baseline albumin and LDH, among others. Bevacizumab added only incremental toxicity to the IFL regimen. The overall grade 3/4 toxicity rate was increased from 74% to 84%, mostly due to an increase in grade 3 hypertension (ie, requiring the addition or modification of blood pressure medications), and small increases in leukopenia and diarrhea.

More recently, several additional studies have demonstrated the safety and activity of bevacizumab in combination with other 5-FU based chemotherapy regimens. These studies include the 209 patient randomized phase II study of 5-FU/LV +/- bevacizumab in patients who were not ideal candidates for irinotecan. This study demonstrated statistically superior progression free survival (9.2 months vs. 5.5 months, p = 0.0002), and response rate (26% vs. 15%, p = 0.0552), and a strong but not statistically significant trend for improved survival (16.6 months vs. 12.9 months, p = 0.159). The efficacy was seen across all prespecified subgroups in this study as well as the IFL +/- bevacizumab study above, suggesting that anti-VEGF therapy is broadly active in colorectal cancer patients, even in older and less robust populations, groups traditionally not helped by many cytotoxic agents, including irinotecan.

Bevacizumab has also been combined in the 1st line setting in modest size trials using FOLFOX6 and capecitabine plus oxaliplatin (Xelox or Capox), where these regimens seem both well tolerated and active. Similar 5-FU/oxaliplatin and capecitabine/oxaliplatin based regimens are now also in testing in large cooperative group
trials. In the second line treatment of metastatic colorectal cancer, the addition of bevacizumab to the FOLFOX 4 regimen also conferred significant benefits in terms of survival, progression free survival, and response rate without changing the side effect profile expected for FOLFOX and bevacizumab alone. Taken as a whole, these data strongly support the use of the VEGF inhibitor bevacizumab in combination with standard chemotherapy for patients with metastatic colorectal cancer. Additional studies designed to build upon these findings are now ongoing.

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Because many patients with liver metastases of colorectal cancer (CRC) are unsuitable for hepatic resection, interest has focused on alternative treatment options. Direct ablation treatments include thermal ablation by use of lasers, microwaves and radiofrequency. Radiofrequency ablation (RFA) provides an effective technique for minimally invasive tissue destruction. An alternating current delivered via a needle electrode causes localised ionic agitation and frictional heating of the tissue around the needle. RFA with modern equipments effectively ablates tumours and is not associated with some of the side-effects of other ablative techniques.

The majority of investigators uses RFA percutaneously without general anesthesia. Some groups guide the procedure during laparoscopy or direct open while laparotomy. Studies have shown the practical difficulties in assessing the adequacy of ablation, and contrast-enhanced computed tomography (CT), ultrasound, or magnetic resonance imaging (MRI) are used to assess necrosis. These difficulties, together with short follow-up periods in most studies, should be considered alongside the reported low rates of local recurrence. Randomised controlled trials of CRC metastases treated with RFA reporting survival data are lacking.

In summary, radiofrequency ablation is an interesting and promising therapy for the treatment of liver metastases of CRC. But the available evidence does not support use of this technique as a single treatment modality for patients with non-resectable liver metastases.

References:


Adjuvant and neoadjuvant chemoradiotherapy for locally advanced rectal cancer

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**Background:** Standard treatment for patients with rectal cancer is surgery. For locally advanced disease (TNM stage II and III), postoperative chemoradiotherapy significantly improved both local control and overall survival as compared with surgery alone. Thus, postoperative chemoradiotherapy has been recommended for pT3/4 and or pN+ tumors. In recent years, encouraging results have been reported for preoperative radiotherapy. We compared the effect of preoperative versus postoperative chemoradiotherapy in a randomized trial.

**Methods:** Patients with clinical stage T3 to T4 or node positive disease were randomly assigned to preoperative or postoperative chemoradiotherapy. The preoperative treatment consisted of 5040 cGy at 180 cGy per day. Fluorouracil was given by a 120 hours intravenous infusion at a dose of 1000 mg per square meter every 24 hours during the first and fifth week of radiation. Surgery was performed six weeks after completion of chemoradiotherapy. One month after surgery four five-day cycles of fluorouracil (500 mg per square meter per day) were given. Chemoradiotherapy was identical in the postoperative arm except for a boost of 540 cGy. Primary end point was overall survival.

**Results:** 421 patients were randomly assigned to preoperative and 402 patients to postoperative chemoradiotherapy. The overall 5-year survival was 76 percent and 74 percent, respectively (P = 0.80). The 5-year cumulative incidence of local relapse was 6 percent for patients assigned to preoperative chemoradiotherapy, as compared with 13 percent in the postoperative arm (P = 0.006). The 5-year cumulative incidence of distant metastases was 36% and 38% (P = 0.84), respectively. Grade 3 to 4 acute and long-term toxicity occurred in 27 and 14 percent in the neoadjuvant arm, versus 40 and 24 percent in the postoperative arm (P = 0.001 and P = 0.012, respectively).

**Conclusions:** Preoperative chemoradiotherapy significantly improved local control and was associated with less acute and long-term toxicity. Although no survival benefit was achieved, we suggest that preoperative chemoradiotherapy is the preferred treatment for patients with locally advanced rectal cancer. Further progress in the prevention of distant recurrences might be accomplished with more effective chemotherapy.
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POSTER ABSTRACTS

Poster Numbers 1 - 63
Inhibiting two key pathways for tumour growth can significantly reduce polyp burden in a mouse model of intestinal cancer

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**Introduction:** ZD6474, an orally active inhibitor of both vascular endothelial growth factor receptor-2 (VEGFR-2) and epidermal growth factor receptor (EGFR) tyrosine kinase activity, was used to study the effect of blocking angiogenesis and EGFR signalling in multiple intestinal neoplasia (Apc\textsuperscript{Min/+}) mice. These mice develop numerous benign polyps due to a mutation of the adenomatous polyposis coli (APC) gene, as occurs in familial adenomatous polyposis (FAP) in humans.

**Methods:** In the first study, ZD6474 (0, 12.5, 25 or 50 mg/kg/day) was administered daily by gavage to 6-week old Apc\textsuperscript{Min/+} mice for 28 days. In the second study, 10-week old Apc\textsuperscript{Min/+} mice were given ZD6474 0 or 50 mg/kg/day for 28 days. The number and size of polyps in the small and large intestines were scored.

**Results:** In the first study, all doses of ZD6474 reduced polyp number in the small bowel and colon (46\% and 76\%, respectively, with 50 mg/kg/day; p < 0.05). Polyp diameter was also significantly reduced in the small bowel, thus decreasing mean burden by 75\%. In the second study, small bowel polyp number and diameter were both reduced, thus decreasing polyp burden by 72\% (p < 0.01). ZD6474 halved the proportion of cells showing nuclear localisation of \(\beta\)-catenin staining also halved the number of cells expressing VEGFR-2 (p < 0.001). The reduction in number and size of polyps was similar in the two studies.

**Discussion/Conclusion:** VEGFR-2/EGFR signalling plays a key role in the development of intestinal adenomas, and that inhibiting this activity can markedly reduce polyp burden at both early and late stages.
Chemotherapy in patients with nonresectable cancer of the biliary system or advanced gallbladder cancer: Do elderly have benefit at all?

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Introduction: Adenocarcinomas of the bile ducts (BSCa) and gallbladder (GBCa) are highly malignant neoplasms with a very poor prognosis. Chemotherapy (cht) plays only a palliative role and responses are transient without prolonged survival. We aimed to evaluate effectiveness of gemcitabine (G) and cisplatin (CP) combination protocol in achieving Clinical Benefit Response (CBR) for patients (pts) with nonresectable BSCa and with advanced GBCa and to evaluate the differences between younger and elderly patients. For this type of tumors, we took 60 years as borderline for elderly.

Methods: During five years period we cured 38 patients with BSCa and GBCa with mean age of 52.4 ± 4.8 years. There were 23 females with mean age of 50.2 ± 3.8 years, and 15 men with mean age 53.7 ± 4.3 years. In the group of elderly, there were 9 females with mean age of 61.8 ± 2.9 years, and 6 men with mean age of 62.4 ± 2.9 years. 25 patients (65.8%) had locally advanced disease and 13 patients (34.2%) metastatic disease. Patients achieved G 1000 mg/m² intravenous once per week for seven times. CP 100 mg/m² IV was administered at day 1, 15, 29 and 43. Number of applied cycles was 1-10 (median 4 cycles).

Results: Partial response rate achieved 22 patients - 57.9%. 36 patients - 94.7% achieved CBR. Pain measured at VAS scale before cht was 5.1 and after first cht cycle 2.1. Karnofsky Performance Status before chemotherapy was 62.4% and after first cycle 78.8%. Median duration Clinical Benefit was 21 weeks. 29 patients (76.3%) developed fatigue gradus II-III (WHO); 20 patients (52.6%) expressed nausea gradus II (WHO); elderly had stronger fatigue (gradus III) versus younger; also more serious leucopenia and thrombocytopenia (gradus III) than younger (gradus II).

Discussion/Conclusion: There was no difference between younger and elderly patients in number of applied cycles; also no difference in achieving partial response rate and CBR. There was difference in developing side effects; elderly had more serious fatigue, leucopenia and thrombocytopenia than younger. These results indicate that the treatment of GBCa and BSCa with gemcitabine and cisplatinum is effective and well tolerated also to elderly patients and leads to Clinical Benefit for both groups. The role of chemotherapy in these malignancies is palliation and achieving better Quality of Life more than prolonging survival time.
Nutritional and pharmacologic support in patients with pancreatic carcinoma - Our results

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Introduction: Patients (pts) with carcinoma are often malnourished. Cachexia occurs in 8-84% of cases, depending on primary site of carcinoma (predominantly in gastric and pancreatic cancers; 84% and 73% respectively). We aimed to assess the impact of nutritional and pharmacologic support on weight gain and its influence on general well-being of our pts.

Methods: During 18-month interval, we examined 44 patients with pancreatic carcinoma (18 females, and 26 males, with mean age of 66 ± 2.4 years). Metastatic disease was found in 21 and locally advanced disease in 24 pts; metastatic and locally advanced had 17 pts. In 34 pts some kind of operative procedure was performed, but only 3 pts were radically operated. Dietary intake (Nottingham Screening Tool Score), body weight (BMI), appetite, food intake and Karnofsky Performance Status (KPS) were monitored at baseline (visit 0) and after every 2 weeks during 2-month interval (visits 1, 2, 3, 4). 44 (100%) of pts underwent nutritional counselling, 33 of them (75%) took supplemental enteral feeding and 44 (100%) took megestrol acetate 400 mg per day.

Results: At baseline, 44 (100%) of pts had decrease in weight gain (low BMI in 32 pts - 72.7%; BMI 18-20 in 22 pts - 50%; < 18 in 10 pts - 22.7%) and poor appetite. In 33 pts (75%) low food intake was recorded. Mean KPS at baseline was 60.2%. After 6 weeks, 38 pts (86.4%) gained their weight and appetite (37-84.1%). KPS was 60.8%.

Discussion/Conclusion: With nutritional counseling, supplemental feeding and pharmacologic support we stopped weight loss in our patients. 86.4% of pts increased their weight. 84.1% improved their appetite. These effects were especially represented 4-6 weeks after initial treatment. Because of the side effects, 13.6% of pts stopped taking enteral supplementation. Although our pts increased weight gain, improved appetite and QoL, KPS remained unchanged.
Gastrointestinal cancers - Assessment of pain in elderly patients

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Introduction: Patients (pts) with gastrointestinal (GIT) cancers suffer from many symptoms during their disease (1-25). Pain is the most prevalent symptom and occurs in 70-90% of pts with advanced cancer. The aim of this study was to compare the pain prevalence in elderly cancer pts versus younger cancer pts.

Methods: During 5 years period we examined 946 pts with cancer of GIT site in our hospital.

Results: 676 of them (71.46%) expressed pain. 120 females and 172 males (292) were elderly with mean age of 77.4 ± 3.2 years. In this group the most common pain was moderate (128 pts - 43.84%). 384 younger pts had mean age of 63.2 ± 4.1 years and also mostly expressed moderate pain. In elderly group 165 (56.51%) of pts respected prescribed pain therapy versus 291 (75.78%) in younger group. Elderly preferred transdermal opioid system for pain relief.

Discussion/Conclusion: There was no difference in pain expression according to age, gender and tumor site. Younger pts respected more pain therapy than elderly did. Elderly preferred transdermal opioid system for pain therapy.
Effects of nutritional support in patients with colorectal cancer during chemotherapy

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Introduction: Cancer cachexia occurs in significant number of patients (pts) with cancer. Benefit of nutrition support still remains unclear. We aimed to examine does nutritional support (counselling, oral liquids and megestrol acetate) has influence on nutritional status and symptoms prevalence in patients with colorectal cancer during chemotherapy.

Methods: We retrospectively (group A) examined data of 173 patients who were treated in 3 years period without nutritional counselling. Prospectively (group B - 215 patients) we offered to our patients individualized nutritional counselling, liquid supplementation, and megestrol acetate. Dietary intake (Nottingham Screening Tool Score) and body weight (body mass index) were monitored before, during and after chemotherapy. The nutritional plan was modified when necessary.

Results: During 6 years period we were treating 388 patients with colorectal cancer. Baseline, in both groups about 55% of patients were moderately or severe malnourished. In group A during chemotherapy 140 (80.9%) of patients decreased in weight gain 2-5 kg and 77 (44.5%) were severe malnourished on the end of chemotherapy. In group B, 184 (85.6%) of patients gain weight. The effect of nutritional support was most expressed in the group receiving megestrol acetate and nutritional counseling after 4 weeks of therapy. Average weight gain was 1.5 kg (0.6-2.8).

Discussion/Conclusion: With nutritional support, our patients with colorectal cancer gain weight and had better quality of life during chemotherapy.
Gastric adenocarcinoma inside an omphalomesenteric infected cyst communicating with a Meckel's diverticulum

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Introduction: Omphalomesenteric cyst is a rare malformation. Malignant tumors arising inside it are very uncommon.

Case Report: A 44-year-old man was admitted with high fever and umbilical discharge of pus. Laparotomy confirmed a 7 cm infected omphalomesenteric cyst, continued by a Meckel's diverticulum. We performed a cuneiform resection of Meckel's diverticulum, together with the cyst and the urachus. The cyst contained enteroliths, and a 1.5 cm protruding tumor. Microscopically, the cyst had ectopic gastric mucosa with G2 adenocarcinoma invading beyond the muscular layer. One lymph node was found to have metastasis. Resection margins were free of tumor. Because there is no standard chemotherapy, we delivered an adjuvant therapy based on Mayo protocol (6 cycles 5-FU + Leucovorin). After 18 months, resection of two solid tumors - 5 and 1.5 cm - (lymph nodes with metastasis of adenocarcinoma) located in the mesentery of the ileum was performed, followed by 6 cycles of Eloxatine standard dose chemotherapy. Patient has no sings of recurrent disease, 28 months after the initial operation.

Discussion/Conclusion: To the best of our knowledge, gastric adenocarcinoma of an omphalomesenteric cyst has not previously been reported. Communication between the cyst and the Meckel's diverticulum was obstructed by enteroliths, leading to a clinical manifest complication (infection) that allowed resection of the tumor at an operable stage. Chemotherapy was performed with drugs used in gastrointestinal cancer adjuvant therapy. Association of the cyst with the diverticulum favored lymphatic spread to the ileum lymph nodes, underlining the need for a segmental resection of the ileum when a malignant tumor with such a location is diagnosed.
Helicobacter pylori infection and risk of gastric cancer

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Introduction: Gastric cancer has a very high incidence in Romania, 28/100,000 of inhabitants. In spite the fact that in the western part of Europe, this incidence has decreased, in Romania is still very high. Helicobacter pylori (HP) is also very frequent, almost 50% of the asymptomatic population have the infection. The purpose of the study is to correlate the high incidence of HP in Romania with the high incidence of the gastric cancer.

Methods: 10570 patients underwent upper digestive endoscopy between five years. For HP were taken biopsies stained with hematoxiline-eosine and Giemsa and rapid urease test was performed. For the neoplastic lesions biopsies were stained with hematoxiline-eosine, Van Gieson and Alcian Blue. This allowed us to found nucleocitoplasmatic atipies and to appreciate the degree of the malignancy.

Results: In 371 (3.50%) patients (69.2% males, mean age 65.3 years) were discovered advanced gastric cancer and in 9 (0.08%) patients (77.7% males, mean age 59.8 years) early gastric cancer. In 241 (64.95%) cases of advanced gastric cancer was found HP and also in 7 cases (77.7%) of early gastric cancer.

Discussion/Conclusion: The infection with HP is statistically significant correlating with both advanced and early gastric cancer (p < 0.05). We can conclude that the high incidence of gastric cancer in Romania is probably due to the high frequency of HP. The eradication of HP together with the improving of the socio-economic status we hope will decrease the gastric cancer.
Evaluation of the combined use of cyclooxygenase-2-inhibitors and tumor antigen-loaded dendritic cells for the immunotherapy of pancreatic carcinoma

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Background: Overexpression of cyclooxygenase-2 (COX-2) plays an important role in gastrointestinal carcinogenesis. Here, we investigated whether COX-2-inhibitors can increase the efficacy of dendritic cell (DC)-based vaccination therapy for pancreatic carcinoma.

Material and methods: DCs were loaded with lysate from the pancreatic carcinoma cell line Panc-1 and cocultured with autologeous T cells. Restimulations with autologeous, tumor-lysate pulsed DCs were performed at weekly intervals. Lysis of Panc-1 cells left untreated or pre-treated with the COX-2-inhibitor NS398 was determined in a standard chromium release assay. In addition, apoptosis of the pancreatic carcinoma cells Panc-1, MiaPaca and BxPc-3 treated with NS398 (20 or 100 μM) or left untreated was induced by CD95-activating antibody CH-11 and measured by Annexin-V/PI-staining. The in vivo effect of the COX-2-inhibitor parecoxib on DC-based vaccination was investigated in a murine model of pancreatic carcinoma. DCs derived from the bone marrow of C57BL/6 mice were pulsed with syngeneic apoptotic Panc02-cells and matured with LPS/IFN-γ. After establishment of subcutaneous Panc02-tumors, DCs were injected into the contralateral flank of C57BL/6 mice at weekly intervals. Parecoxib was administered i.p. daily; tumor size and weight loss were monitored.

Results: Lysate-pulsed DCs primed tumor-specific, IFN-γ-producing T cells in vitro. CTLs derived from cocultures with lysate-pulsed DCs specifically lysed Panc-1 cells. Pre-treatment of Panc-1 cells with NS-398 prior to coculture with CTLs enhanced MHC class I-restricted, specific lysis. Blocking of CD95 on tumor cells prevented this increase in lysis. In vitro, COX-2-inhibitor NS398 sensitized Panc-1 cells to apoptosis (14% apoptotic cells after CD95-treatment in untreated cells versus 23% in NS398-treated cells). In the in vivo-model, DC vaccination and parecoxib therapy were successful in preventing tumor growth and increased overall survival. However, combined therapy with parecoxib was not more effective in preventing tumor progression than DC-based vaccination alone.
**Conclusions**: Pancreatic carcinoma cells can be sensitized *in vitro* to CTL-mediated lysis by treatment with COX-2-inhibitors. Studies using CD95-blocking antibody as well as agonistic anti-CD95 antibody indicate that COX-2-inhibitors specifically sensitize tumor cells to CD95-mediated CTL killing. Currently, studies are performed to analyze whether the observed effects are COX-2-dependent and to characterize alternative downstream mechanisms involved in tumor cell sensitization to CTL-mediated killing.
Colon tumor derived TGF-beta induces the conversion of tumor infiltrating CD4 T cells into FoxP3+ regulatory T cells

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Introduction: We have recently demonstrated that TGF-beta is critical for the peripheral induction of regulatory T cells by inducing the expression of FoxP3. Here we now demonstrate a role for TGF-beta induced regulatory T cells in colon cancer.

Methods: Colitis dependent tumors were induced by a single dose of azoxymethane followed by three weekly periods of DSS in drinking water.

Results: CD4+ cells isolated from colon tumors expressed high levels of FoxP3 mRNA while CD4+ cells isolated from non-dysplastic colon tissue did not. Immunohistochemistry demonstrated that as much as 50% of infiltrating CD4+ cells expressed nuclear FoxP3. In contrast only few FoxP3 positive cells were detectable in the lamina propria of normal colon tissue. Since tumor cells overexpressed TGF-beta, we then investigated mice, transgenic for a dominant negative TGF-beta receptor specifically in T cells. Strikingly, CD4+ T cells isolated from tumors of transgenic mice showed strongly diminished expression of FoxP3 mRNA as compared to cells from wild-type tumors. Furthermore, tumors in transgenic mice were larger that wildtype tumors implicating that in this model of inflammation dependent colon cancer, regulatory T cells may inhibit tumor growth.

Discussion/Conclusion: Colon tumors induce regulatory T cells by releasing TGF-beta. These may in the case of inflammation dependent cancer control tumor growth. However, in spontaneous cancer development, Treg may rather mediate tolerance towards the tumor causing immune evasion.
In vivo imaging of colitis and colon cancer development in mice using high resolution chromoendoscopy

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Introduction: Mouse models of colitis and cancer are indispensable for investigating the pathogenesis of colitis and colon cancer. In the past, mice had to be sacrificed in order to analyze colon pathology. We have developed a method for high resolution endoscopic monitoring of living mice.

Methods: Mice developing colitis or colonic tumors were anesthetized using avertine and repeatedly examined by endoscopy using a novel miniendoscope, denoted Coloview. The endoscope was introduced via the anus and the colon was carefully insufflated with an air pump before analysis of the colonic mucosa. An extra working channel allowed the introduction of biopsy forceps or injection needles as well as the surface staining with methylene blue in order to visualize the surface of the crypts and the pit pattern architecture.

Results: Endoscopic pictures obtained were of high quality and allowed the monitoring and grading of the disease. Scoring of colitis activity as well as tumor size and growth was possible. In addition, pit pattern analysis using chromoendoscopy permitted discrimination between inflammatory and neoplastic changes. Biopsies taken during examinations yielded enough tissue for molecular and histopathological analyses.

Discussion/Conclusion: Chromoendoscopy in mice allows monitoring of the development of colitis and colon cancer with high resolution. Manipulations like local injection of reagents or the taking of biopsies can easily be performed.
TNF-alpha and IL-6 signaling promote tumor growth in colitis associated colon cancer

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Introduction: Patients with ulcerative colitis have a high risk to develop colon cancer. Several lines of evidence suggest an important role for proinflammatory cytokines in cancer development, although the molecular and immunological mechanisms are largely unknown.

Methods: Colitis dependent tumors were induced by a single dose of azoxymethane followed by three weekly periods of DSS in drinking water.

Results: TNF-alpha and IL-6 were overexpressed in the tumor stroma as compared to control tissue. Mice that were deficient for TNF receptor I or II as well as double deficient mice were protected from tumor development as determined by high resolution endoscopy and histopathology. We furthermore provide evidence that IL-6 promotes tumor growth in the colon. Blocking of the IL-6R led to a protection from colon cancer development. IL-6 induced proliferation of dysplastic epithelial cells via trans-signaling. Accordingly the injection of hyper-IL-6 resulted in a stronger proliferation of dysplastic epithelial cells. Conversely, blocking IL-6 trans-signaling protected mice from colon tumor development. We further show that human colon cancer tissue expressed only low amounts of membrane bound IL-6R while expression and activity of the matrix metalloproteinase TACE was increased, implicating a similar mechanism in humans.

Discussion/Conclusion: Our data show that inflammation induced tumor genesis in the colon is highly dependent on signaling by the proinflammatory cytokines IL-6 and TNF-alpha. Targeting of these signaling pathways may be used for future therapy.
Does the site of a K-ras mutation influence the route of tumor cell dissemination from colorectal cancer?

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Introduction: The aim of this prospective study was to relate the incidences of cytokeratin 20 (CK20) and guanylylcyclase C (GCC) in lymph node-, liver-, and bone marrow specimens of 245 colorectal cancer (CRC) patients with the K-ras status of the corresponding primary tumor.

Methods: RT-PCR detection of CK20 and GCC mRNA was used as marker of circulating epithelial cells (CEC). Samples were considered positive for CEC only when both markers were detected concomitantly. For the detection of K-ras mutations, a PCR-RFLP assay was used.

Results: In the group with K-ras mutated primary carcinomas (n = 92), CEC were detected in 62% of lymph node-, 43% of liver-, and 2% of bone marrow samples, similar to the results in patients with K-ras wild-type carcinoma (59%, 46%, and 0%). Interestingly, separate analysis of K-ras codons 12 (n = 75, 82%) and 13 (n = 17, 18%) revealed significantly differing CEC incidences. Lymph node specimens from corresponding K-ras codon 13 mutated carcinomas showed a significantly higher CEC incidence (82%) than those from codon 12 mutation (57%, p < 0.05) or K-ras wild-type sequence (59%, p < 0.05). Unlike these findings in lymph nodes, liver biopsies from corresponding carcinomas with K-ras codon 12 mutation or wild-type sequence were significantly more often positive for CEC (31% and 29%) than specimens from K-ras codon 13 mutated primary CRC (12%, p < 0.04, respectively).

Discussion/Conclusion: Colorectal carcinomas with K-ras codon 12 mutation showed the same pattern of tumor cell dissemination as their K-ras wild-type counterparts. However, the CEC incidence in patients with K-ras codon 13 mutation was significantly increased in lymph nodes and decreased in liver biopsies.
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US detection of focal liver lesions in management patients with cancer

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The purpose of our study was to evaluate the impact of injection on the sonographic detection of focal liver lesions.

US is frequently used in detection of focal liver lesions, but its sensibility is lower than those of CT or MR. Injection of ultrasound contrast agent improves the resolution of focal liver lesions.

Between September 2001 and September 2004 (3 years) all patients referred for cancer staging were examined with fundamental mode and after contrast injection.

99 patients have been included. Injected studies were performed in harmonic mode.

Lesions detected after contrast injection were counted and compared with lesions detected in fundamental mode.

141 focal liver lesions were identified in classic ultrasonography and 163 lesions after contrast injection.

In 39% of cases no lesions was detected with both modalities. In 38% patients, the same number of lesions were seen with both modalities. In 9% more lesions were seen after injection. In 4% of cases no lesions initially detected. In 8% cases, lesions seen in normal Sonography disappeared after injection - were focal steatosis or small hemangiomas.

In 13% of cases, injection of ultrasound contrast agent improved the detection of focal liver lesions.

Injection also enhanced diagnostic in case of focal steatosis.
Inflammation causes G2-M arrest and apoptosis in colorectal cells which depend on MMR proficiency

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Inactivation of the mismatch repair (MMR) pathway may be one mechanism by which chronic inflammation in ulcerative colitis increases the risk for development of colorectal cancer. Here we used an in vitro co-culture system to test for inflammation-induced changes in apoptosis and cell cycle progression in colorectal cells with respect to their MMR status. HCT116 (hMLH1-/-, MMR deficient), HCT116+chr3 (hMLH1wt/-, MMR proficient), Lovo (hMSH2-/-, MMR deficient) and Lovo+chr2 (hMSH2wt/-, MMR proficient) colorectal cell lines were cultured in 6-well plates. Freshly isolated human leucocytes or PMA-activated granulocyte-like HL60 were added 24 h later into the upper chamber that was separated from the target cell by a permeable membrane. Flow cytometry of target cells was done after 24 to 72 hours of co-culture to quantitate apoptosis (Annexin-V staining) or cell cycle progression (PI staining). In all cell lines under investigation, 24 h co-culture with PMA-activated HL60 or freshly isolated leukocytes induced apoptosis at various degrees. MMR-proficient cell lines (HCT116+chr3, Lovo+chr2) showed a significant higher apoptotic response than their MMR-deficient parental cell lines (HCT116, Lovo). As expected, apoptosis was more pronounced with freshly isolated leukocytes than with PMA-activated HL60. Similar effects and an accumulation of apoptotic cells were seen in co-cultures for 48 h and 72 h. Parallel to apoptosis a marked increase in the G2-population and according changes in G1- and S-phase were observed. Again in MMR-deficient cell lines this G2-increase was either smaller (HCT116) or not present at all (Lovo). In this model, inflammation-induced changes cause a G2-M arrest and apoptosis which most likely reflect activation of the MMR system. Such changes are most likely DNA mispairs as a consequence of mutations during replication. Our system will serve as a tool to study the specific processes that are involved in inflammation-associated carcinogenesis.
Serum lipids and fasting plasma glucose levels of patients with colorectal carcinoma

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Introduction: There has long been an argument whether low serum lipids are related to colorectal cancer risk. This hypothesis was derived from the observation of positive relation between colorectal cancers and western dietary. This study was planned to investigate the associations between serum lipids, glucose and other risk factors and colorectal cancer.

Methods: This case-control study was conducted with 330 cases with colonic or rectal carcinoma and 310 age and sex matched healthy controls who were examined for check-up. Blood samples were collected from all the cases and controls after 12 hr fasting; blood was collected one week before surgery so as to avoid measuring serum biochemical parameters during pre-operative bowel preparation and fasting. Total cholesterol, other lipids and glucose levels were determined. Body weight and body mass index were also evaluated. Tumor staging was dependent upon the histological features and the clinical findings at the time of resection. Questions about life style were asked.

Results: The mean serum cholesterol level was 167.1 ± 43.9 mg/dl for cases and 210.1 ± 30.7 mg/dl for controls. There is a significant difference in fasting plasma glucose and serum lipid levels except LDL-C. Serum total cholesterol level was lower in advanced stages of cancer.

Discussion/Conclusion: Our results suggest that there is an association between low serum total cholesterol level and colorectal cancer. Our belief is that low level of serum cholesterol was a consequence of colorectal carcinogenesis. The association with hypertriglyceridemia and high fasting plasma glucose levels suggest the role of hyperinsulinemia in colorectal carcinogenesis.
The effect of the cathepsin B expression on disease progression in gastric cancer

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Background and aim: The objective was to evaluated the role of cathepsin B expression in invasion and tumor progression in advanced gastric cancer.

Material and methods: A standard avidin-biotin immunoperoxidase (Novostain Super ABC Kit mouse) method was used for the detection of cathepsin B (NCL-CATH-B, Novocastra Laboratories Ltd; dilution 1:40) protein expression. The reaction products were visualized with diaminobenzidine DAB (DAKO S3000, DAKO, Poland).

Results: The immunohistochemical expression of cathepsin B in primary tumor and lymph node metastasis was studied in 91 specimens of gastric cancer. Positive staining for cathepsin B in primary tumor was observed in 34/91 cases. The expression of cathepsin B in primary tumor was associated with the presence of lymph node metastasis (p < 0.001) and depth of invasion (p = 0.05). But we have not found such association for expression of cathepsin B in lymph node metastasis. We also have not observed correlation between expression of cathepsin B in primary tumor and lymph node metastasis and sex, localization, Lauren's classification and histological differentiation.

Conclusion: On the bases of our results we can concluded, that expression of cathepsin B is related with lymph node metastasis and depth of tumor invasion in gastric cancer.

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Functional assessment of multidrug resistance in colorectal malignancies and their lives metastases

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Introduction: MDR1 and MRP1 proteins seem to have clinical role, although performed studies revealed contradictory results on their prognostic relevance in CRC.

Our aim was to determine the functional MDR1 and MRP1 activity of colorectal cancers and their metastases.

Methods: Surgical samples were taken from more than 50 primary and more than 30 metastatic colorectal cancers. The samples were brought into one-cell solution and calcein-assay was performed with MDR1 and MRP1 blockers following the protocol described in details at www.solvobiotech.com. Viable epithelial cells were gated and their change in mean fluorescence activity was evaluated with flow cytometry.

Results: Increased ABC-MDR transporter function was found equally in 18% of cases of colorectal cancerous samples and in metastatic cancers. The proportion of CRCs with primarily elevated MDR transporter activity was not different in metastatic and locally spread cancers.

Conclusion: Only a small proportion of patients undergoing surgery without prior chemotherapy had elevated MDR1 and MRP1 activity in the studied disease groups based on cut-off values previously demonstrated to correlate with clinical resistance to chemotherapy. Assessment of transporter function during and subsequent to chemotherapy may be necessary for the objective evaluation of MDR function.
Role of hepatitis and other factors precursor to originating the liver cancer

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The purpose: To learn influencing hepatitis A, B, C and some other factors in originating the liver cancer (LC).

With this purpose the outcomes of 430 patients with the primary LC, which were in the department of abdominal surgery of clinic from 1996 to 2004. the men - 264 (61.4%) and women 166 (38.6%). The age oscillated from 13 till 78 years. The methods of testing included: X-ray inspection of gastrointestinal tract, gastroscopy, ultrasonography, computer tomography, laparoscopy inspection with biopsy, morphological and clinical - biochemical researches.

305 (70.9%) patients among 430 before addressed in medical entity concerning this or that pathology of liver.

The diagnosis histological was established in 408 (95%) patients. From them hepatocellulare cancer is established in 310 (76%) and cholangiocellulare in 96 (24%) patients. T3N1M0 in 96 (22%), T4N0-1M0 in 334 (77.7%) patients. Careful data acquisition on the transferred diseases, kind of work, nature of a feed, the ecological features showed that in 195 (45%) patients in anamnesis took place the transferred different forms o hepatitis, from them hepatitis A in 65 (15.1%) patients, hepatitis B in 105 (24%), which one confirmed also by availability in a blood HBsAg. Hepatitis C in 14 (3.3%) patients. The small number observation hepatitis C is connected that identification of this virus started since 2000. duration of transition of hepatitis in cancer gives from 8 till 26 years.

The alcoholic cirrhosis of liver with transition to cancer is marked in 38 (8.8%) patients. The long and constant contact with agricultural chemicals is found at 42 (9.7%) patients with liver cancer. In 14 (3.2%) patients professional work was connected with manufacturing color products. 85 (19.7%) patients were hospitalized from an ecological ill-behaved zone - Aral sea coastal region, where the poor maintenance of water, reinforced contamination of water and soils by nitrogen-containing agricultural chemicals. Burdened a heredity is established in 8 (1.8%) patients and in 48 (11.2%) patients precursor factors were not established.

Under the data of testing methods from 430 patients for 365 (84.8%) apart from a tumoral lesion in the rest segments are found cirrhotic changes 55.4% and in 44.6% phenomenon of chronic hepatitis, that eliminated a capability of surgical treatment. Only in 65 (15.1%) patients rest segments of liver were in a normal or rather normal condition permitting to execute this or that kind of surgical intervention.

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Combined treatment with chemoembolisation and radiofrequency ablation improves the prognosis of patients with hepatocellular carcinoma

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Introduction: Hepatocellular carcinoma (HCC) is a common and serious complication of chronic liver disease. Despite multimodal treatment including liver resection, chemoembolisation, percutaneous ethanol ablation and systemic therapy with tamoxifen or chemotherapy, the outcome remains unsatisfactory for the majority of affected patients. However, recent studies suggest that radiofrequency ablation (RFA) may improve the prognosis of patients with HCC.

Methods: We applied a newly constructed Cox proportional hazard model (Vienna survival model for HCC = VISUM - HCC) to identify three disease stages with different prognosis. Patients suitable for liver transplantation were excluded.

Results: One hundred nine patients presented in stage 1, 33 patients in stage 2 and 20 patients in stage 3 at the time of HCC diagnosis. Median survival in patients with HCC was 12 months (stage 1), 3 months (stage 2) and 2 months (stage 3). Combined treatment with chemoembolisation and subsequent RFA was performed in 16 patients with stage 1 (median survival 34 months). Further 17 patients with stage 1 were treated by liver resection (median survival 10 months) and 76 patients with stage 1 received chemoembolisation alone or systemic therapy (median survival 10 months). Thus, an improved survival time was observed in the subgroup of stage 1 patients treated with chemoembolisation and RFA.

Discussion/Conclusion: The inclusion of chemoembolisation with subsequent RFA in the multimodal treatment of patients with HCC improves markedly the prognosis in patients with stage 1 according to the Vienna survival model for HCC. These results support the application of the combined treatment of chemoembolisation and RFA in patients with HCC and VISUM stage 1 in larger study populations.
Angiogenin in hepatocellular carcinoma: Correlation with tumor vascularity and proliferative activity

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Introduction: Angiogenesis is an essential process for growth and metastasis of various cancer cells. Since hepatocellular carcinoma (HCC) is a typical hypervascular tumor, the purpose of this study was to evaluate the relationship between angiogenin (ANG) (which is best known for its in vivo angiogenic activity) and tumor vascularity, differentiation and proliferative activity in HCC.

Methods: To clarify this relationship, serum ANG levels were measured in 30 cirrhotic patients with HCC of variable sizes, 30 patients with cirrhosis and 20 healthy subjects using enzyme-linked immunosorbant assay. Tumor vascularity of HCC was assessed by color Doppler sonography in terms of the pulsatile flow pattern, the resistive index, the pulsatility index and the hepatic tumor index. Also, tumor vascularity was histologically quantitated as the intratumor microvessel area (MVA) using a computerized image analysis system in core liver biopsy specimens obtained from 18 HCC patients. Moreover, tumor differentiation classified according to Edmondson's grading system and tumor proliferative activity assessed by proliferating cell nuclear antigen-labeling index (PCNA-LI), were studied in all liver biopsies.

Results: In patients with HCC, the serum ANG levels were significantly higher than those in patients with cirrhosis and healthy subjects and showed a positive correlation with the intratumor MVA (p < 0.05). Moreover, the serum ANG levels and the intratumor MVA were well correlated with Edmondson's grade, PCNA-LI and tumor size in HCC patients (p < 0.05). Among color Doppler measurements, the hepatic tumor index (but not the resistive index or the pulsatility index of tumor vessels) showed direct correlations with serum ANG levels, intratumor MVA, Edmondson's grade and PCNA-LI (p < 0.05). Meanwhile, these parameters showed significant increases in patients with peritumoral and intratumoral pulsatile flow compared with those in patients with peritumoral pulsatile flow only (p < 0.05). On the other hand, there were no significant correlations between serum ANG levels and age, hepatitis virus status, Child-Pugh score and serum alpha-fetoprotein levels in HCC patients (p > 0.05).

Discussion/Conclusion: Excessive ANG production in HCC may contribute to tumor angiogenesis, differentiation and proliferative activity suggesting a potential role for the use of ANG antagonists in the treatment of HCC. Serum ANG may be a useful tumor marker for the assessment of prognosis in HCC.
Introduction: Clinical trials of anti-EGFR agents in treatment of advanced colorectal cancer are currently being conducted, but criteria for patient selection and predictors of response are lacking. The expression and cellular distribution of EGFR in tumours as detected by immunoprofiling does not correlate consistently with response to therapy. The aim of this study was to test the hypothesis that the immunoprofile of EGFR may vary on using antibodies to different domains of the protein.

Methods: We assessed the expression of EGFR in 30 colorectal carcinomas (CRC) by the avidin-biotin immunodetection complex technique using antibodies to the extracellular and cytoplasmic domains of EGFR.

Results: EGFR was expressed in 28/30 CRC, with cytoplasmic expression of varying intensity. 6/28 cases showed membranous expression and 10/28 showed nuclear localisation. In 20/28 cases cytoplasmic localisation was similar using both antibodies. In 2/28 there was no cytoplasmic localisation using the antibody to the extracellular domain and in 6/30 there was a difference in intensity between the 2 antibodies. Membranous localisation was detected mainly using the cytoplasmic domain antibody. Nuclear localisation was detected only on targeting the cytoplasmic domain and was more frequent with advanced tumour grade and stage.

Discussion/Conclusion: This is the first study reporting nuclear localisation of EGFR in sporadic CRC. The significance of nuclear intracellular domain EGFR and its potential as a predictor for response to therapy need further evaluation. Detection of the level of expression and cellular distribution of EGFR varies on using antibodies to different domains of the protein.
Total colonoscopy in colorectal cancer prevention

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Introduction: Italian health system allows all persons aged ≥ 45 years to undergo a free colonoscopy every five years for preventing colorectal cancer. We have evaluated the impact of this policy on the burden of our activity and its efficacy.

Methods: From February 2002 to March 2005 demographic and anamnestic data from all our outpatients submitted to colonoscopy were prospectively collected. The rate of advanced adenomas and carcinomas diagnosed in asymptomatic patients examined for colorectal cancer prevention has been matched with that of neoplastic lesions found in symptomatic patients. Differences among proportions were compared using Chi square test and a value of p < 0.05 was considered statistically significant.

Results: A total of 1250 colonoscopies were performed in the study period on 1142 persons. Of them, 170 (15%; 81 males, mean age 52.3, and 89 females, mean age 50.5) underwent colonoscopy for colorectal cancer prevention, and 972 (85%; 492 males, mean age 54.7, 480 females, mean age 55.5) because of symptoms. Only one case of adenocarcinoma was found in the prevention group versus 59 in the symptomatic group: 1/170 (0.6%) versus 59/972 (6%); p < 0.002. Advanced adenoma was found in 165 patients, 15/170 (8.8%) in the prevention group and 150/972 (15.4%) in the symptomatic group (p = n.s.). Complications after polypectomy had an incidence < 1% and were resolved conservatively, with endoscopic therapy.

Discussion/Conclusion: Our data show that prevention colonoscopy, at the present time, represents about 15% of the work load. The finding that 10% of persons undergoing prevention colonoscopy had an adenoma, together with the diagnosis of one cancer case, confirms that this procedure is crucial to early detect neoplastic lesions of the colon. Among these, carcinoma could be diagnosed in a more easily curative stage, and advanced adenoma may be removed with a minimal incidence of complications.
The Czech national program of colorectal cancer screening

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Introduction: Czech Republic has the highest incidence of colorectal cancer (CRC) worldwide (79.4 per 100,000 inhabitants of general population in 2002). The arrangement and results of the national screening program in the years 2001-2004 are reviewed.

Methods: The program was started on July 1st 2000. Screening is performed as part of the free-of-charge preventive check-up at 2-years interval. Asymptomatic persons since 50 years of age are eligible. The guaiac fecal occult blood test (FOBT, 3 slides) is the initial method. The tests are developed by general practitioners (GPs) at their offices. In FOBT-positive subjects total colonoscopy is recommended. The individual interview of the GP with the eligible person is the most important procedure in recruiting probands.

Results: 20% of GPs accepted fully the program. The remaining GPs perform screening to a lesser extent. Indirect data at half-year intervals display in comparison with the prescreening period: huge increase of FOBT use (up to values 19-times higher), increase of total colonoscopies (+75%), endoscopic polypectomies (+169%), and curative colectomies (+10%). Direct data: In 2004 the proportion of procedures and findings due to positive FOBTs amounted to: total colonoscopy 10.7%, CRC 16.7%, polyps 28%.

Discussion/Conclusion: The requirements of FOBT-based population CRC screening include: 1. the key-role of GPs and their associates, 2. motivation and education of health professionals and the public, 3. continuous and intense media campaign addressing both the public and the professionals, 4. constitution of a managing body of medical representatives and health care system authorities aimed at monitoring, co-ordination, and evaluation of the program.
Radiofrequency ablation of liver metastases from colorectal cancer

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Aim: To assess the effectiveness of radiofrequency ablation (RFA) in the treatment of unresectable liver metastases from colorectal cancer (CRC).

Methods: 87 patients with 139 metastases from CRC (age 29-81, tumor size 0.5-11 cm) were treated with percutaneous RFA under i.v. or general anesthesia in a 4-year period. 1-3 sessions per lesion were performed with 20-130 min duration per session. Overall 2.7-7.5 kJ/ml neoplastic tissue were applied with overlapping insertions of the electrode. The hospital stay was 1-3 days after RFA. The achieved destruction was assessed using power-Doppler, contrast-enhanced Doppler, CT and cytological analysis. Patients were followed-up for 3-37 months (mean 14 months). The recurred lesions were treated when possible.

Results: Complete destruction was achieved in 88.3% of metastases < 3.0 cm (83/94), in 57.6% of lesions sized 3.0-5.0 cm (19/33) and in 16.7% of tumors > 5.0 cm (2/12). Major complications occurred in 8% of the patients (abscesses, biliary fistula with peritonitis, cholecystitis, seeding, non-Q myocardial infarction), with no-mortality. The local recurrence rate was 34.6% (23 small, 11 medium and 2 large lesions). The 1-year survival was 87.3% (76/87).

Discussion/Conclusion: The effectiveness of RFA in CRC liver metastases depends on the size of the lesions. Frequency of intrahepatic recurrence increases with the tumor size. Future studies are needed to confirm the benefit of post-RFA chemotherapy in patients with medium-sized and large metastases.
Analysis of molecular markers related to progression of colorectal carcinoma

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Introduction: The aim of this study was to determine the incidence of KRAS and BRAF mutations in a series of colorectal carcinomas obtained from patients of the National Oncological Centre Hospital in Sofia, Bulgaria. In addition, the expression of the cystatine-like metastasis-associated protein (CMAP) was assessed in the same tumours.

Methods: PCR-RFLP assays were used for tracking point mutations of the KRAS oncogene at codons 12 and 13, and for detecting mutations of BRAF codon 599. In addition, a multiplex RT-PCR was used for detecting the expression of CMAP and that of GAPDH, used as control.

Results: Tumour tissues were examined from 23 patients (9 females, median age: 61.5 years, and 14 males, median age: 63.2 years) with colorectal carcinoma. The tumours were derived from the colon (n = 8) and the rectum (n = 15). KRAS mutations were found in 8 of 23 patients (codon 12: n = 7, 30%; codon 13: n = 1, 4%). BRAF mutations were found in 3 of 23 tumour samples (13%), all of which were KRAS wild type. Seven of 23 tumour samples showed a 1.5- to 2-fold increase in CMAP expression. There was no obvious relationship between CMAP expression and KRAS/BRAF mutation status.

Discussion/Conclusion: We conclude that CMAP expression is a prognostic factor that is independent of KRAS/BRAF mutation status.
Genetically induced pancreatic adenocarcinoma is highly immunogenic and causes spontaneous tumor-specific immune responses

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Introduction: Treatment options for pancreatic cancer are limited and often ineffective. Immunotherapeutic approaches are one possible option that need to be evaluated in appropriate animal models. The aim of the present study was to analyze tumor-specific immune responses in a mouse model of pancreatic cancer, which mimics the human disease closely.

Methods: C57BL/6 TGF-α Trp53-/- mice were generated. Tumor-specific CD8+ T cell responses and IgG responses were analyzed in TGF-α Trp53-/- during tumor development and compared to mice with subcutaneously growing pancreatic tumor. Intratumoral cytokine secretion was determined and adoptive T cell transfer into tumor bearing mice was performed using tumor-specific lymphocytes.

Results: TGF-α Trp53-/- mice developed spontaneous pancreatic tumors with pathomorphological features close to the human disease. Spontaneous tumor-specific CD8+ T cell and IgG responses were detected in these mice. In contrast to spontaneous pancreatic tumors, cell lines generated from these tumors were rejected after subcutaneous injection into wild-type mice but not in nude or RAG knockout mice. Direct comparison of spontaneous tumors and subcutaneously injected tumors revealed an impaired infiltration of CD8+ T cells in spontaneous pancreatic tumors, which was also evident after adoptive transfer of tumor-specific T cells.

Discussion/Conclusion: Our data provides clear evidence for a tumor-specific immune response in a genetic mouse model for pancreatic carcinoma. Comparative analysis of subcutaneously injected tumors and spontaneous tumors demonstrated significant differences in tumor-specific immune responses, which will help in improving current immune-based cancer therapies against ductal adenocarcinoma of the pancreas.
Introduction: It has already been well established that angiogenesis is crucial in the process of tumor progression and metastasis. The aim of the current study was to assess the microvessel density (MVD) and the number of mast cells (MCs) in primary colorectal cancers and to elucidate their prognostic significance for survival of the patients after the surgery.

Methods: Histochemistry with toluidine blue (TB) and immunohistochemistry (IHC) with antibodies recognizing MC tryptase and CD 31 (PECAM) were used for evaluation of MCs and blood vessel in the "hot" spots in the invasive front of 89 biopsies obtained from 47 patients with primary colonic and 42 with rectal cancers.

Results: With IHC for MC tryptase there were detected significantly higher numbers of MCs than with TB-staining (40.5 vs. 14.5 in p < 0.0001, paired t-test). The MVD varied between 13 to 187 vessels/mm² with a mean value of 65.9 and median of 54 blood vessels/mm². Strong (r = 0.613) and moderate (r = 0.466) statistically significant correlations were observed between the MVD and MCs detected by IHC and TB-staining, respectively (p < 0.0001, linear regression analysis). The survival analyses showed that patients with higher numbers of MCs stained with IHC for MC-tryptase had worse prognosis than those with smaller numbers (p = 0.009, log-rank test). Analogous strong association was observed between the higher MVD and shorter survival of the patients after the operation (p < 0.0001, log-rank test).

Discussion/Conclusion: In conclusion we suggest that the number of mast cells and microvessel density assessed by IHC for mast cells tryptase and CD31, respectively, are valuable prognostic factors for patients' survival.
Claudins in esophageal neoplasms and Barrett’s esophagus

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Introduction: Claudins (CLDN) are the main transmembrane proteins of tight junctions (TJ), key molecules participating in cell adhesion and polarity. Several studies suggested that changes in CLDN expression pattern might play role in carcinogenesis. The aim of the present study was to investigate the changes in the pattern of CLDN 1, 2, 3, 4 and 7 expression of the Barrett’s esophagus (BE) and adenocarcinoma (ACC) in comparison to foveolar glandular epithelium (FOV).

Materials and methods: Formalin fixed, paraffin embedded samples of 25 human BEs, 25 ACCs and 25 glandular epithelia from the lower esophageal tract were studied by immunohistochemistry. Antibodies for CLDN 1, 3, 2, 4, and 7 were analyzed and scored for areas of positive cells. Significant alterations were analyzed for mRNA expression by Real-Time RT-PCR, using relative quantification with GAPDH as internal control.

Results: CLDN1 and 7 were highly expressed in all groups without significant differences. CLDN 3 and 4 were increased in BE and ACC compared to FOV (p = 0.0022, p = 0.0064, p = 0.0107). CLDN2 was higher in ACC than in BE and FOV (p = 0.0001, p = 0.0022, resp).

Conclusion: CLDN 3 and 4 show increased expression in BE and ACC, which differentiates these lesions from FOV. In addition elevated CLDN 2 expression is characteristic to the carcinoma stage. Similarities and differences of CLDN pattern in BE and ACC might indicate a sequence of molecular alterations in carcinogenesis, and support the view that BE is a precancerous stage of ACC.

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Inhibition of RICK/NF-κB and p38 signaling attenuates intestinal inflammation in a murine model

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Introduction: Studies indicate that NF-κB is essential for promoting inflammation-associated cancer, and a potential target for cancer prevention in chronic inflammatory diseases (Nature 2004; 431: 461-6). Our study investigates the molecular anti-inflammatory mechanisms of SB203580, an inhibitor of both, the MAP kinase p38 and RICK. RICK has been shown to interact with IKK and subsequently leading to NF-κB activation.

Methods: The murine TNBS-induced colitis and DSS-induced colitis were used as established models for human intestinal inflammation.

Results: We show that SB203580 improved the clinical condition, reduced intestinal inflammation, and suppressed mRNA levels of pro-inflammatory cytokines. Besides p38 kinase activity, the "classical" IκB-dependent NF-κB pathway was strongly up-regulated during colitis induction in both models, whereas the "alternative" was only in the DSS-colitis model. The molecular analysis of NF-κB activation revealed that the inhibitory effect of SB203580 on NF-κB activation is to a large extend mediated by RICK inhibition.

Discussion/Conclusion: RICK is the effector kinase of the intracellular receptor of bacterial peptidoglycan NOD. Because bacterial products are suggested to be an important pathogenic agent triggering chronic inflammatory intestinal conditions (e.g. ulcerative colitis, Crohn disease) leading to a greatly increased risk of colorectal cancer, inhibition of the NOD/RICK pathway may serve as a novel target of NF-κB-inhibiting and therefore cancer- preventive therapy with little side effects.
Iron and nutritional status of Helicobater pylori-infected children

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Introduction: H. pylori infection has been reported to be associated with various extradigestive manifestations in childhood. The aim of this study was to assess the relationship between H. pylori infection and iron and nutritional status in children, correlated with socio-economic factors.

Methods: 359 consecutive children (244 girls; mean age 12.9; range 3-18 years) underwent upper endoscopy, predominantly for dyspeptic symptoms. All had urease test and histological examination for H. pylori infection. Health and dietary history, anthropometrical data and socio-economic status were analysed. Known possible causes for iron deficiency anaemia were excluded.

Results: Of the 359 children, 179 (49.86%) had documented H. pylori infection (sex ratio F/M 1.75; mean age 13.2 years; 72.5% with low socio-economic status). The incidence of H. pylori increased with age from 11.73% for 3-6 years to 42.45% for 15-18 years. H. pylori colonization was inversely correlated with socio-economic status (p < 0.005). Iron deficiency anaemia was found most frequently in infected patients (43.8%) compared with uninfected ones (19.5%), p = 0.01. Infected children with iron deficiency had lower height for age (p = 0.006) compared with those with H. pylori infection alone. The differences between the H. pylori-positive and -negative patients were less significant for BMI.

Discussion/Conclusion: H. pylori is associated with iron deficiency anaemia and both, coupled with socio-economic factors, can be considered as a cause of growth deficit. This affects particularly the height, especially in older and disadvantaged children. The clinical evaluation of iron deficiency anaemia must also include diagnostic test for H. pylori infection.
Erosive esophagitis in children: A clinicopathological study

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Introduction: Erosive esophagitis (EE) is a severe esophageal manifestation of gastroesophageal reflux disease because it may be a precursor of Barrett's oesophagus. This study aims to assess prevalence and clinicopathological findings of EE in children older than 3 years without neurological disease or other risk factors hereto.

Methods: We reviewed the clinical, endoscopic and histological reports of all children aged 3-18 years, who underwent upper endoscopy (EGD) in our university hospital during the past 4 years. Endoscopic grading of esophageal mucosa was performed using the Hetzel and Dent criteria. Esophageal biopsy was taken only in the presence of visible mucosal architecture alterations. H. pylori status was assessed in all children.

Results: Of the 359 children who underwent EGD predominantly for dyspeptic symptoms, 57 (15.87%) had EE (ratio F/M 1.85, mean age 12.9 years). The prevalence of EE increased with age from 10.09% for 3-6 years to 46.63% for 15-18 years. The most common symptom was epigastric pain (71.92%). Hiatal hernia was found in 36.84% of children with EE, compared with those without (8.2%), p < 0.0001. H. pylori was diagnosed in 31.57% of patients with EE, compared with those without (49.86%). All children with EE had reflux esophagitis on histology. Specialized intestinal metaplasia was identified in 15.78% cases.

Discussion/Conclusion: Erosive esophagitis is an endoscopic finding observed frequently in children undergoing EGD which must be considered a predictive factor for histologic esophagitis. Its prevalence increases with age. Hiatal hernia is a predictive factor for erosive esophagitis. Barrett's esophagus is unexpectedly frequent in adolescents with severe esophagitis.
Chemopreventive mechanisms of 5-aminosalicylic acid in colorectal cancer: Cell growth inhibition through mitotic arrest and apoptosis

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Epidemiological studies suggest that 5-aminosalicylic acid (5-ASA) has cancer-chemopreventive properties in vivo, e.g., 5-ASA medication in ulcerative colitis is associated with a reduced colorectal cancer incidence. Due to its few and relative mild side effects 5-ASA is therefore an attractive candidate in a chemopreventive/treatment strategy to colorectal cancer. Inhibition of proliferation and induction of apoptosis are two widely recognized mechanisms of action of such agents. Our studies aimed to evaluate 5-ASA's potential on these features to colorectal cancer cells in vitro, within a physiological achievable range. 5-ASA was found to inhibit cell growth at low concentrations (< 35 mM), and reduced cell numbers to even below starting values at higher concentrations (> 35 mM) in HT29, Colo205 and Caco2 cells (IC$_{50}$ range 17-34 mM). Interestingly, 5-ASA enemas (2 and 4 g) had similar effects at the same 5-ASA concentration range (IC$_{50}$ 27-32 mM). Microscopic examination showed that 5-ASA induced cell arrest in the mitotic phase, with condensed chromosomes dispersed throughout the cytoplasm. This mitotic arrest did lead to a significant cell growth inhibition after 72 hours. Replating 5-ASA-treated cells in control medium revealed that this arrest was fully reversible, i.e., similar growth as non-treated cells. Isolating the mitotic cells and replating in 5-ASA showed that all mitotic phases are increased (also binucleated cells) and that there is no block at a particular mitotic phase. Features of mitotic catastrophe, i.e., giant cells containing micronuclei or multilobular nuclei, were frequently seen after arresting the cells in mitosis for longer time at higher dose. A dose-dependent increase in apoptosis by 5-ASA was also found, as assessed by immunohistochemical detection of active caspase-3 and a caspase-cleavage product of cytokeratin 18 (M30), in addition to the classical morphological features of apoptosis, like chromatin condensation and cytoplasmic shrinkage.

These observations clearly indicate that prolonged exposure to higher doses of 5-ASA, also in enema formulation, induces mitotic arrest, slower growth, apoptosis and cell death in colorectal cancer cells in vitro.
Local recurrence following high vs. low anterior resection for rectal cancer

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Introduction: The Dutch rectal cancer study demonstrated a significant reduction in local recurrence rate in the TME-plus preoperative short-course radiotherapy group compared to the TME-alone group. However, high rectal cancers greater than 10 cm from the anal verge did not show the same benefits in terms of reduced local recurrence compared to low rectal cancers. To explain this variation, we analyzed the clinical and histological features of rectal cancers treated with curative intent by either high anterior resection (HAR) or low anterior resection (LAR).

Methods: Patients undergoing either HAR or LAR were identified from a prospective database. Clinical details (age, sex, tumour site, tumour size, tumour fixation, and distal resection margins) and histological features (tumour differentiation, T-stage, N-stage, venous and perineural invasion) for the 2 groups were subjected to univariate analysis. Predictors for local recurrence were identified using binary logistic regression modelling.

Results: 861 patients with rectal cancer were analysed, 415 of which had undergone HAR and 446 LAR. Local recurrence occurred in 7.7% and 7.6% of the HAR and LAR groups respectively, despite tumours in the LAR group being associated with worse prognostic features on univariate analysis. T-stage and distal resection margin were identified as independent predictors of recurrence in the HAR group, and tumour fixation and differentiation as independent predictors in the LAR group.

Discussion/Conclusion: Cancers of the upper and lower rectum differ in their clinical and histological features. Separate predictors for local recurrence following HAR and LAR suggest that different factors are involved in local recurrence in the two groups.
Importance of long-term observation of patients with ulcerative colitis considering the occurrence of colorectal carcinoma

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Introduction: Chronic course of inflammatory bowel disease manifests by recurring relapses with relatively high occurrence of complications. The most serious complications include colorectal carcinoma (CRCa) in patients with long-term ulcerative colitis (UC) of extensive type.

Methods: In 1995-2004, 63 patients with UC of extensive type lasting longer than 10 years were observed within the follow up of the 2nd Internal Clinics of the Palacky University Medical Faculty. In the whole set, colonoscopic examinations were carried out in regular two-year (or shorter) intervals with follow-up biopsy and histological examination focused on the occurrence of dysplastic changes and (CRCa).

Results: In 4 patients CRCa was found as complications of long-term course of UC, in 35 patients, dysplastic changes have been detected and monitored their development and seriousness. In 24 persons, no dysplastic changes have been found during the whole period of observation.

Discussion/Conclusion: Regular colonoscopic examinations should be commenced within 10 years from the date when the UC of extensive type was found. If no dysplastic changes are found during biopsy, we continue in endoscopic examinations in two-year intervals. When dysplasia of low grade is found, we perform another colonoscopy with the follow-up biopsy within one year. High-grade dysplasia is also an indication for colectomy as CRCa is found in the resectate in 40% of cases.
The analysis of presence of H. pylori infection in patients with gastric cancer based on the material examined in years 2002-2004

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**Introduction:** Gastric cancer is the second leading cause of cancer deaths worldwide. One factor associated with it is infection with the H. pylori. It has become evident that the presence of H. pylori increases risk for development of adenocarcinomas of the stomach. We analyzed the association of H. pylori infection with the gastric cancer using the histochemical method of detection of bacteria in the tissue samples.

**Methods:** We obtained the parrafin blocks and clinical data of 178 patients with gastric cancer examined in years 2002-2004 from our department. Patients were 24-96 years of age (mean 67.5; 63 women and 115 men. The samples were obtained either from the biopsy (77 patients; 43.25%) or from the total gastrectomy (101 patients; 56.74%). The slides were stained with H+E and Warthin - Starry to visualized H. pylori.

**Results:** H. pylori was present in 76 of 99 (54.45%) patients with the intestinal type of carcinoma according to Lauren's classification, in 29 of 38 patients (78.3%) with diffuse type and in 29 of 50 patients (69.04%) with mixed type. Total number of patients with positive reaction for the presence of H. pylori was 153 (85.9%). In 8 cases (5.2%) the blood test for the presence of H. pylori was negative.

**Discussion/Conclusion:** These data indicate that there is an association of the gastric cancer and the H. pylori infection in examined material in our region. We may suggest that there should be histochemical examination of the presence of H. pylori in the mucosa done in each examined gastric biopsy.
Chronomodulated administration of capecitabine reduces side effects of therapy in patients with colorectal cancer

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Introduction: The oral fluoropyrimidine capecitabine was developed as prodrug of 5-FU with the goal to improve tolerability. However, still common dose-limiting adverse effects associated with capecitabine monotherapy exist as diarrhea, hand-foot-syndrome, myelosuppression, fatigue, weakness and nausea. As shown previously chronomodulated infusion-regimes of 5-FU that consider the circadian rhythm were better tolerated. Therefore, the purpose of our study was to investigate whether chronomodulated administration of capecitabine would also reduce toxicity of the drug.

Methods: 27 patients with advanced colorectal cancer were randomized to receive capecitabine (2500 mg/KOF daily) either in standard administration with 50% of the doses in the morning and 50% in the evening separated by 12 hours or as chronomodulated dose-regime with 25% morning-doses and 75% late evening-doses. Treatment was continued until thirteen treatment-cycles were finished or progress of cancer or limitation of therapy due to side effects occurred.

Results: Overall, response rates to chemotherapy were similar in both treatment groups (p = 0.296). However, within the group of patients with chronomodulated application of capecitabine, reduction of drug-doses due to side effects was less frequently necessary (14.8 vs. 19.3%, p = 0.026) and more patients were able to finish all thirteen chemotherapy-cycles (41.7 vs. 7.1%, p = 0.007). While there was no statistically significant difference in the frequency of hand-foot-syndrome in both treatment-arms (51.7 vs. 41.5%, p = 0.119) other side effects as nightly nausea, tiredness and sleeplessness were significantly reduced in patients receiving chronomodulated therapy (p = 0.035, p = 0.001 and 0.009, respectively). Additionally, there was a trend to lower frequency of nausea, diarrhea and stomatitis within this group compared to standard treatment (9.0 vs. 19.5%, 19.1 vs. 25.6%, and 4.5 vs. 8.5%)

Discussion/Conclusion: The chronomodulated capecitabine schedule achieved similar response rate and better tolerability regarding various side effects compared with standard regimens in patients with colorectal cancer and enabled patients to sustain on capecitabine therapy for a longer time-period.

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The usefulness of immunohistochemical methods in prognosis and follow-up of patients with colon cancer

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The aim of this study was evaluation of (immunohistochemical) IHC methods as predictors of post-surgery therapy in colon cancers.

The studies were done on 89 patients (33 women and 56 men) aged from 31 to 83 years (mean 58.74 years), who undergo surgery because of colon cancer. The follow-up lasted 10 years. In all cases we performed routine histological studies as well as IHC localization of CEA, Nm23, CA19.9, bcl-2, c-ERB-2, Ki-67, PCNA, p53, UEA-I in paraffin slides applying ABC/HRP method. The results were also studied using statistical methods based on the theory of fuzzy sets.

Application of statistical methods allowed for reduction of sets of attributes only for those attributes which were significant. IHC significant attributes included: Ki-67, Nm23, UEA-I, CA19-9, PCNA. Using statistically significant attributes decision-making algorithms with determining rules were developed. They described relations between IHC studies results, selected clinical data and patients' survival. Significant attributes in this comparison included: primary tumor localization, TNM classification, age, sex, level of maturation of tumor cells, Dukes' classification, c-ERB-2, bcl-2, Nm23, UEA-I.

Relations between IHC results and level of tumor cells maturation (significant attributes: UEA-I, Nm23, Ki67, CA19.9, p53, CEA) and tumor localization (significant attributes: Nm23, UEA-I, p53, Ca19.9, CEA, PCNA) and histological diagnosis (significant attributes: UEA-I, Nm23, p53, CA19.9, c-ERB-2). Developed decision-making algorithms could be used by clinicians for selection of patients for post-surgery adjuvant chemotherapy. It is extremely important for patients with high risk of cancer recurrence but previously included in groups with good prognosis, and for patients with low risk of cancer recurrence but previously placed in group with bad prognosis. However, analysis of decision-making algorithms gave no clear-cut model for our results, which suggests influence of other not yet defined attributes.

Conclusion: IHC studies allow for developing of decision-making algorithms, that could be eventually used by clinicians for selecting patients for adjuvant post-surgery therapy.
Conjugated linoleic acid (CLA's) isomers reduce colonic tumour number but mixtures increase tumor size in a mouse model of intestinal cancer

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Introduction: Dietary conjugated linoleic acids (CLA) are involved in fat metabolism and may have a role in the (prevention) of intestinal neoplasia and colitis. The predominant forms of CLA are the cis-9, trans-11 (c9t11 present in ruminant products) and trans-10, cis-12 isomers (t10c12). The effects of various forms of CLA were investigated in multiple intestinal neoplasia (Apc\textsuperscript{Min/+}) mice, which have a mutation of the APC gene, equivalent to FAP in man.

Methods: Five-week-old Apc\textsuperscript{Min/+} mice were fed a control diet or diets with 1% of c9t11, t10c12 or a mix of the isomers (n = 20 per group). After 28 days the mice were killed and the intestines opened, mounted on card and fixed in Carnoy's. The number and size of polyps in the small and large intestines were scored. Cell proliferation and crypt fission were also determined.

Results: t10c12 and the mix significantly reduced polyp number in the proximal small intestine (p < 0.001). However, t10c12 and the mixture increased polyp diameter by 20% (p < 0.05 to 0.001) and this was reflected in the polyp burden which was significantly increased (p < 0.05). All the CLA's reduced polyp number in the colon (from 9.1 ± 1.6 to 4.4 ± 0.7, 3.6 ± 0.8 and 3.0 ± 0.6, p < 0.01) but the mixture significantly increased polyp diameter in the colon.

Discussion/Conclusion: CLA's could reduce polyp number, especially in the colon, however t10c12 and the mixture (the commercially available version) increased polyp diameter and thus burden. c9t11 did not alter diameter, lending weight to the suggestion that this natural isomer of CLA is the more likely to be beneficial.
Flow cytometric analysis of T-lymphocyte subsets in human liver from patients with colorectal cancer

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Introduction: Tumor infiltrating lymphocytes (TIL) play an important role in antitumor immunity. The data about TIL in liver metastases are scarce.

Aim: In the present study we examined the three main intrahepatic lymphocyte subsets - CD3⁺ CD56⁻ conventional T-lymphocytes, CD3⁺CD56⁺ natural killer (NK) lymphocytes, and CD3⁺CD56⁺ NKT lymphocytes in patients with colorectal cancer. In addition CD4⁺ and CD8⁺ cells were investigated as well.

Methods: Twenty two patients were studied (10 patients with and 12 without liver metastases). Fresh liver samples were obtained after surgical resection of metastatic or liver tissue for diagnostic or curative purpose. The proportion of each hepatic lymphocyte subset was evaluated by flow cytometry after lymphocyte isolation by density centrifugation over Ficol gradient. Frozen liver sections were examined immunohistochemically using anti-CD4, CD8, and CD56 monoclonal antibodies.

Results: Immunohistochemically CD4⁺, CD8⁺, and CD56⁺ cells were gathered at the periphery of metastases. Flow cytometry revealed a dominance of CD8⁺ lymphocytes with a lower number of CD4⁺ lymphocytes in both patients groups. The mean percentage of T, NKT, and NK lymphocytes in liver tissue was as followed: 35.4, 13.1, and 11.7 around metastasis and 31.1, 23.9, and 17.5 in liver tissues without metastases, respectively. NK and NKT lymphocytes in livers without metastases were more as compared to those with metastases.

Discussion/Conclusion: The decreased number of NK and NKT lymphocytes in liver around metastases might be responsible for their development. A suppression of the innate immune system could be a reason for the escape of metastatic cells from liver sinusoidal tumor surveillance.
Bleeding jejunal GIST diagnosed by capsule endoscopy: Report of a case

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In the majority of gastrointestinal bleeding episodes, the source of bleeding is located in regions that can be reached by standard upper gastrointestinal endoscopy or colonoscopy. However, approximately 5% of bleeding sites are thought to be located in the small bowel, a proportion that increases in those patients classified as obscure gastrointestinal bleeding (OGB), because standard endoscopic examinations of the upper and lower gastrointestinal tract are negative. The development of the capsule endoscope (M2A; Given Imaging Ltd., Yoqneam, Israel) has, for the first time, allowed simple and safe examination of the entire small intestine. 26-year-old male was admitted to University Clinic for gastroenterology due to painless melaena in last three months. He was dependent on frequent transfusions of packed red cells. Previous examinations included upper endoscopy, enteroscopy, colonoscopy as well as barium follow-through did not reveal the source of bleeding. Angiography was negative, too. The second-look colonoscopy and enteroscopy were inconclusive. Finally, capsule endoscopy was done and disclosed subepithelial mass in the distal jejunum, around 15 mm in the diameter. The tumor was round shaped, well defined and covered with the normal appearing mucosa. The tumor was spontaneously bleeding by oozing. On surgery, that finding was confirmed and the tumor was resected on. The histology and immunochemistry revealed the benign GIST (gastrointestinal stromal tumor). The recovery was complete, and a year after the operation, the patient is symptom free. Capsule endoscopy has become the investigation of the choice for the patients with GI bleeding of obscure origin, either overt or occult with iron deficiency anemia.
Etiology of gastric stump cancer

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Introduction: Gastric stump is considered to be an important pre-cancerous lesion in gastric cancer.

Aim: Endoscopical evaluation of the patients with gastric stump; we tried to find some predictive factors for gastric stump cancer.

Methods: Between first January 2004 and first January 2005 we performed upper digestive endoscopy in 206 patients with gastric resection for duodenal or gastric ulcer.

Results: There were 62% men and 38% women, aged between 26 and 84 years. The main symptoms were: abdominal pain, anemia or upper digestive bleeding, nausea and vomiting, weight loss. The histological diagnostic of GSC was confirmed in 16 cases (8%). We compared the patients with GSC (lot A) with those with other lesions (stump gastritis, peptic ulcer etc) - lot B. GSC was more frequent in men; the time between gastric resection and diagnosis was about 14 years for A and 6 years for B. Hemoglobin < 8 g% and upper digestive bleeding are the most important predictive factors for GSC. Helicobacter pylori infection was present in 3 patients in A group (18.7%) and in 87 B-patients (45.78%).

Discussion/Conclusion: The time over 10 years, anemia, upper digestive bleeding and not Helicobacter pylori seem to be the most important predictive factors for GSC.
Methotrexate induces intestinal inflammation and mucosal barrier dysfunction which is not ameliorated by ethyl pyruvate

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**Introduction:** Methotrexate (MTX) is a chemotherapeutic agent which when administered parenterally induces acute intestinal injury. Ethyl pyruvate (an anti-oxidant agent) has been shown benefit in a number of in vivo models of intestinal injury. The aim of this study was to investigate the effects of ethyl pyruvate in an animal model of methotrexate-induced mucositis.

**Methods:** Adult male Sprague-Dawley rats were randomised to one of five groups (n = 16); group 1 - saline only (sham chemotherapy); group 2 - MTX plus Ringer’s lactate solution (treatment control); group 3 - MTX plus 20 mg/kg ethyl pyruvate (EP); group 4 - MTX plus 40 mg/kg EP; and group 5 - MTX plus 60 mg/kg EP. Mucositis was induced by the administration of 5 mg/kg MTX daily by subcutaneous injection for three consecutive days, treatment was given for the same time period. The experiment was ended on day five when the following parameters were assessed; intestinal permeability to $^{14}$C-labelled polyethylene glycol 4000, small bowel histological injury score, mucosal myeloperoxidase activity and serum endotoxaemia (using EndoCAb assay).

**Results:** Rats treated with methotrexate demonstrated significantly increased gut permeability ($p = 0.027$), increased histological injury score ($p < 0.0001$), increased mucosal myeloperoxidase activity ($p < 0.0001$) and decreased serum EndoCAb concentrations ($p < 0.0001$) when compared to sham animals. Compared with treatment controls, the treatment of rats with ethyl pyruvate did not significantly ameliorate the intestinal hyperpermeability, histological injury, mucosal myeloperoxidase activity and the consumption of EndoCAb.

**Discussion/Conclusion:** Methotrexate induces small bowel mucositis which is not ameliorated by the administration of ethyl pyruvate solution in this model.
Hepatitis viruses and hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is a frequent malignant tumor worldwide that is associated with chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection. Its incidence in Western countries has significantly increased during the last decades and is now in the range of 10-30 cases per 100,000 population and year.

The increase in HBV and HCV induced liver cirrhosis accounts for the high HCC incidence: HCV related HCC incidence has increased about three times from 2.3 per 100,000 population and year during the period from 1993-1995 to 7.0 per 100,000 population and year during the period from 1996-1998. During the same period HBV related HCC incidence has increased only slightly from 2.2 to 3.1 and alcohol-related HCC incidence from 8.4 to 9.1 per 100,000 population and year.

Apart from the development of novel therapeutic strategies, prophylactic and therapeutic measures aimed at the prevention of hepatitis or its progression from acute to chronic hepatitis or from chronic hepatitis to cirrhosis and HCC are of high clinical priority. In this context existing measures of primary prophylaxis should be implemented: vaccination against hepatitis B, prevention of HCV transmission, cessation or reduction of alcohol consumption, early detection and treatment of inherited liver diseases, such as hemochromatosis and Wilson's disease, as well as early and effective treatment of chronic hepatitis B and C.

Indeed, HCC incidence has already declined in children after the beginning of the program for newborn vaccination. Based on this scientific basis, in Turkmenistan there was a program for newborn vaccination developed and implemented with HBV vaccination of all newborns since January 2002. Through this program it should be possible to reduce the incidence of HBV infection in Turkmenistan and at the same time the morbidity and mortality associated with HBV-induced chronic hepatitis, cirrhosis and HCC. An expansion of the HBV vaccination program to other risk groups for HBV infection is expected.
High-density microarray analysis of inducible regulatory T-cells - Identification of new targets for anti-tumor immunotherapy

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Introduction: Regulatory T-cells (Tregs) can dampen the immune response towards malignant cells. Recent data suggest a selective accumulation of Tregs at sites of neoplasms. Recent findings of our group suggest that tumor derived TGF-beta may locally induce Tregs (TiTregs) from infiltrating T-cells. The molecular mechanisms giving rise to TiTregs are poorly understood. To address that topic, we have profiled the gene expression patterns of TiTregs by highdensity microarray analysis.

Methods: CD4+CD25- cells were isolated from spleens from balb/cJ mice. Cells were stimulated and grown in the presence or absence of TGF-beta for 5 days. Whole genome analysis was performed by high-density microarrays (Affymetrix). Validations of differential gene expressions were carried out by quantitative PCR resp. FACS.

Results: We have identified more than 350 genes that were differentially expressed with a significance level of p < 0.05 and an increase resp. decrease of > 4-fold in TiTregs. About three fourth of the genes showed lowered expression levels. Many cytokines and their receptors belonged to the most repressed genes (up to 200-fold). In contrast, FoxP3 as the master regulator of Tregs was induced 50-fold and ranked among the most increased genes. In general, the expression profile of TiTregs showed similarities to phenotypes reported in association with T-cell quiescence.

Discussion/Conclusion: The further evaluation of differentially expressed genes in TiTregs can help to shed light upon the mechanisms of Treg induction by tumors and might reveal new targets for anti-tumor immunotherapy.
High level of CA 19-9 in a patient with combined hepatocellular and cholangiocarcinoma

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Introduction: Combined Hepatocellular and Cholangiocarcinoma (CHCC) is a rare subtype of primary hepatic carcinoma. Hepatitis B virus is one of the most important factors in the etiology of hepatocellular cancer and cholangiocarcinoma.

Case: A 63-year-old man was admitted to the hospital because of abdominal pain and weight loss during the last two months. On physical examination, there were hepatomegaly and splenomegaly. Blood chemistry studies yielded the following: alanine aminotransferase 60 U/l, aspartate aminotransferase 298 U/l, gamma-glutamyltransferase (GGT) 396 U/l, alkaline phosphatase (AP) 1729 U/l, total bilirubin 0.96 mg/dl, direct bilirubin 0.34 mg/dl, and prothrombin time 16.2 seconds. The patient was positive for hepatitis B surface antigen, antiHBe, and antiHBc IgG. Other serologic viral markers were negative. Other biochemical tests and complete blood count were in normal limits. He did not have a history of alcohol drinking. Abdominal ultrasonography showed liver length 210 mm, liver contour irregularities, multiple hypoechoic nodules between 11 to 55 mm in diameter and splenomegaly.

Results: The patient was investigated for a metastatic malignancy but studies did not show any primary site. The serum alpha-fetoprotein level was 51178 IU/l (N = 0-4), CA19-9 27758 (N = < 4), and CEA 1.5 IU/l (N = < 37).

Liver biopsy was performed. Histopathological appearance was adenocarcinoma set in abundant fibrous stroma. It had both hepatocellular and cholangiocellular components. There were both bile and mucin production (confirmed by PAS stain). Immunohistochemical examination revealed alpha-fetoprotein and polyclonal CEA positivity.

The patient was started weekly 5-fluorouracil. During the days after chemotherapy, patients serum total bilirubin increased to 6.8 mg/dl, and direct bilirubin to 4.2 mg/dl. The patient's condition failed to improve, and he died with hepatic encephalopathy on the 14th day after the first dosage of chemotherapy.

Discussion: CA19-9 is a tumor marker useful in pancreatic carcinoma, and additionally has diagnostic value in cholangiocarcinoma, bile duct tumors, and liver metastases. Cholestasis affects serum CA19-9 levels by reducing its hepatic metabolism, and CA 19-9 level correlates with aminotransferases, GGT, and AP. At the time of diagnosis our patient's bilirubin levels were normal. Both increased production of CA 19-9 by biliary epithelial cells and decreased clearance may be contributing to the elevation of CA19-9 in the blood stream. We report this patient because extremely high levels of both AFP and CA 19-9 in CHCC was very rare in the literature.
Microarray technology for the identification and prognostic impact of histopathological response after neoadjuvant radiochemotherapy of locally advanced rectal carcinoma

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**Introduction**: Patients with locally advanced rectal carcinoma have a significant improved local control rate after neoadjuvant radiochemotherapy. We examined, if histopathological response after neoadjuvant radiochemotherapy has a prognostic impact and is predictable pretherapeutically using gene expression profiles.

**Methods**: 99 patients with uT3 rectal carcinoma were operated after neoadjuvant radiochemotherapy between 1997 and 2001. The patients were treated with a radiation dose of 45 Gy and continuous 5-FU chemotherapy. The median follow-up time was 55 months. Histopathological response according to Becker was evaluated and correlated with overall survival. Gene expression profiles were available of 33 pretherapeutical tumor biopsies using microarray technology, which were correlated with histopathological response and survival.

**Results**: 7-year overall survival and incidence of local recurrence was 80% and 6%, respectively. Stratification of the patients according to the histopathological response revealed, that the 42 patients with response grade 1 according to Becker (no or less than 10% residual tumor) had a significant improved overall survival (5-years: 89% ± 8%) than the 56 patients with histopathological non-response (Becker grade 2/3) and a 5-year overall survival of 72% ± 7% (p < 0.05). All microarray gene expression profiles of the histopathological responder and non-responder besides of one patient were classified correctly in 2 different gene clusters.

**Discussion/Conclusion**: Patients with histopathological tumor response after radiochemotherapy of a locally advanced rectal carcinoma have a significantly improved overall survival and represent the "winner" of the therapy. Our promising data for the use of microarrays for the identification of histopathological response and for response prediction has to be further evaluated.
The expression of interleukin-8 (IL-8) and IL-8 receptors, CXCR1 and CXCR2 in colorectal carcinoma

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Introduction: IL-8, a potent chemoattractant for neutrophils, is also implicated in mediating tumour growth and progression. We and others have demonstrated the expression of IL-8 and IL-8 receptors in colorectal carcinoma cell lines. The aim of this study was to investigate the expression of IL-8 and its receptors, in colorectal adenocarcinomas.

Methods: Fifty colorectal adenocarcinomas of different grades and stages were studied by the avidin-biotin complex immunocytochemical technique for the expression of IL-8 and IL-8 receptors.

Results: IL-8 immunoreactivity was detected in epithelial cells of only 3/50 tumours, but was strongly expressed in macrophages and some neutrophils in the stroma of all tumours. All tumours expressed CXCR1 at varying intensities in the membrane and cytoplasm of tumour cells. CXCR1 expression showed correlation with Dukes' stage, being strongest in Dukes' C tumours. CXCR2 was only weakly and focally expressed in occasional tumours.

Discussion/Conclusion: This is the first in vivo study of IL-8 and its receptors in colorectal cancer. CXCR1 is overexpressed in colorectal carcinoma as compared to normal colonic mucosa, and appears to be the receptor through which the effects of IL-8 are mediated on colonocytes. CXCR1 may represent a potential immunotherapeutic target in advanced colorectal cancer.
The role of mast cell tryptase in neoangiogenesis in colorectal cancer patients - Preliminary study

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Introduction: Close relationship between the formation of blood vessels in the vicinity of tumor cells and the development and spreading of tumors, strongly suggests that angiogenesis might be a prerequisite for tumor development. Neovascularization is regulated by complex interactions among growth factors and cytokines and influenced by proteolytic enzymes such as plasminogen activators and matrix metalloproteases, expression of adhesion molecules, and distribution of extracellular matrices. Angiogenesis plays a major role not only in tumor growth but also in metastasis development. There is evidence suggesting that this process is related to poor prognosis in many tumors including colorectal cancer. Recently, mast cell tryptase has been identified as another potent proangiogenic factor in tumors, along with fibroblasts and vascular endothelial growth factors. The aim of our study was to estimate a semiquantitative correlation between the number of immunohistochemically determined mast cells (MCs) and tumor angiogenesis.

Methods: The number of MCs at the cancer borders - on the border of tumor and healthy tissue - in the buffered formalin-fixed and paraffin-embedded postoperative material collected from 38 patients with colorectal carcinoma in Duke's grade C was analysed. Sections obtained from the primary lesion were subjected to the immunohistochemical reaction using antibodies against tryptase, which is a mastocyte marker (DAKO, mouse anti-human mast cell tryptase, clone AA1, M7052). The number of MCs per ten fields at a magnification of 200x was counted under light microscope, and the average count was determined. A three-grade scale was used for analysis.

Results: The current study showed that the number of peritumoral cell tryptase-positive mast cells increased with tumor progression and the cells were close to newly formed blood vessels. We found a significant correlation between mast cell count and highly angiogenic areas. The cells were always accompanied by intensified tumor cell budding, i.e. the presence of conspicuously isolated cells or small clusters of cancer cells which do not show a typical adenocarcinoma texture yet. The number of MCs adjacent to the areas of neovascularization at the cancer borders compared to the area without it was statistically significantly higher (p < 0.01).

Discussion/Conclusion: Our findings suggest that there appears to be a direct correlation between the number of mast cells and tumor angiogenesis in patients with colorectal cancer; MC tryptase may upregulate neoangiogenesis in carcinogenesis of the colon.
Colorectal carcinoma: Comparison of various screening methods in populations with the highest incidence of colorectal carcinoma in the world by Markov chain based model

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Introduction: Czech Republic takes the prime in incidence of colon cancer worldwide. Therefore it is necessary to carry out a study regarding the use of different screening methods considering health impact and cost-effectiveness

Methods: We created a Markov chain based model of colon cancer evolution investigating both colon cancer mortality and costs of three screening methods (first coloscopy, second coloscopy, FOBT). The screening starts at the age of fifty. For a given population age numbers of mortalities, polypectomies and costs are reported.

Results: First coloscopy excludes healthy individuals and second coloscopy (10 years later) applies the screening programme again for excluded individuals of chosen age. In both variants patients excluded for colon cancer and after polypectomy are examined again after 5 years. In case of no screening for 100,000 inhabitants between 50 and 85 years mortality would be 11,337 (100%). If first coloscopy is applied, mortality decreases to 7535 (66.5%) cases, number of polypectomies is 18,665 and the cost is $3.75 million. In case of second coloscopy, mortality decreases (6521 cases, 57.5%), number of polypectomies is 27,809 and the cost increases to $6.2 million. With FOBT, mortality is 8273 (73%), there are 35,473 polypectomies, the cost being $ 4.7 million.

Discussion/Conclusion: According to this Markov chain based model for impact evaluation of different screening methods mortality decreases the most with second coloscopy, however, cost effectiveness is the lowest. Coloscopy seems to be a good tool for primary screening method.

IGA, CR, MH 7878/3
Colonic adenomatous polyposis and metachronous small intestinal cancer

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We are presenting a case report about a patient with colonic adenomatous polyposis and metachronous small intestinal cancer.

**Introduction:** 72-year-old female with complaints of recurrent abdominal pain and constipation. Two colonic polyps of the sigma were observed and removed endoscopically. Histology: tubular adenoma with moderate dysplasia, grade of invasion was not evaluated. The patient was followed up for five years and annual colonoscopic investigation was performed, which revealed recurrent small polyps. The latter were removed by polypectomy, and examined histologically- mixed features: adenomatous and hyperplastic. Short-time chemoprevention was performed.

In 2004 the same old female patient was presented with recurrent episodes of acute upper abdominal pain, nausea, vomiting (subileus symptoms). The performed endoscopy did not find any pathological features in the stomach, duodenum and colon. US: picture of small intestinal subileus. Abdominal contrast CT scan showed small intestinal obstruction. The patient has undergone surgical treatment, which revealed small bowell adenocarcinoma (probably due to tubulovilous adenoma), as well as several small adenomatous polyps. Further the small bowell cancer was surgically resected.

**Discussion/Conclusion:** Metachronous intestinal tumors are not rare. The adenomatous polyps have high malignant potential. Synchronous existing adenoma and carcinoma of the small and large intestines is often observed. Therefore follow-up of such patients by prophylactic colonoscopy is necessary and mandatory. This again raises the question for the need of adequate chemoprevention of the large bowell polyposis.
Risk of colorectal cancer in ulcerative colitis in India

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Background: The risk for colorectal cancer (CRC) in ulcerative colitis (UC) in India is not known. Setting: Retrospective cohort from a tertiary level hospital in South India.

Methods: Analysis of archived records of all patients with ulcerative colitis who underwent colonoscopy and segmental biopsies over the last 25 years. Incidence densities and risk of developing high grade dysplasia or CRC was calculated and Chi-square test was performed for risk factors of interest.

Results: Complete records were available for 532 patients, 336 (63.2%) male. The mean (± SEM) duration of illness was 6.04 ± 0.29 years. 234 patients (44%) had pancolitis, 121 (22.7%) had left sided colitis and 177 (33.3%) had proctitis or proctosigmoiditis. Overall 5 (0.94%) patients developed carcinoma and 1 (0.19%) patient had high grade dysplasia. The incidence density and risk of developing either CRC or high grade dysplasia was nil in the first 10 years of disease. In those with disease duration of 10-20 years, incidence density was 2.34 per 1000 person years’ duration (PYD) for all patients with colitis and 4.5 per 1000 PYD for patients with pancolitis alone. This corresponded to risks of 2.3% and 4.4% respectively. For those with disease duration longer than 20 years, incidence density was 2.73 per 1000 PYD for all patients and 4.9 per 1000 PYD for patients with pancolitis. This corresponded to risks of 5.8% and 10.2% respectively. Duration of disease beyond 10 years and extent of colitis were the only risk factors significantly associated with CRC.

Discussion/Conclusion: The risk of developing CRC is Indian patients with UC is lower than that reported from the West. Strategies for cancer surveillance in Indian patients with UC need to be tailored accordingly.
Toxicity profile of adjuvant bolus 5-FU treatment in elderly colorectal cancer patients

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Introduction: We analysed the tolerability of the adjuvant bolus 5-FU treatment according to the age distribution of the patients.

Methods: 104 stage II/III CRC patients were treated according to Mayo protocol at our department between January 2004 and January 2005. The patients were analyzed according to their age, 79 patients under the age of 70 (group A) and 25 patients older than 70 years (group B).

Results: Difference between groups A and B was observed regarding stomatitis (32% vs. 48%) and anemia requiring transfusion (5.1% vs. 24%). In relationship with other side effects no major differences were observed between the two groups. Grade 2-4 neutropenia 12.8% vs. 16%, diarrhea 56% vs. 48%, nausea/vomitus 56% vs. 56%, hand and foot syndrome 3.8% vs. 4%, flatulence/abdominal dyscomfort 18% vs. 28%, dyspnea/angina-like complaints 22% vs. 28%, constipation 15.3% vs. 16%. In each group one case of deep vein thrombosis occurred. One patient of group B developed sepsis and died. Due to severe side effects in 2 cases we had to interrupt the treatment permanently, in 8 patients dose reduction was performed.

Discussion/Conclusion: Comparing the group of patients under and above 70 years, the Mayo protocol can be characterized by an equally inconvenient toxicity profile. In the adjuvant treatment of CRC the more convenient continuos infusion 5-fluorouracil/leucovorin should be administered.
Endoscopic resection of large colorectal polyps with endoloop

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Introduction: Endoscopic resection of colorectal polyps is technically complicated with high risk of complications. In order to lower the risk the treatment of colorectal polyps undergone a significant progress and new instruments have been introduced. Besides vascular clips and argon coagulation, polyp stalk ligation with endoloops has been frequently used. The study aims at evaluating the results of endoscopic resection of colorectal polyps over 2 cm in diameter using endoloops.

Methods: Between 2000-2004, 3435 lower gastrointestinal endoscopies were performed. Of these 108 patients underwent resection of large polyps after polyp stalk ligation with endoloop on the same day (96 cases) and on the following day (12 cases). A group consisted of 57 men and 51 women (mean age 64.1 years, age range 35-92). Treatment results, complications and resection radicality were analysed.

Results: Complications included bleeding in 6 patients that were endoscopically controlled in 4 cases, and the remaining 2 required surgical intervention. None of the patients developed intestinal perforation. The diameter of resected polyps was 27 mm (18-48 mm) on average. Mean time of hospitalization was 1.1 days.

Discussion/Conclusion: Endoscopic polypectomy of colorectal polyps using endoloop is effective and may be a method of choice in the treatment of large polyps.
Clinico-pathological significance of inflammatory infiltrate in colorectal carcinoma: Correlation with prognosis and survival

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\textbf{Introduction}: The infiltration of inflammatory cells in cancer tissues is considered an important aspect of the host response in cancer. We investigated the prognostic potential of lymphocytic inflammation present in and around colorectal carcinomas in relation to TNM stage and survival.

\textbf{Methods}: The patients included 31 men and 32 women aged between 31 and 78 years. The median rate of 5-year survival was 52.4\%. Routine HE stained sections and immunohistochemical reactions with anti-CD\textsubscript{45RO} (UCHL clone, Carpinteria, Dako, USA) were used to determine the peritumoral lymphocytic inflammation with or without Crohn's like reaction (present, absent) and tumor infiltrating lymphocytes (present, absent).

\textbf{Results}: The presence of peritumoral T cells was correlated with the colonic tumors (62.2\%), adenocarcinomas (51.9\%), anaplastic carcinomas (60\%) and early stages in the TNM classification (I and IIA). For these colorectal carcinomas we observed higher survival rate (78.9\%) when compared with tumors without peritumoral infiltrates (28.1\%). Results indicated that CLR is more likely to occur in transmurally invasive carcinomas than in those confined to the colonic wall, especially in the right side. An intense CLR is associated with colonic carcinomas (73.3\%), adenocarcinomas (86.6\%), pT2 (46.6\%) and pT3 (33.3\%) tumors, a lower incidence of nodal metastases and a significant increase in 5-year survival (80\% compared to 43.5\% for tumors without CLR).

\textbf{Discussion/Conclusion}: The specific immune response (T cells) demonstrated prognostic value. Our results suggest that the CLR around colorectal carcinomas may be a favorable host response, similar to lymphocytic infiltrates at the advancing tumor edge.
The estimation of angiogenesis in benign and early malignant colorectal neoplasia

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Introduction: We investigated by immunohistochemistry the role of angiogenesis in the adenoma-carcinoma sequence and the relation between microvessel density (MVD) and clinico-pathological factors.

Methods: MVD was assed by counting intratumoral microvessels immunostained for CD-34 antigen (QB-END 10). Specimens were composed of 10 hyperplastic polyps, 14 adenomas with low grade dysplasia, 20 adenomas with high grade dysplasia, 20 carcinomas in adenoma (malignant polyps) and 4 normal colonic mucosa. MVD was expressed as the mean number of microvessels/x 200 field.

Results: The hyperplastic polyps presented a uniform spatial distribution of microvessels between cripts (MVD = 21.4). A gradual increment of MVD was noted in the adenoma-carcinoma sequence. MVD increased to a mean value of 35.2 in adenomas with low grade dysplasia, 51.4 in adenomas with high grade dysplasia and 58.5 in malignant polyps. The morphological changes of microvessels displayed a great aberrance in adenomas with high grade dysplasia and in malignant polyps, as characterized by considerable diversity in the lumen and irregular spatial distribution. There was a significant correlation between MVD and the size of polyps (< 1.5 cm MVD = 32.1; > 1.5 cm MVD = 54.2). Sessile tumors presented higher MVD (55.7) when compared with pedunculated lesions (MVD = 36.4). MVD does not presented important differences regarding to sex of patients, tumor site and histological type.

Discussion/Conclusion: Our results suggest that tumor angiogenesis was initiated during the transition of adenomas from low dysplasia to high dysplasia and carcinoma as reflected by the significant rising of MVD and aberrant morphological changes of vessels.
Downregulation of Eomesodermin levels in tumor infiltrating T cells is associated with poor tumor prognosis

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Introduction: As shown recently, T-box factor Eomesodermin plays a critical role in differentiation of murine CD8⁺ T cells towards cytolytic effector cells. A better understanding of Eomesodermin mediated effects in human CD8⁺ T cells and in cancer development might provide new insights into the mechanisms of immune mediated tumor-defense.

Methods: Expression of Eomesodermin was analysed in tissue probes from 88 human colorectal cancers by PCR and results were compared with clinical data (UICC, TNM, Tumor Grading, lymph vessel and venous invasion). Human CD8⁺ T cells were transfected with siRNA, designed to silence the Eomesodermin gene. Eomesodermin mediated effects on the expression of IFN-gamma, perforin and granzyme B in these cells were analysed by RT-PCR.

Results: A significant inverse correlation between Eomesodermin expression in human colorectal cancers and infiltration of tumor cells into lymph nodes (p < 0.02) was found. Furthermore, Eomesodermin expression in tumors correlated with improved prognosis based on the UICC-classification (p < 0.03). To find out, whether these effects might be caused by an increased Eomesodermin-triggered effector function of CD8⁺ T cells, the role of Eomesodermin in human cells was analysed. In human CD8⁺ T cells transfected with siRNA, designed to silence the Eomesodermin gene, the expression of IFN-gamma and perforin was decreased, whereas the expression of granzyme B was unchanged.

Discussion/Conclusion: Our data suggest that IFN-gamma production and expression of perforin in human CD8⁺ T cells is critically dependent on Eomesodermin function. Downregulation of Eomesodermin levels in tumor infiltrating T cells is associated with poor tumor prognosis and the presence of lymph node metastases.
Changes in lymphocyte subpopulations during intraperitoneal IL-2 applications in patients with different malignancies

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Introduction: Interleukin 2 (IL-2) is being considered as a potent growth factor that amplifies lymphocyte responses in vivo. Recently it has also been established that IL-2 is critical for the development of regulatory T cells with high expression of CD25 (CD4+CD25⁺high). These cells promote self-tolerance by suppressing T cell responses in vivo. Increased percentage of these cells was found in tumour patients and their suppressive effect on anti-tumour immune response had been assumed.

Methods: This study was aimed to investigate blood and ascitic changes in lymphocyte subpopulations and the percentage of CD4+CD25⁺high regulatory cells in 6 patients with different malignancies treated with intraperitoneal applications of low-doses IL-2. Lymphocytes were analysed by double-coloured flow cytometry at baseline (0h), 24h, 48h, 168h, 192h and 216h after the 1st IL-2 application. Simulset IMK(BD) kit for measurement of CD3+CD4+, CD3+CD8+, CD3+CD19+, CD56+CD16+, CD3+CD56+CD16+ lymphocytes and CD4-FITC and CD25-PE-conjugated antibodies (Bul-Bio, Bulgaria) for regulatory T cells were used.

Results: Substantial increases in blood and ascitic CD3CD4, CD3CD8, CD3CD19, CD56CD16 and D3CD56CD16 lymphocytes were registered in 3 of the patients. Significant increases in the percentage of CD4CD25⁺high cells were observed both in blood and ascites.

Discussion/Conclusion: Local application of low doses IL-2 in cancer patients led to systemic and local changes in lymphocyte subpopulations. Increase of T cells with regulatory phenotype (CD4CD25⁺high) in our study needs further investigation pointed at suppressive effects of these cells in patients undergoing IL-2 immunotherapy in vivo.
Complex postoperative follow-up of patients operated on for colon cancer

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Background: Generally 70 colon resection is performed in our surgical department due to colon malignancies. Most frequent types of resections are: left and right side hemicolectomy, abdomino-perianal rectum extirpation.

Aims: Periodic clinical and imaging follow up examinations can ensure early detection of disease recurrence or propagation. We made up our protocol adapting the international follow up guidelines. Here we summarize our results of more than 300 patients during the follow up period of 2000-2005.

Patients and methods: Peri- and postoperative chemo- and radiotherapy is processed in close cooperation with another institute. In our department control colonoscopy is performed 3, 6 and 12 months after surgical procedures, parallely with other imaging and laboratory examinations.

Results: Due to the close follow up protocol our patients are returning for control examinations in elevating number. In average every second patient presents for control. Polyps were found in 5.2% of patients at 3 months, in 4.9% of patients at 6 months and in 6.2% of patients at 12 months after surgery. Because of recurrence of malignancies, every year 3-5 patients are reoperated.

Conclusions: Regular colonoscopy may result in early detection and prevention of recurrence of malignancies (early polypectomy) thus may significantly ameliorate the prognosis of patients. With our follow up protocol we found synchronous/metachronous mucosa changes and polyps in a relatively high percent of cases and according to the adenoma-carcinoma sequence early polypectomy can be an effective method in preventing disease recurrence.
Effect of 5-aminosalicylate use on colorectal cancer and dysplasia risk: A systematic review and metaanalysis of observational studies

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Objectives: We performed a systematic review with meta-analysis of observational studies evaluating the association between 5-ASA use and colorectal cancer or dysplasia among patients with ulcerative colitis.

Methods: We conducted a search of Medline, Biosis, Web of Science, Cochrane Collaboration, manually reviewed the literature, and consulted with experts. Studies were included if they (1) evaluated and clearly defined exposure to 5-aminosalicylates in patients with ulcerative colitis, (2) reported colorectal cancer or dysplasia outcomes, (3) reported relative risks or odds ratio or provided data for their calculations. Quantitative analysis using a random-effects model is presented.

Results: Nine studies (3 cohort, 6 case-control) containing 334 cases of colorectal cancer, 140 cases of dysplasia, and a total of 1932 subjects satisfied all inclusion criteria. Five studies reported colorectal cancer outcomes alone, two studies reported separate cancer and dysplasia outcomes, and two studies reported a combined outcome of colorectal cancer or dysplasia. All primary estimates are homogenous. Pooled analysis showed a protective association between use of 5-aminosalicylates and colorectal cancer (OR = 0.51; 95% CI: 0.37-0.69) or a combined endpoint of colorectal cancer/dysplasia (OR = 0.51; 95% CI: 0.38-0.69). 5-ASA use was not associated with a lower risk of dysplasia, although only 2 studies evaluated this outcome (OR = 1.18; 95% CI: 0.41-3.43).

Conclusion: Pooled results of observational studies support a protective association between 5-aminosalicylates and colorectal cancer or a combined endpoint of colorectal cancer/dysplasia in patients with ulcerative colitis. Additional studies analyzing the effect of 5-ASA on risk of dysplasia are needed.
Magnifying chromoendoscopy in gastric mucosal lesions

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Introduction: Magnifying endoscopy can provide high resolution mucosal detail of the gastrointestinal tissue. Only a few studies on clinical application of magnifying endoscopy have been focused on gastric mucosa lesions. The aim of this study was to assess the clinical significance of pit pattern mucosal distribution and architecture of collecting venules for detection and characterization of different gastric mucosal lesions.

Methods: From November 2004 to June 2005 a total of 900 patients with upper GI symptoms underwent magnifying chromoendoscopy with different stainings plus directed biopsies from the specific areas. Standard histological examination was done. Helicobacter pylori infection was confirmed by rapid urease test and histology.

Results: Magnifying pit pattern gastric mucosal distribution and architecture of collecting venules significantly correlated with degree of inflammation and atrophy. Intestinal metaplasia and dysplasia were found predominantly in type D and E. The detection of dysplasia and gastric ectopia in duodenal mucosa was more frequent after magnifying chromoendoscopy. H. pylori infection rate in type R was significantly lower than that in other two types (p < 0.01). There was relationship between the size of metaplastic area and Helicobacter pylori gastritis. The combination of two stains increased the distinguishing of metaplastic from dysplastic lesions and early gastric cancer.

Conclusion: Magnifying chromoendoscopy with different stainings is useful for detection and characterization of gastric mucosal lesions.
Introduction: Colorectal cancer is one of the most malignancies. However, the molecular pathogenesis of colorectal cancer is poorly understood. In order to investigate the functional role of IL-4-associated transcription factor NFATc2 (nuclear factor of activated T-cells), in colon carcinogenesis, we used a previously established murine colon carcinoma model based on the mutagenic agent azoxymethan (AOM).

Methods: Accordingly, mice were treated with AOM followed by three consecutive cycles of orally administrated dextran sulfate sodium (DSS) over a period of 7 days. To monitor tumorigenesis in mice in vivo, we used our novel mini-endoscopic system.

Results: By using this system together with methylene blue, we were able to detect aberrant crypt foci in DSS plus AOM-treated wild-type mice at early time point before macroscopically visible lesions were seen. Small visible lesions first appeared around day 20, which were followed by the development of large tumors until day 80. In contrast, NFATc2 deficient mice are protected against tumor development and showed no colitis-similar symptoms. The possibility, that T cells play a regulatory role in the development of colon tumors led us to perform a screening of the expression of T cell-derived cytokines in colons and tumors of AOM plus DSS-treated wild-type and knockout mice.

Discussion/Conclusion: Our data encourage further work of NFAT signaling in colon cancer to establish a novel mechanism for regulation of tumor cell growth.
EBI3/IL-27 deficiency protects mice from colitis-associated cancer

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Introduction: IL-27 is a novel IL-12-related cytokine. Although initial studies have described the IL-27/WSX-1 pathway primarily as a promoter of Th1 differentiation in CD4+ T-cells, it has been shown recently that IL-27 is a pleiotropic cytokine with distinct proinflammatory and regulatory functions in different phases of the immune response. Furthermore, data obtained by two new studies demonstrated that expression of recombinant IL-27 was associated with T cell dependent and independent tumour rejection. On the other hand a selective overexpression of IL-27-EBI3 but not IL-27-p28 by human transformed cells suggests that IL-27-EBI3 may be involved in tumour evasion by antagonizing Th1 responses. Here we analyzed the role of EBI3/IL-27 in a rodent model of colitis-associated cancer.

Methods: IL-27-EBI3KO and controls were analyzed in a colon-carcinoma model based on the treatment with azoxymethane followed by three cycles of oral DSS. Inflammation/tumorigenesis was monitored by high-resolution colonoscopy, histology and myeloperoxidase determination. Cytokine levels were determined by ELISA and real-time-PCR.

Results: Both control and EBI3KO mice displayed DSS dependent colitis, although the KO gained weight earlier after DSS removal. Controls had strong neo-/dysplastic alterations until and developed large tumours until day 60, whereas EBI3KO mice did not display tumorigenesis. MPO levels and infiltrations with polymorphonuclear and mononuclear cells were much lower in EBI3 KO. Isolated and stimulated LPMC from wildtype mice produced much more proinflammatory cytokines such as IL-1, TNF and IL-6 than those of EBI3 KO mice.

Discussion/Conclusion: EBI3/IL-27 deficiency is protective in a model of colitis-associated cancer and targeting of this molecule may also be beneficial in human disease.
Role of endoarterial chemotherapy at nonresectable liver cancer

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Material and methods: The direct and remote results of endoarterial chemotherapy (EC) are analysed at 32 patients with nonresectable liver cancer (NLC). Hepatocellular cancer was - at 19 and cholangiocellular - at 13. The process was located in the right part at 20 patients, in left part - at 12. For undertaking EC was produced catheterisation of a. hepatica propria by Celdinger's method. At bilaterial defeat of liver catheter established in a. hepatica communis. The treatment was conducted with 5-fluoruracil (5 g) and doxorubicin (80-90 mg) depending on masses of body during 120 hours continuously. In control group was 26 patients with NLC, which rate systemic chemotherapy with same preparations and same dozes was carried out.

Results: efficiency of treatment were estimated under the recommendation of WHO (1978). The complete effect is not marked at anybody, partial effect - at 28 and stabilization - at 4 patients. Progression is not marked. The subjective improvement is marked at 27 patients, without changes at 3 and deterioration at 2. The average survival has made 12.1 months. In control group complete effect is not marked at anybody, partial - at 11, stabilization - at 9 and progression - at 6 patients. The subjective improvement is marked at 12 patients and deterioration at 14. The average survival has made 6.4 months.

Conclusion: The ES is method of choice at NLC, as it authentically prolongs life of patients and improves quality of life.

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