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Prospective randomised trial comparing multimodal therapy with surgery alone for oesophageal adenocarcinoma: a long-term follow-up

P. Naughton[1], S. Tormey[2], A. Kelly[3], P.W.N. Keeling[4], N. Noonan[5], T.P. Hennessy[6], T.N. Walsh[7]. [1] *James Connolly Memorial Hospital, Surgery, Dublin, Ireland;* [2] *James Connolly Memorial Hospital, Surgery, Dublin, Ireland;* [3] *St.James Hospital, Surgery, Dublin, Ireland;* [4] *St.James Hospital, Gastroenterology, Dublin, Ireland;* [5] *St.James Hospital, Surgery, Dublin, Ireland;* [6] *St.James Hospital, Surgery, Dublin, Ireland;* [7] *James Connolly Memorial Hospital, Surgery, Dublin, Ireland*

Background: A randomized trial showed a survival advantage for neoadjuvant chemoradiotherapy prior to surgery over surgery alone at 3 years followup. The aim of this study was to assess if this survival advantage was durable. We performed long term follow-up on patients randomized to neoadjuvant chemoradiotherapy versus surgery alone and all patients were followed-up for more than 7 years.

Method: Patients were recruited between May 1990 and September 1995 inclusive (n=113). Patients assigned to the multimodal group received two courses of chemotherapy in weeks 1 and 6 (fluorouracil, 15mg per kg for five days and cisplatin, 75mg per kg on day 7) and a course of radiotherapy (40Gy, administered in 15 fractions over a 3 week period beginning concurrently with the first course of chemotherapy), followed by surgery. Long-term follow-up was performed via chart review and family doctor contact. Survival was measured from the date of randomization to the date of death or most recent follow-up. Estimates of median survival are based on the Kaplan-Meier method: group comparisons of survival involving individual variables were based on the log-rank test.

Results

	Multimodal Therapy	Surgery Alone
No. Patients	58	55
Mean survival (Months)	54	19
Median Survival	17	12
Downstaging (% node negative at resection)	55	18
Complete Response (%)	22	0
Median Survival for Complete Responders (months)	67	-
Stage 2a median survival (months)	67	11.5
Survival Node+ (months)	9	15

Discussion: The survival advantage conferred by neoadjuvant chemoradiotherapy is durable with a significant difference maintained at 7 years allaying fears that the benefit seen at three years is transient.

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Evaluation of messenger RNA of Beta-1,6-N-acetylglucosaminyltransferase V as a molecular marker for micrometastasis in gastrointestinal cancer.

M. Valladares-Ayerbes[1], M. Reboredo[1], P. Iglesias[2], I. Brandon[3], M. Haz[3], M. Lorenzo[2], L. Calvo[1], S. Antolin[1], G. Alonso[1], L.M. Anton-Aparicio[1]. [1] *Juan Canalejo Hospital, Medical Oncology, La Coruña, Spain;* [2] *Juan Canalejo Hospital, Pathology, La Coruña, Spain;* [3] *Juan Canalejo Hospital, Research Unit, LA Coruña, Spain*

Background: Deregulated glycosylation patterns are characteristic of metastatic phenotype. Malignant transformation is accompanied by increased beta-1,6-GlcNAc branching of N-glycans attached to Asn-X-Ser/Thr sequences in mature glycoproteins. Beta-1,6-N-acetylglucosaminyltransferase V (GNTV) catalyzes the addition of beta-1,6-GlcNAc to N-glycan on glycoproteins. GNTV functions seem to be involved in focal adhesion turnover and signaling through PI3K/Akt.The amount of GNT-V correlates with disease progression.

Aims: In order to detect micrometastasis (MM) in patients with gastrointestinal cancer (GC) we have developed a model system based on reverse transcriptase (RT)-PCR amplification of GNTV mRNA.

Methods: As a surrogate model to asses sensitivity, GNTV mRNA expression was analyzed in a panel of 9 human GC cell-lines (CL), including 2 CL developed in our laboratory: pancreatic carcinoma MBQ-OJC1 and colon carcinoma JJPF-OJC4. The specificity of GNTV mRNA was examined by PCR amplification of cDNA from normal lymph nodes (LN), bone marrows (BM) and peripheral blood (PB, cellular and plasmatic mRNA).

Results: Specific amplicon for GNTV was demonstrated by RT-PCR in all the GCCL tested. Using a hot-start PCR and defined amounts of input RNA, GNTV transcripts were not detected in normal LN (pooled, n=34) and BM (pooled, n=83). GNTV mRNA expression in PB was detected on 1 among 14 healthy donors analyzed.

Conclusions: We have developed a model system based on RT-PCR for GNTV mRNA in order to detect isolated and circulating micrometastatic cells in patients with gastrointestinal cancer. GNTV could serve as a sensitive and specific molecular marker for targeting micrometastasis in PB, LN and BM. Support: Xunta de Galicia PGIDT01PXI90001PR.

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Thymidylate synthase is a predictor for response and survival of patients with isolated unresectable liver tumors receiving hepatic artery infusion chemotherapy

A. Hillenbrand[1], A. Formentini[1], L. Staib[1], K. Danenberg[2], P. Danenberg[2], M. Kornmann[1]. [1] *University of Ulm, Visceral and Transplantation Surgery, Ulm, Germany;* [2] *University of Southern California, Biochemistry, Los Angeles, USA*

Introduction: Fluoropyrimidine (FP)-based hepatic artery infusion (HAI) chemotherapy is one promising approach when a primary or secondary liver tumor cannot be resected surgically. Thymidylate synthase (TS) is a key enzyme for DNA synthesis and targeted by FPs. Several studies have shown that high TS levels are associated with resistance to systemic 5-fluorouracil (5-FU) based chemotherapy. The aim of this study was to investigate the influence of TS mRNA levels on response and survival of patients receiving FP-based HAI.

Patients and methods: Fifty-one consecutive patients with liver tumors receiving HAI with available tumor tissue for TS quantitation were entered between 1991 and 2001 into this study. Fourty-one patients suffered from colorectal cancer, 4 from primary liver cancers, and 6 from other cancers. Patients (29 men and 22 women) had unresectable metastases limited to the liver or primary liver tumors with a mean age of 58 years (range: 28 to 76 years). Tumor tissue was obtained at laparotomy for the intra-arterial infusion device implanation. Relative TS mRNA quantitation was performed by RT-PCR using beta-actin as internal standard.

Results: Median TS expression was 2.2 with high variation between tumors ranging from 0.1 to 27. Twenty-two out of 51 patients responded to HAI (CR + PR). The median TS level of the responders was 1.6 and more than two-fold lower than the level of the non-responders (n = 29) with 3.3. In the subgroup with TS $\leq$ 3.0 18 out of 29 patients responded, whereas in the subgroup with TS > 3.0 only 4 out of 22 responded (p = 0.04). Moreover, all patients with very high TS > 4.5 (n = 13) did not respond (NC or PD). The median survival from start of HAI for all patients was 18 months (range: 3 to 97). Patients with TS levels $\leq$ 3.0 showed with 24 months a longer median survival duration compared to patients with TS levels > 3.0 wit 15 months (p = 0.016).

Conclusion: Patients with lower TS levels showed a better response rate and had a longer survival duration compared to patients with higher TS levels. Thus, TS levels also seem to influence the prognosis of patients receiving FP-based HAI. Additionally, patients with very high TS levels do not seem to profit from FP-based HAI.

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Immunohistochemical characterization of human hepatocellular carcinomas

P. Nagy[1], D. Görög[2], J. Regöly-Mérei[3]. [1] *Semmelweis University, Ist. Institute of Pathology & Exp. Cancer Res., Budapest, Hungary;* [2] *Semmelweis University, Dept. of Transplantation and Surgery, Budapest, Hungary;* [3] *Semmelweis University, III.rd Dept.of Surgery, Budapest, Hungary*

Background: It has been known for a long time, that alfafetoprotein (AFP), an oncofetal marker is expressed in approximately half of hepatocellular carcinomas (HCC). However its production is not perceived to be related with the clinical behaviour of the tumors. Recently two independent microarray studies described different expression profiles for AFP positive and negative HCC cell lines (Hepatology 2001, 33, 676, Hepatology 2002, 35, 1134). We decided to compare immunohistochemical characteristics of AFP positive and negative clinical HCC samples.

Material and methods: Immunohistochemical staining for different antigens (AFP, β-catenin, P53, CD44, MSH-2, MLH-1) were done on 31 paraffin embedded tumor samples. The results were compared with the most important clinical parameters.

Results: Sixteen tumors was AFP positive. Gender and age of the patients, etiological factors, histological type of the tumors, MSH-2, MLH-1 expression did not segregate between the AFP positive and negative tumors.

However CD 44, P53 staining, high tumor grade occurred preferentially among the AFP expressing tumors. All but one of the 10 HCC-s showing nuclear positivity for β-catenin were AFP negative.

Conclusions: Our results indicate that AFP expression in HCC-s is more frequently associated with several unfavourable prognostic factors, while nuclear β-catenin positivity, suggesting constitutive activation of the Wnt signal pathway is more common among the AFP negative liver tumors. This observation supports the microarray data on *in vivo* human tumors and implies that the re-evaluation of the signifinance of AFP production in HCCs may be required.

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The use of a novel chromatographic molecular method for the detection of the membrane cancer antigen Ep-CAM (17-1A) in peripheral blood and bone marrow of patients with metastatic colorectal cancer.

I. Skiadas[1], S. Georgakopoulou[1], C. Karanikiotes[2], M. Karina[3], C. Mitropoulos[4], I. Roukas[2], P. Didaggelos[2], G. Fountzilas[3], E. Georgakopoulos[1], E. Prinarakis[1]. [1] *Pegasus Genomics S.A, Biotechnology, Athens, Greece;* [2] *424 General Military Hospital, Medical Oncology, Thessaloniki, Greece;* [3] *AHEPA University Hospital, Medical Oncology, Thessaloniki, Greece;* [4] *Medicon Hellas S.A., Biotechnology, Athens, Greece*

A new method for the molecular detection of PCR amplified product has been developed (Medicon Hellas S.A. and Pegasus Genomics S.A.). The outcome of this research project is a strip that can give fast and highly reliable results for the detection of specific PCR products utilizing a chromatographic method. According to this method, biotin-labeled products are applied to a strip and their presence can be analyzed chromatographically due to a chromogenic reaction. In our study we used this product for the detection of the Ep-CAM gene expression in human peripheral blood and bone marrow samples of patients with metastatic colorectal cancer and heavy tumor load and we confirmed the results by the standard method of agarose gel electrophoresis. 30 patients with participated in this study providing 27 peripheral blood samples and 26 bone marrow samples. Total RNA extraction (Abgene, UK) was performed followed by RT using oligo(dT)s as primers (Promega, USA) according to standard protocols. The cDNAs produced by this procedure were used as templates in PCR with selected Ep-CAM primers to amplify specifically 540bp of the Ep-CAM mRNA. PCR products were then detected using the strip and the results were confirmed by 1,5% agarose gel electrophoresis. 23 patients provided both blood and bone marrow samples. 17 patients (74%) expressed the Ep-CAM both in blood and in bone marrow whereas only 1 (4%) was negative in both. The remaining 5 patients (22%) were positive for Ep-CAM expression in their blood and negative in their bone marrow. 2 of the 3 patients who provided only bone marrow samples were positive for Ep-CAM. All 4 patients who provided blood samples, were found positive for Ep-CAM. Totally 19 of the 26 bone marrow samples (73%) and 26 of the 27 blood samples (96%) were found positive for Ep-CAM expression using the strip detection method and the standard 1,5% agarose gel electrophoresis. The new strip detection method is at least as reliable as agarose gel electrophoresis and certainly easier to handle, faster and safer for the user (no ethidium bromide staining needed). It is also highly specific for the PCR product under investigation due to the use of the internal probe. We recommend the use of our method in the molecular detection of circulating micrometastatic cancer cells.

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Evaluation of tumor board recommendations at the center of gastrointestinal oncology (ZGO) at Tübingen University

C. Burkart[1], N. Höppel[2], W. Budach[3], B. Klump[4], R. Teichmann[5], J. Hartmann[6], P. Pereira[7], L. Kanz[6], C. Bokemeyer[6]. [1] *Center of Gastrointestinal Oncology, Center of Gastrointestinal Oncology, Tübingen, Germany;* [2] *University Hospital Tübingen, University Hospital Tübingen, Tübingen, Germany*

Background: The center of Gastrointestinal Oncology (ZGO) is operated by a disease oriented interdisciplinary working group within the context of the Interdisciplinary Cancer Center at Tübingen University. Data from patients treated within the university hospital or at associated medical facilities are collected and individual patient management is discussed at interdisciplinary tumor boards with members of all departments twice weekly.

Material and methods: We have evaluated medical data and diagnostic and therapeutic procedures in all patients presented at the ZGO tumor boards in 2001.

Results: 393 of 445 (88%) pts. were evaluable. The medium age of the pts. was 60 years. Pts. had on average 2 accompanying illnesses (mostly cardiac or pulmonary diseases). The majority of the pts. (73%) was male. Localization of malignancies of patients discussed at the board was distributed as follows: 26% rectum, 21% colon, 20% esophagus, 16% stomach, 5% pancreas, 4% hepatocellular, 3% cholangiocellular, 5% other gastrointestinal malignancies. The majority of the pts. had a malignancy with the initial TNM-stages as follows: T3 (51%), N1 (48%), M0 (77%) and G2 (51%). On average interdisciplinary management was discussed 3 month after first diagnosis. 74% of pts. were presented once, 26% twice at the ZGO tumorboard. 60% of the questioning occurred in the palliative setting, 26% were on an adjuvant, and 14% on a neoadjuvant approach. 89% of the pts. were treated within the university hospital. The surgical department presented the majority of pts. (61%) at the tumorboards. The recommendations were mainly therapeutic (76%) or diagnostic (12%) or both (12%). Participation in a clinical study was suggested to 7% of the pts. 85% of all recommendations given by the tumor board were followed. Reasons why recommendations were not followed were analysed: 47% of these pts. received an alternative treatment strategy, in 18% no therapy was chosen and 25% of these pts. declined the recommendation. 10% of pts. died prior to further treatment.

Summary: This analysis describes the distribution of patients in routine care discussed in a GI-specialized interdisciplinary tumorboard at a university hospital. Quality control needs to include the assessment whether interdisciplinary recommendations were followed. The overall outcome must be further analysed to validate the impact of multidisciplinary management strategies.

Colorectal cancer

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A phase I study of irinotecan (CPT-11) and capecitabina (XL) as second line treatment in advanced colorectal cancer

P. Garcia-Alfonso[1], A. Munoz[1], M. Méndez[2], S. Garcia-Adrian[1], P. Lopez-Criado[1], R. Alvarez[1], I. Siso[1], G. Pérez-Manga[1]. [1] *H. General Gregorio Maranon, Madrid, Spain;* [2] *H. Mostoles, Servicio de Oncologia Medica, Madrid, Spain*

Objective: To determine the MTD of XL administered during 7 days, associated with CPT-11 175 mg/m^2 day 1, every 2 weeks.

Material and Methods: Patients (p) with histological diagnosis of CCR, with previous chemotherapy with oxaliplatin for metastatic disease, ECOG $\leq$ 2, adequate bone marrow, renal and hepatic function.CPT-11 175 mg/m^2 IV 30 min. day 1, followed by XL o.r. taken b.i.d. days 2-8, every 2 weeks. Dose escalation: XL- Level 1: 500 mg/m^2 b.i.d.; Level 2: 750 mg/m^2; Level 3: 1.000 mg/m^2 b.i.d.; Level 4: 1.250 mg/m^2 b.i.d.

Results: 26 p were included (L1/2/3: 3/5/18; M/F, 13/13), median age 58 (range 40-79) and ECOG 0-1 (85%). Primary tumour sites: colon (17p) and rectum (9p). Histology: Adenocarcinoma. Previous treatment: surgery (73.1%), adjuvant chemotherapy (38.5%), radiotherapy (30.8%). Median number of tumoral lesions was 1. 157 cycles were administered (L1/2/3: 11/31/115; median 4.0/7.0/5.5) with a median relative dose intensity in L1/2/3 of 92/87/90% for CPT-11 and 92/90/87% for XL. Dose levels were escalated if toxicity grade 3/4 was not observed. No toxicity grade 3/4 was observed in levels 1-2. In L3, DLT was observed in 3 of 18 p: diarrhea G3, neutropenia G3 and vomiting G3. Global toxicity in L3 (per patient/cycle) was neutropenia (2.5/16.7%), anemia (1.7/11.1%), leucopenia (3.4/11.1%), diarrhea (5.9/33.3%), vomiting (1.7/11.1%), asthenia (1.7/11.1%), mucositis (0.8/5.6%) and infection without neutropenia (0.8/5.6%). Responses were only obtained in L3: 5 PR (27.8%) CI 95% (7.1 48.5%).

Conclusions: MTD has not been established, however the schedule administered in L3 is recommended, as it presents an adequate and manageable toxicity profile. Preliminary data show that this is an effective and well tolerated combination.