was to evaluate the usefulness of FNAC and IFSE in patients with NSTN undergoing surgery.

Patients and Methods: We retrospectively analyzed a series of 606 patients with a SNTN who underwent both preoperative FNAC and IFSE prior to partial or total thyroidectomy. There were 118 (19.5%) men and 488 (80.5%) women, with an overall median age of 44 years (range 16-81 years). Final pathologic examination showed 500 (82.5%) benign nodules, including 239 (39.4%) follicular adenomas, and 106 (17.5%) thyroid carcinomas, of which 80 (75.5%) papillary, 18 (17.0%) follicular, 5 (4.7%) undifferentiated, and 3 (2.8%) medullary carcinomas. Patients with benign tumors were significantly ($p<0.05$) younger

Results: In the preoperative differential diagnosis between hyperplastic thyroid nodules and thyroid tumors, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of FNAC was 93.6%, 98.9%, 92.1%, 99.4%, and 95.9%, respectively. In the detection of malignancy sensitivity, specificity, PPV, NPV, and accuracy were 94.3%, 99.8%, 98.8%, 99.0%, and 98.8% for FNAC, and 95.3%, 100%, 99.0%, 100%, and 99.2% for IFSE (p=NS, chi-squared test). The combination of FNAC and IFSE did not improve significantly (p=NS) the results. In fact, IFSE suggested a thyroid cancer in only one of the 6 patients with false negative FNAC, and failed to detect malignancy in 5 of 18 (27.8%) follicular carcinomas.

Conclusions: In patients with NSTN and an adequate FNAC suggesting malignancy IFSE may be unnecessary, and in those with follicular tumors the results of both FNAC and IFSE should not affect the final intraoperative decision-making.

399 POSTER

Results of Interferon alpha 2b (Introna) treatment in 22 patients with metastatic progressive differentiated endocrine tumors

C. Baiseau Corrieri[1], J.Y. Scoazec[2], O. Boillot[1], C. Dumanoir[3], C. Barel[1], J.C. Saurin[1], J. Dumortier[1], J.A. Chayvialle[1], C. Lombard-Bohas[1].
[1] Hopital Edouard Herriot, Digestive Diseases Federation, Lyon, France; [2] Hopital Edouard Herriot, Pathology Department, Lyon, France; [3] Schering Plough, Laboratory, Paris, France

Digestive endocrine tumors (ET) are uncommon tumors; treatment of metastatic disease is not well established. Oberg and Collegues first described the potential role of Interferon alpha 2-b in the treatment of non curable progressive disease. We report here the results in 22 consecutive patients (pts) with progressive histologically proven ET treated with interferon alpha 2b (Introna): 14 men - 8 women, median age 57,8 years (40 - 75). Eleven were enterochromaffin ET (1 bronchial, 1 rectal, 8 ileal); 8/11 patients had carcinoid syndrom and 11 high serotonin levels. Eleven were pancreatic ET, 5/11 with clinical functional syndrome and high hormonal blood levels (1 vipoma, 1 glucagonoma, 1 insulinoma, 2 gastrinomas). All had documented progressive disease in the past six months before Interferon treatment. They all had been pretreated: surgery (20) including liver transplantation (2), octreotide (13), chemotherapy (10) and hepatic chemoembolisation (7). The median time beetween first diagnosis and interferon treatment was 3.9 years (1 month - 17.4 years). Interferon was given subcutaneouly three times a week first at 1.5 MU per injection and escalated to 5 MU until progression.

Results: Median follow -up was 23 months (6 - 80). Among 12 patients with secretory syndromes, 10 (83%) had objective responses (OR): 3 complete (CR) and 7 partial (PR), including 7/8 carcinoid syndromes (2 CR) and 3 pancreatic ET (1 CR). Hormonal responses were evaluated in 13 patients, with 8 OR (3 CR). Effects on tumeur burden could be assessed in 20 patients. These were 2 OR (9%), 11 stable disease (SD, 50%), 7 progressive disease (PD, 41%), with similar profiles in enterochromaffin and pancreatic ETs. Eleven patients are still on treatment. As regards toxicity, 2 patients discontinued Interferon at 2 and 6 weeks, respectively. Other effects included neutropenia, anemia, elevated transaminases, dysthyroidism, sexual dysfunction and flux-like syndrome, each in 4 patients at most.

Conclusion: were observed 59% stabilisation with 9% OR in patients with progressive endocrine tumors after previous therapy. Tolerance was fair. Interferon in useful in a substantial proportion of patients with ETs, and should be prospectively evaluated against other treatment modalities.

Tumour biology/Human genetics

400 POSTER

Isolation of human leukocyte antigen (HLA)-associated peptide(s) in the absence of HLA-restricted specific cytolytic T lymphocytes (CTL)

A.M.E. Nouri, N. Torabi-Pour, R.T.D. Oliver. *Departments of Medical Oncology, Bart's and The London Trust, London, UK*

The human leukocyte antigens (HLA) class I and class II are critical molecules for T cell recognition of endogenous and exogenous non-self antigens and hence are the major players in immune surveillance. As bladder cancer is one of the most immune sensitive tumours, based on their response to BCG, a unique combination of an in-housed established bladder tumour cell lines Fen (the original class I negative and class I positive Fen cells after the restoration of the missing class I antigen by β2-m gene transfection) were used for detail biochemical analysis of the nature of the corrected class I antigens and the associated peptides using various approaches. These included: immuno-precipitation, dot blot, immunocytochemical staining, SDS PAGE and high performance liquid chromatography (HPLC).

The results showed that.

(1) Trasfection of Fen cell line with normal β2-m gene resulted in restoration of missing class I antigens as assessed by HPLC and dot blot assay.
(2) Both interferon alpha (IFNα and IFNγ stimulation of cells led to an up-regulation of class I antigens, more so in the case of IFNγ.
(3) The intact class I antigens could be isolated from lysate of the β2-m gene transfected cells using sepharose CNBr-W6/32 beads and DEA as a dissociation reagent.
(4) Dissociation of class I antigens from heads by DEA and analysis by the SDS PAGE showed the presence of both free heavy and light chains of class I antigens.
(5) More than 20 class I-associated peptides with molecular weight of 700 to 3000 Daltons could be isolated from W6/32-loaded beads but only from lysate of HLA positive Fen cell line. The data also showed that 1×10^6 of positive Fen cells contained about 200 ug total protein of which about 0.10ug was class I of which about 2 ng was class I-associated peptides.

These findings demonstrated that gene transfection approach could be used to restore missing class I antigens on otherwise a class I negative bladder tumour cell line. The results also showed the feasibility of using various immuno-biochemical techniques to isolated HLA-associated peptides form lysate of a class I positive tumour cell line in the absence of specific cytolytic T lymphocyte (CTL). These approaches may provide a realistic possibility for extraction and identification of putative tumour specific peptide(s) from tumour specimens with the aim to use such peptide(s) for immunotherapy in cancer patients.

401 POSTER

Profile of p53 expressions in human tissue biopsies of bladder and head and neck tumours: Effects of various in vitro manipulations of p53 on tumour cell behaviour in vitro

A.M.E. Nouri, H. Cannell, R.T.D. Oliver. *Depts. of Medical Oncology and Maxillofacial Surgery; The Royal London Trusts Hospitals, London E1, UK*

In this investigation the profile of p53 expression in tumour tissue biopsies from bladder and from oro-phamgeal tumours was investigated using immunocytochemical staining method. In addition, various techniques including SDS page gel electrophoresis, colorimetric assay and gene transfection were used to investigate the influence of p53 on the behaviour of established human tumour cell lines in vitro. The results showed that:

(a) positive p53 expression was present in more than 40% of cases from both regions, although their profile of the expression differed.

(b) both gamma radiation and cisplatin treatment of tumour cell lines showed induction of p53.

(c) the susceptibility of two cell lines, one with constitutive expression of p53 and one with no p53 expression, showed that the expressing cells were more sensitive to the gamma radiation.

(d) the insertion of wild type and therefore non-mutated p53 into a bladder tumour cell line showed that the inserted cells apoptosed very rapidly whereas the cells inserted with the mutated p53 survived.

If these data could be translated to an in vivo setting, it would be possible that the introduction of wild type p53 gene by gene transfection into tumour cells independent of their p53 gene mutational status, would prove to be beneficial in that if the cellular p53 gene is mutated, the introduction of the

normal p53 gene would induce the tumour cells to apoptose. Alternatively if the p53 is normal then the level of the p53 expression would enable the cells to become more chemo-sensitive to agents such as cisplatin.

Infections and cancer

402 POSTER

Haematological colony-stimulating factors (CSF) in febrile neutropenic patients. A systematic review of the literature with meta-analysis

T. Berghmans[1], M. Paesmans[1], J.J. Lafitte[2], A.P. Meert[1], C. Mascaux[1], E. Steels[1], F. Vallot[1], J.P. Sculier[1]. [1] *Institut Jules Bordet, Internal Medicine, Bruxelles, Belgium;* [2] *CHU Calmette, Pneumology, Lille, France*

Purpose: To assess the role of G-CSF and GM-CSF in the treatment of febrile neutropenic cancer patients, we conducted a systematic review of the randomised trials published as full papers on this topic.

Methods: A methodological evaluation using a specifically designed quality scale was performed before meta-analysis. The effect of CSF on mortality was measured by the odds ratio, estimated in each individual trial. A combined odds ratio was obtained using the method described by Peto.

Results: Eleven trials were eligible of which 8 were meta-analysable (962 febrile neutropenic episodes). The median quality score for the 11 pooled trials was 58.3% (range: 33.3%-68.8%). The lack of significant quality difference ($p = 0.36$) between positive (CSF more effective) and negative trials allowed us to perform a quantitative aggregation of the individual studies results. No advantage on mortality due to febrile neutropenia was detected for the use of CSF with an odds ratio of 0.69 (95% CI 0.42-1.15; $p = 0.98$). The odds ratio was 0.61 (95% CI 0.34-1.09; $p = 0.43$) in the G-CSF subgroup and 1.05 (95% CI 0.36-3.05; $p = 0.99$) in the GM-CSF subgroup. No other meaningful quantitative aggregation could be performed due to the lack of adequate data in the publications.

Conclusions: On the basis of this review, we cannot recommend the routine use of G-CSF or GM-CSF in established febrile neutropenia.

403 POSTER

Infectious complications after autologous peripheral blood progenitor cell transplantation (PBPCT) in breast cancer (BC) patients

A. Zambelli, G.A. Da Prada, L. Velutti, L. Ponchio, G. Robustelli della Cuna. *Salvatore Maugeri Foundation, Medical Oncology Division, Pavia, Italy*

We retrospectively analyzed the infectious complications in 148 patients, median age 46 yrs (range 23-64), who underwent high dose chemotherapy and PBPC autologous support plus G-CSF, for breast cancer both in primary (pBC) and metastatic setting (mBC). Mobilizing regimen and myeloablative treatments varied according to the setting of pts. PBPC mobilization was obtained with high-dose CTX plus G-CSF regimen in the 102 pBC pts while Paclitaxel+Epirubucin plus G-CSF regimen was used in the 46 mBC pts.

Myeloablation included high-dose alkylators-based regimen: TioTepa + LPAM for pBC, and CTX + TioTepa + Mitoxantrone in the mBC setting.

Pts were isolated in a germ-free room after myeloablative treatment and received antimicrobial iv prophylaxis with quinolone, fluconazolo and acyclovir. Median time for neutrophils (ANC>0.5x10e9/L) and platelets (>20x10e9/L) recovery were respectively 10 and 9 days. One hundred and twenty eight patients (86%) developed fever (>38.5 °C, median 4 days, range 2-10); bacteremia occurred in 28 pts (22%): 23 of them (81%) had Gram positive while 5 (19%) had Gram negative bacterial infections. There were no fungal infections or infection-related deaths.

The development of bacteremia was strongly associated with grade IV mucositis ($P<0.001$; odds ratio 11.8) and with neutropenia ANC<0.1x10e9/L lasting more than 5 days ($P<0.01$; odds ratio 4.5), without any significant difference between the 2 setting of pts. Febrile women received a second line antimicrobial therapy with amikacin, ceftazidime and teicoplanin. In most of these cases the severity of the infections was moderate and no life-threatening infectious complications were observed. In our experience, the severe but short-lasting neutropenia and mucositis are significantly associated with incidence of bacterial infections after PBPCT but, with appropriate anti-infective treatment, these infections can be managed without sequelae.

Angiogenesis

404 POSTER

X rays affect the extracellular matrix in a way that favours both normal and tumour-induced angiogenesis

E. Giannopoulou[1], P. Katsoris[2], M. Hatziapostolou[1,2], D. Kardamakis[3], E. Kotsaki[1], C. Polytarchou[1], A. Parthymou[1], S. Papaioannou[1], E. Papadimitriou[1]. [1] *University Of Patras, Pharmacy, Patras, Greece;* [2] *University Of Patras, Biology, Patras, Greece;* [3] *University Of Patras, Medicine, Patras, Greece*

Purpose: Previous studies, using the chicken embryo chorioallantoic membrane (CAM) model of in vivo angiogenesis, have shown that X rays have an antiangiogenic effect. In the present study, we tried to clarify some of the mechanisms through which X rays regulate angiogenesis.

Methods: Apoptosis was studied using DNA fragmentation and acridine orange staining. The amounts of the proteins of the ECM and their mRNAs were quantitated using image analysis of the corresponding Western blots and RT-PCRs respectively. An ELISA test has been used to quantitate the amounts of integrin alpha1 β3 and zymography was used for MMP-2. Tissue localization of ECM proteins or tumor cells implanted onto CAM, has been performed by immunohistochemistry and histochemistry, using paraffin sections of CAMs.

Results: Apoptosis was evident within 1-2 h, but not later than 6 h after irradiation. Fibronectin, laminin, collagen type I, integrin alpha1 β3 and MMP-2 protein amounts were all decreased 6 h after irradiation. In contrast, collagen type IV, which is restricted to basement membrane, was not affected by irradiation of the CAM. There was a similar decrease of gene expression for fibronectin, laminin, collagen type I and MMP-2, 6 h after irradiation. The levels of mRNA for integrin alpha1 β3 and collagen type IV were unaffected up to 24 h after irradiation. The decrease in both protein and mRNA levels was reversed at later time points and 48 h after irradiation, there was a significant increase in the expression of all the genes studied. When C6 glioma tumour cells were implanted on irradiated CAMs, there was a significant increase in the angiogenesis induced by tumour cells, compared to that in non-irradiated CAMs.

Conclusion: Although X rays initially have inhibitory effect on angiogenesis, their action on the ECM enhances normal and tumour-induced vessel formation.

405 POSTER

The angiogenic role of harp, a novel growth factor

E. Papadimitriou[1], A. Polycratis[2], M. Heroult[3], G. Kokolakis[2], J. Courty[3], P. Katsoris[2]. [1] *University of Patras, Department of Pharmacy, Patras, Greece;* [2] *University of Patras, Department of Biology, Patras, Greece;* [3] *Universite Paris Val de Marne, Laboratoire de Recherche sur la Croissance Cellulaire, la Reparation et la Regen, Paris, France*

Purpose: HARP (Heparin Affin Regulatory Peptide) is a 18 kDa secreted protein with distinct lysine-rich clusters within both the NH2- and COOH-terminal domains. It is a growth factor that exhibits a high affinity for heparin and is localized in the extracellular matrix, where it interacts with glycosaminoglycans. In the present work, we studied the angiogenic action of HARP and two peptides representing the termini of the molecule (HARP residues 1-21 and residues 121-139), which are rich in lysine and have high affinity for heparin.

Methods: Angiogenic action was studied in the in vitro models of matrigel and collagen gels. As an in vivo model, we used the chicken embryo chorioallantoic membrane (CAM). Quantification of the vessel networks was performed with image analysis of digitized images. Migration studies were performed using Boyden chamber tests. Human recombinant HARP was expressed and purified from E. coli.

Results: HARP induces migration and stimulates endothelial cells to form tubular, capillary like structures in several in vitro models of angiogenesis (matrigel, collagen and fibrin gels). Using the in vivo model of chicken embryo CAM, we found that HARP has an angiogenic effect. This biological action was seen whether or not HARP has the three amino acid extension of the N terminus region and the exact role of those amino acids remains unclear. The two HARP peptides tested are also angiogenic in most of the assays used. They both stimulate in vivo angiogenesis and in vitro endothelial cell migration and tube formation on matrigel.

Conclusions: We conclude that HARP has an angiogenic activity and its NH2 and COOH termini seem to play an important role.