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RNAi mediated downregulation of bcl-2 and xIAP may have therapeutical potential in human breast adenocarcinoma

R.T. Lima[1], L.M. Martins[2], J.E. Guimarães[1,3], C. Sambade[1,3], M.H. Vasconcelos[1,4]. [1] *IPATIMUP-Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal;* [2] *Cancer Research UK, London, UK;* [3] *Faculdade de Medicina da Universidade do Porto, Porto;* [4] *Faculdade de Farmácia da Universidade do Porto, Porto, Portugal*

Purpose: Resistance to cytotoxic drugs may be partly due to resistance to apoptosis, which may be conferred by genes such as bcl-2 or xIAP. Therefore, we investigated if downregulation of bcl-2 or xIAP gene expression with siRNAs sensitised MCF-7 human breast adenocarcinoma cells to etoposide and doxorubicin.

Methods: Cells were transfected either with control siRNAs or with siRNAs designed against bcl-2 mRNA or against xIAP mRNA. For the chemosensitisation studies, cells were treated with the IC50 dose of etoposide or doxorubicin. Uptake of the FITC-siRNAs was studied by fluorescent microscopy and bcl-2 or xIAP downregulation was verified by Western blotting. Cellular proliferation studies were carried out with the BrdU incorporation assay and apoptosis was verified with the TUNEL assay. The number of viable cells following treatment with siRNAs and/or chemotherapeutical drugs was verified with the Trypan blue exclusion assay.

Results: Both siRNAs were taken up by the MCF-7 cells. RNA interference was confirmed, protein downregulation being stronger at 48 hours following transfection. Both siRNAs caused an inhibition of cellular proliferation and an increase in apoptosis. RNA interference of bcl-2 sensitised cells to etoposide and doxorubicin. However, siRNAs for xIAP did not have a significant effect on sensitisation of cells to either of these drugs.

Conclusion: RNA interference was possible in human MCF-7 breast adenocarcinoma cells. Downregulation of bcl-2 or xIAP inhibited cellular proliferation and induced apoptosis. Downregulation of bcl-2 sensitised MCF-7 cells to etoposide and doxorubicin.

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Which complement regulatory proteins could be a good target for a breast cancer vaccine?

Z. Madjd[1], S. Pinder[2], I. Spendlove[3], L. Durrant[4]. [1] *University of Nottingham, CRUK Clinical Oncology, Nottingham, United Kingdom;* [2] *University of Nottingham, Histopathology, Nottingham, United Kingdom;* [3] *University of Nottingham, CRUK Clinical Oncology, Nottingham, United Kingdom;* [4] *University of Nottingham, CRUK Clinical Oncology, Nottingham, United Kingdom*

Introduction: To avoid self-attack by complement, cells express complement regulatory proteins CD59, CD55 and CD46. Invasive ductal breast carcinomas showed variable expression of these proteins. Targeting complement regulatory proteins is potentially a very attractive approach to tumour therapy, as these vaccines can eliminate any cell over-expressing a complement inhibitor.

Aim and methods: We have investigated the correlation between CD55 and CD59 expression and tumour characteristics, patient features and outcome in a series of primary operable breast cancers diagnosed between 1987 and 1992. 500 tumour samples were stained using an anti-CD55 monoclonal antibody RM1 (developed in the department) and anti-CD59 (clone MEM-43). As there are no commercially available anti-CD46 antibodies which react on paraffin sections, we have recently developed a monoclonal antibody specific to CD46 that looks promising on material processed in this way.

Results: 95% of the tumours showed positive immunoreactivity for both CD55 (RM1) and CD59. High expression of CD55 and CD59 was significantly associated with low histological grade ($p<0.001$) and good prognosis tumours (Nottingham prognostic Index <3.4) ($p<0.001$). There was a significant relationship between CD55 and CD59 expression and overall survival showing that loss of CD55 and CD59 in breast tumours correlates with poor survival ($p<0.001$, $p=0.006$ respectively). The anti-CD46 antibody is now being used to screen the same breast tumour tissue arrays as assessed with anti-CD59 and anti-CD55 (RM1) antibodies to complete the picture of the role of these complement inhibitory proteins in tumour prognosis.

Conclusion: These data indicate unexpectedly that loss of CD55 and CD59 is associated with aggressive breast tumours. There appears, however, to be an inverse association between loss of CD55 and increased expression of CD46 in malignant breast tumours and over-expression of CD46 breast tumours could potentially be a good target for a breast cancer vaccine.

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Additive cytotoxic and proapoptotic effects of external radiation and Rituximab on B cell lymphoma cell lines in vitro.

H. Schmidberger[1], S. Dingeldein[2], M. Rave-Fraenk[1], J. Hasenkamp[2], W. Jung[2], A. Rokita[2], O. Pradier[1], B. Glass[2]. [1] *University Goettingen, Radiotherapy and Radiation Oncology, Goettingen, Germany;* [2] *University Goettingen, Haematology and Oncology, Goettingen, Germany*

Background: The combination of the anti-CD20 antibody Rituximab with chemotherapy including anthracylin and steroids has been shown to be effective against B cell lymphoma cells in vitro and in vivo. However, little is known about combinations of Rituximab and external radiation.

Material and Methods: The cytotoxic effect of rituximab (1 microg/ml and 10 microg/ml, respectively) given 20 h after radiation (10, 20 and 40 Gy, respectively) was investigated in transformed lymphoma cell lines: 2 follicular lymphoma (SU-DHL4 and Karpas 422), 2 Burkitt lymphoma (Daudi and Ramos) and 1 diffuse large cell lymphoma (Balm3). Endpoints were: antibody-dependent cell-mediated cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), and direct apoptotic effects. ADCC and CDC were assessed by by propidium jodid (PI) staining in a flowcytometric analysis; direct cytotoxic effects were determined by Annexin V externalization.

Results: Although all cell lines expressed high levels of CD20 antigen, the susceptibility to Rituximab differed significantly between cell lines. In general, if a CD20+ B-NHL cell line showed susceptibility to a certain effect of Rituximab, this effect added to the cytotoxic effect of radiation. Supra-additive (synergistic) effects were not observed in these experiments. For example, SU-DHL4 cells were susceptible to the direct apoptotic effect of Rituximab with 29% of the cells being Annexin V positive. After 10, 20, and 40 Gy of radiation, 11%, 12%, and 19% of the cells stained positive for Annexin V, respectively. After combined treatment, (10 microg/ml Rituximab + 10, 20, or 40 Gy) 36%, 37%, and 38% of the cells became apoptotic, respectively. Daudi cells were grossly resistant to direct apoptotic effects of Rituximab but susceptible to ADCC exerted by the antibody. These effects added to radiation induced cell damage: 34% of the cells were PI-positive after incubation with NK cells and Rituximab solely, radiation with 10, 20, and 40 Gy resulted in 15%, 18%, and 27% PI-positive cells, respectively. After combined treatment, 46%, 50%, and 53% of the cells became PI-positive. Ramos cells were typical targets for CDC obtained with Rituximab. 22% of the cells were PI-positive after incubation with complement and Rituximab. Radiation with 10, 20, and 40 Gy resulted in 13%, 18%, and 28% PI-positive cells, respectively. After combined treatment, 47%, 50%, and 58% of cells became PI-positive. The reaction of Karpas 422 cells and Balm3 cells was comparable to Daudi cells.

Conclusion: Incubation with Rituximab twenty hours after irradiation resulted in an additive interaction in all cell lines and at all end points tested. In current experiments, different time scheduling and the mechanisms of interaction are under investigation.

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A phase I/II single arm trial to determine the safety, tolerability, and biological activity of intrahepatic delivery of doxorubicin hydrochloride adsorbed to magnetic targeted carriers (MTC-DOX) in patients with metastatic tumors in the liver

R. Just[1], C. Hoh[2], T. Vogl[3], P. Neese[4], J. Doemeny[5], M. Schechter[5], R. Varney[5], W. Stanton[8], M. Schiemann[3], P. Goldfarb[7]. [1] *Scripps Clinic and Research Foundation, Hematology/Oncology, Escondido, USA;* [2] *University of California, San Diego, Nuclear Medicine, San Diego, USA;* [3] *University Hospital Frankfurt am Main, Interventional Radiology, Frankfurt, Germany;* [4] *Scott and White, Interventional Radiology, Temple, USA;* [5] *Scripps Mercy Hospital, Interventional Radiology, San Diego, USA;* [6] *Scripps Mercy Hospital, Hematology/ Oncology, San Diego, USA;* [7] *Scripps Mercy Hospital, Surgery, San Diego, USA*

Purpose: To test the safety and metabolic tumor activity following a single selective arterial infusion of doxorubicin adsorbed to Magnetic Targeted Carriers (MTC-DOX) under magnetic guidance in patients with metastatic disease in the liver from various primary tumor types.

Materials and Methods: A phase I/II dose escalation study was undertaken in up to 20 patients. MTC-DOX (a combination of doxorubicin bound to magnetic targeted carrier 1:8.3 w:w) was delivered regionally to the tumor via arterial catheterization. An external magnet (field strength of 5 KG) was positioned over the tumor to both guide the MTC-DOX into the proper location and to extravasate the material into the tumor parenchyma. Tumor localization of MTC-DOX was confirmed by MRI post administration. A

range of metastatic tumor types was treated. Hepatic PET and CT imaging was obtained prior to and 21 days following therapy and analyzed for tumor response.

Results: Data is available on 15 patients. Regional localization of MTC-DOX to the tumor was achieved in patients with metastatic disease even though many of the lesions were not hypervascular. Dose escalation was completed to a maximum dose of 1 mg DOX, 8 mg MTC /cm^2 of tumor cross sectional area up to a total dose of 60 mg DOX, 500 mg MTC. Adverse events reported to date were predominantly mild to moderate in severity. The most common adverse events (% of reported events) were transient abdominal pain (10%), nausea (10%), malaise (7%), anorexia (7%), and fever (7%). Three patients were not analyzed by PET due to poor uptake of FDG. The treated tumors in six patients demonstrated a significant reduction in FDG activity (between 20-60% of baseline) at 21 days following a single administration of MTC-DOX. Tumor types demonstrating a response by PET imaging following MTC-DOX treatment included adenocarcinoma of unknown origin, breast carcinoma, bladder, cholangiocarcinoma, renal cell, and colon carcinoma. The treated tumors in six patients did not demonstrate any reduction in FDG activity (between 75-116% of baseline).

Conclusion: MTC-DOX can be localized regionally following intra-arterial administration without clinically significant toxicities. MTC-DOX demonstrates possible activity against some hepatic metastases as measured by FDG activity and PET imaging. Further studies are warranted in order to better ascertain the potential therapeutic role of MTC-DOX in patients with metastatic tumors to the liver.

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Stromal contribution to elevated YKL-40 in human cancer

N. Junker[1], J.S. Johansen[2], P.E.G. Kristjansen. [1] *University of Copenhagen, Laboratory of Experimental Oncology, Copenhagen;* [2] *Herlev University Hospital, Department of Medicine, Herlev, Denmark*

High serum levels of YKL-40 are found in patients with late stages of colorectal, breast, ovarian, and malignant glioma, and high serum YKL-40 is a prognostic marker of short survival. YKL-40 is a secreted 40 kD extracellular matrix glycoprotein belonging to the mammalian chitinase-like proteins but without chitinase activity. YKL-40 is expressed by chondrocytes, macrophages, and neutrophils as well as several cancer cell lines. The expression pattern of YKL-40 suggests involvement in tissue remodeling. YKL-40 stimulates proliferation of fibroblasts, endothelial cell migration and tube formation.

We have investigated the expression pattern of YKL-40 on mRNA and protein level in 20 human small cell lung cancer (SCLC) and 3 human malignant glioma lines *in vitro* and *in vivo*. None of the SCLC lines had measurable YKL-40 protein expression in cell culture or when grown as solid tumors on nude mice. The three glioma lines had YKL-40 mRNA production but only U87 secreted the protein in measurable amounts both when grown in culture and as solid tumors. Stromal (mouse) YKL-40 mRNA expression was found by RT-PCR in all tumors.

To further investigate the origin of elevated YKL-40 levels in human cancers, human umbilical cord endothelial cells (HUVEC) and primary human fibroblast GM38B were tested for YKL40 expression when grown in culture and after ionizing radiation. Neither HUVECs nor GM38B expressed YKL-40 under these conditions.

Our data on 23 cancer cell lines (SCLC and malignant glioma) suggest that stromal production of YKL40 is responsible for some of the elevated serum YKL-40 levels associated with poor prognosis in several cancer types. However, our data also indicates that neither stromal fibroblasts nor endothelial cells are responsible for the YKL-40 production. Further investigations are needed to determine the possible role of myofibloblasts, macrophages, neutrophils, and other cell types in YKL-40 expression during malignant cancer progression.

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Phase I/II trial evaluating blockade of tumour blood supply and tumour cell proliferation with combined bevacizumab and erlotinib HCl as targeted cancer therapy in patients with recurrent non-small cell lung cancer.

R.S. Herbst[1], E. Mininberg[1], T. Henderson[1], E. Kim[1], W.K. Hong[1], R. Mass[2], W. Novotny[2], B. Garcia[3], D. Johnson[3], A. Sandler[3]. [1] *University of Texas, M.D. Anderson Center, Houston, TX, USA;* [2] *Genentech, S. San Francisco, CA, USA;* [3] *Vanderbilt University, Nashville, TN, USA*

Introduction: Non-small cell lung cancer (NSCLC) has resisted all therapeutic approaches for decades and its incidence is increasing. Bevacizumab (Avastin") is a recombinant humanised monoclonal antibody against vascular endothelial growth factor (VEGF) that neutralises' VEGF and prevents it from mediating tumour blood vessel growth and maintenance. Erlotinib HCl (Tarceva") is an epidermal growth factor receptor (HER1/EGFR)-tyrosine kinase inhibitor that optimally blocks HER1/EGFR activation and downstream cell signaling pathways, inhibiting tumour growth. Tumours are biologically heterogenous so the rationale for this study exploits this fact by giving these two targeted cancer therapies in combination to patients with recurrent NSCLC.

Materials and methods: Phase I objectives are to establish maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs) for these two agents combined in 18 patients, and to use this information to establish a regimen for subsequent phase II evaluation of efficacy and tolerability at MTD. Pharmacokinetic and pharmacodynamic parameters will be monitored throughout both study phases to look for any interaction. A standard 3+3 design was used with no intrapatient dose escalation allowed. Regimens comprised: dose level 1, bevacizumab 7.5mg/kg iv q3w + erlotinib 100mg/day po; dose level 2, bevacizumab 15mg/kg iv q3w + erlotinib 100mg/day po; and dose level 3, bevacizumab 15mg/kg iv q3w, erlotinib 150mg/day po. DLT is defined as a NCI-CTC grade 3 toxicity or greater, not adequately controlled with appropriate therapy. All patients with advanced or recurrent non-squamous NSCLC who had previously failed at least one chemotherapeutic treatment were eligible for the trial.

Results: To date, 12 patients have been enrolled in phase I of whom three each have been treated at dose levels 1 and 2, respectively, and six at dose level 3. No patients developed DLTs, and of nine evaluable patients, three showed partial responses, including one at dose level 1 and one at dose level 2, and two showed minor response. Preliminary pharmacokinetic analysis revealed no interaction between bevacizumab and erlotinib. Final phase I data will be presented.

Conclusion: Preliminary data from this phase I trial of a combination of two targeted cancer therapies blocking different aspects of cell biology pivotal to tumour growth and development are encouraging. The bevacizumab with erlotinib combination is well tolerated with no unexpected adverse events.

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Microvascular transfers in the elderly in oncology.

P. Vico[1], D. Dequanter[2], B. Nokerman[1]. [1] *Clinique Generale Saint Jean, Plastic Surgery, Brussels, Belgium;* [2] *Institut Jules Bordet, Head And Neck Surgery, Brussels*

Background: Microsurgery is often considered as a serious surgical procedure, especially in the elderly population. This group is considered to be at a high surgical risk and on the hold receive substandard treatment.In selected cases, a microsurgical free flap is the last chance of a good quality life for those patients.

Material and methods: Thirteen patients over 70 years were operated on from July 1994 to April 2002 (13 patients - 15 flaps - mean age: 74 years; range: 70-87 years - 7 males-6 females). Indications were wide excisions for skin carcinoma or melanoma (N=3), abdominal wall defect after irradiated bladder cancer (N=1), soft tissue sarcoma (N=2),and head and neck cancer (N=7). Musculo/cutaneous (n=9), fascio /cutaneous (n=4) and osteoseptocutaneous (n=2) free flaps were performed under general anesthaesia during the same operative time than cancer resection in 10 cases, to treat a late complication of the medical treatment of the cancer (radiotherapy) in 6 cases or of an early complication in 1 case (flap necrosis). Co-morbidity was studied and evaluated following the ASA criteria.

Results: Complications were: Complete flap necrosis (n=1 (6.5%) - leading to a second successful free flap), skin paddle necrosis in a fibular osteoseptocutaneous flap conservatively treated (n=1). Minor complications were seroma of the donor site (n=2 (13%)). In the other cases, patients recover without any further complications. The mean operative time was 8 hours for the whole procedure (resection and reconstruction) (range: 5h30 - 10h10); the mean blood loss was 950 ml (range: 250 - 3000). Seven patients are still alive without any reccurrence of the cancer except in one case (mean follow-up 57 months), 8 died from a generalization of the cancer except in one case 10 months after microsurgery (range of 1-20 months).

Conclusions: In our limited experience, microsurgery can be the ultimate surgical option in order to resolve difficult problems in a selected group of elderly people with oncological disease. The success rate is about 93.5% without any major complications. In almost all cases, the clinical problem was primarily under treated because the age of the patient was considered as a contra-indication factor for radical surgery. Survival rates with and without recurrence, lead us to conclude that age is not a contra-indication factor and that this surgery can be ethically proposed in a selected number of cases.