of specific molecular/genetic pathways in diseases such as cancer (e.g., FDG PET). Biomarker imaging is very likely to be less specific and more limited with respect to the number of molecular-genetic processes that can be imaged. Nevertheless, it benefits from the use of radiopharmaceuticals that have already been developed and are currently being used in human subjects. This application strategy is most dramatically illustrated by the use of [18F]FDG PET to image the response, recurrence and progression of particular tumors (e.g., Gleevac treatment of GIST). The translation and application of biomarker imaging paradigms into patient studies, using clinically-approved radiopharmaceuticals or contrast agents, will be far easier than either the direct imaging or reporter transgene imaging paradigms.

Reporter gene imaging studies will be more limited in patients compared to that in animals, due to the necessity of transducing the target tissue or cells with specific reporter constructs, or the production of transgenic animals bearing the reporter constructs. Ideal vectors for targeting specific organs or tissue (tumors) do not exist at this time, although this is a very active area of human gene therapy research. Each new vector requires extensive and time-consuming safety testing prior to regulatory approval for human administration. Nevertheless, reporter gene imaging, particularly the genetic labeling of cells with reporter constructs, has several advantages. There are now three well-defined human genes (*hNIS, hNET and hSSTR2*) with complimentary, clinically approved, radiopharmaceuticals for PET or gamma camera imaging in patients. These complimentary pairs (gene + probe) are excellent candidates for future reporter gene imaging in patients. Importantly, these human genes are less likely to be immunogenic compared to the reporter genes currently used in animals (e.g., viral thymidine kinases, luciferases, fluorescent proteins). It should also be noted that a single reporter gene – reporter probe pair can be used in different reporter constructs to image many different biological and molecular-genetic processes. Once a complimentary reporter-pair (gene + probe) has been approved for human studies, regulatory issues will focus will shift to the particular backbone and regulatory sequence of the reporter construct.

The major factor limiting translation of reporter gene imaging studies to patients is the "transduction requirement"; target tissue or adoptively administered cells must be transduced (usually with viral vectors to achieve high transduction efficiency) with reporter constructs for reporter gene imaging studies. At least *two* different reporter constructs will be required in most future applications of reporter gene imaging. One will be a "constitutive" reporter that will be used to identify the site, extent and duration of vector delivery and tissue transduction or for identifying the distribution/trafficking, homing/targeting and persistence of adoptively administered cells (the "normalizing" or denominator term). The second one will be an "inducible" reporter that is sensitive to endogenous transcription factors, signaling pathways or protein-protein interactions that monitor the biological activity and function of the transduced cells (the "sensor" or numerator term). The initial application of such double-reporter systems in patients will most likely be performed as part of a gene therapy protocol or an adoptive therapy protocol where the patients own cells are harvested (e.g., lymphocytes, T cells or blood-derived progenitor cells), transduced with the reporter systems and expanded *ex vivo*, and then adoptively re-administered to the patient. For example, adoptive T cell therapy could provide a venue for imaging T cell trafficking, targeting, activation, proliferation and persistence. These issues could be addressed in a quantitative manner by repetitive PET imaging of the double-reporter system described above in the same subject over time.

Once in place, Cancer Clinical Trials and Personalized Medicine will be able to benefit from the noninvasive imaging paradigms described above; similar to the benefits of sequential FDG PET scans performed today in order to monitor GIST tumor response and recurrence. The ability to visualize transcriptional and post-transcriptional regulation of endogenous target gene expression, as well as specific intracellular protein-protein interactions in patients will provide the opportunity for new experimental venues in patients. They include the potential to image the malignant phenotype (e.g., signal pathway activity) of an individual patient's tumor at a molecular level and to monitor changes in the phenotype over time. The potential to image a drug's effect on a specific target molecule or signal transduction pathway in an individual patient's tumor provides the opportunity for monitoring treatment response at the molecular level.

207 INVITED

Causes and consequences of glycolysis and acid pH in tumors

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Over the last decade, we have developed and improved MR-based techniques with which to measure the pH of tumors. These have included 31-P MRS and 1-H MRSI approaches and, most recently, pH-dependent relaxometry using pH-dependent contrast agents. This latter approach has been particularly challenging as it required simultaneous and independent quantification of relaxation rates and contrast agent concentration. All of these approaches have shown that the extracellular-interstitial pH (pHe) of tumors is unequivocally acidic, reaching as low as pH 6.7. This low pHe is caused by high glucose metabolism in tumors coupled with poor perfusion. The high glucose metabolism occurs in the presence or absence of oxygen, also known as the Warburg Effect., WE. There is evidence, by us and others, that the WE is hardwired in the most aggressive tumors, and that this can occur through the oncogenic activation of at least 6 different pathways. Darwinian evolution selects for phenotype, not genotype and thus, we have proposed that the glycolytic phenotype is evolutionarily selected early during the in situ stage of carcinogenesis, when it is an avascular disease. This does not explain, however, why this phenotype continues to be selected later in carcinogenesis, when invasive and metastatic cells have access to the vasculature. Examining the sequelae of glucose catabolism yields a finite number of consequences that could lead to further selection, including acid production. Acid production could be selected because it has been shown to induce invasion and exacerbate metastasis. We have proposed that this occurs by induction of cathepsin release and export of H^+ from growing tumors into surrounding parenchyma, thus facilitating their ability to invade host tissue. Notably, tumor acidity can be inhibited with oral buffers, such as bicarbonate, and we have shown that this inhibits spontaneous metastasis in some, but not all, animal tumor models.

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209 INVITED

MR imaging of angiogenesis

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Angiogenesis has been validated as a target in multiple randomized clinical trials that tested the advantage of adding VEGF inhibitors to conventional treatment. There remains a clear need to identify the patients who most benefit from this class of drug as the data demonstrate only a modest improvement in overall survival if all patients in a defined disease population are treated; some pre-clinical and clinical data suggest that maintenance therapy is required; the drugs can be toxic; and because the development of combination regimens that include VEGF inhibitors can only occur once we have learned how to identify the patients who most benefit from this class of drug.

Biomarker science is evolving to address the issue of treatment individualisation. Imaging offers the advantage of allowing serial measurements of tumour vascular pathophysiology and has been implemented throughout the development of anti-angiogenic agents. To date multiple clinical trials have evaluated VEGF inhibitors with Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI) and have demonstrated a relationship between drug dose and reductions in DCE-MRI parameters and secondly between the reduction in DCE-MRI parameters and patient benefit. These relationships are confounded by heterogeneity. However, histogram analysis of imaging data, to examine vascular heterogeneity in greater detail demonstrates clinically useful information that is otherwise overlooked.

One of the parameters that evolved from the analysis of heterogeneity was the enhancing fraction, which reflects the vascularity of the tumour. In several clinical trials using MR or CT, in patients treated with anti-vascular agents, cytotoxic drugs or radiotherapy we have demonstrated the clinical value of measuring the vascular enhancing fraction and have shown that this parameter augments traditional prognostic factors. These data led to further clinical trials which demonstrated that VEGF inhibitors reduce the

enhancing fraction and that in patients receiving VEGF inhibitors whose disease is progressing, the tumours manifest an increasing enhancing fraction. Together, these results highlight the potential of the vascular fraction as a potential predictive biomarker for VEGF inhibitors and we are now testing the additional information gathered by measuring imaging and blood borne biomarkers of angiogenesis.
Taken in conjunction with emerging imaging technologies (e.g. ASL) it is now appropriate to test the predictive value of imaging to determine which patients most benefit from anti-angiogenic agents.

Scientific Symposium (Wed, 23 Sep, 09:00−11:00) FLIMS Symposium and ECCO/EJC Young Investigators Award

210 INVITED

The FLIMS Workshop

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The 1st Annual Workshop "Methods in Clinical Cancer Research" was held in Flims, Switzerland in 1999 and was based on a similar training workshop held at Vail, USA. This Workshop had been borne out of a concern about the shortage of young clinical researchers and the increasing need to conduct clinical trials with new anticancer agents. The European Workshop was run under the auspices of FECS (now ECCO), AACR and ASCO). Funding was obtained from these organisations and a grant from NCI together with contributions from the pharmaceutical industry. Ten courses have been held with almost 800 graduates attending the Workshops. Each Workshop comprises 35 to 40 highly experienced international clinical investigators and several innovative techniques and teaching methods are utilised aimed at intensively guiding and supervising the students through the process of completing a protocol concept sheet and developing a finished protocol by the end of the Workshop week.
Four educational formats are used. Protocol development sessions involve small groups of students with at least 3 dedicated faculty members and this constitutes the core activity of the Workshop. Support is provided by Faculty members to each student so that the final protocol can be developed. Small group discussion sessions are held during the week to cover specialised topics relating to clinical trials development. Lectures and panel discussions are held on a daily basis to cover a variety of specific topics presented by experts in the field. These give an essential overview of the design and conduct of high-quality clinical trials. Where appropriate, lectures on related topics are followed by a panel discussion or round table sessions. One on one sessions are held during the week for individual counselling and advice on protocol and career development. The selection of participants is highly competitive and undertaken after submission by applicants of a trial concept sheet together with CV and letter of recommendation. International peer review is undertaken and successful candidates are selected following this. Constant monitoring of the success of the Workshop is undertaken. A set of objectives were determined at the outset of the Workshops in 1999 and, encouragingly all have been exceeded. Greater than 80% of protocols written during the Workshop were subsequently submitted, approved and funded for candidates in 2001, 2004, 2005 and 2006. Greater than 80% of protocols have subsequently been submitted and approved by Ethics Committees every year except 2001. The Workshop has proven extremely popular with universal positive feedback and the perception by many that this is one of the highlights of their careers. Several important trials have been developed at Flims and have been published in high impact journals.

211 INVITED

Presentation of a Flims study: The diagnostic value of PET/CT for primary ovarian cancer – a prospective study

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Background: To prospectively evaluate the diagnostic value of combined PET/CT for detecting a malignant tumor in patients with a pelvic mass and to identify PET/CT predictors of incomplete/suboptimal primary cytoreduction in advanced ovarian cancer patients.

Methods: From September 2004 to August 2007, 201 patients (median age=61 years, range=21–91 years) with a risk-of-malignancy index (RMI)>150 based on serum CA-125, ultrasound examinations, and menopausal state, underwent PET/CT within 2 weeks prior to standard surgery/debulking of a pelvic tumor. Histological diagnoses were compared to the PET/CT results to calculate the diagnostic value of PET/CT in differentiating between malignant and borderline/benign tumors. In 94 ovarian cancer patients the FIGO stage was compared with the stage indicated on PET/CT. Ten PET/CT features were identified and evaluated as predictors of cytoreduction in 66 patients with advanced ovarian cancer.
Results: The sensitivity of PET/CT for diagnosing a malignant pelvic tumor was 95% (107/113) and the specificity was 91% (80/88). FIGO stage IV was found in 11% (10/94) of ovarian cancer patients. In 44% (41/94) of ovarian cancer patients, PET/CT demonstrated areas of abnormally increased metabolic activity that indicated stage IV, metastatic disease. Complete cytoreduction (no macroscopic residual disease) was achieved in 38% (25/66) of patients with advanced ovarian cancer. Using univariate analysis, predictors of incomplete cytoreduction were large bowel mesentery implants (LBMI) ($P < 0.001$), peritoneal carcinosis ($P < 0.001$), pleural effusion ($P < 0.003$), ascites ($P < 0.01$) and small bowel mesentery implants ($P < 0.02$). Using multivariate analysis, LBMI was the only independent predictor of incomplete cytoreduction ($P = 0.004$).
Conclusion: Combined PET/CT demonstrated high diagnostic value in identifying primary ovarian cancer in patients with a pelvic mass of unknown origin and RMI>150. In patients with advanced ovarian cancer PET/CT located metastases unrecognised by standard staging procedures. In addition, PET/CT predictors of cytoreduction were found. However, those predictors should be used with caution until prospective randomised trials have clarified which subgroup of ovarian cancer patients benefits in terms of survival from neoadjuvant chemotherapy followed by interval debulking.

212 INVITED

Presentation of a Flims study: Randomized phase II study of docetaxel/oxaliplatin and docetaxel in previously treated non-small cell lung cancer patients

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The development of a research protocol is the core activity at the workshop on Methods in Clinical Cancer Research, sponsored by ECCO-AACR-ASCO and held annually in Flims, Switzerland. At the end of the workshop, the "Flims graduates" return to their home institution with an approved protocol to be implemented in the real world! Obtaining Ethics approval, granting financial support, having the study up and running can be challenging and the successful completion of the study up to a publication should not be taken for granted. This presentation will be given by an Italian Medical Oncologist who attended the Workshop in 2004 and whose life has definitely changed as a consequence of this experience. The talk will chronicle the development and implementation of her Flims protocol for a randomized phase II study evaluating the activity of docetaxel plus oxaliplatin in second-line non-small cell lung cancer (NSCLC); the comparator arm was single agent docetaxel. The study was designed as a one-stage, three-outcome phase II trial (Sargent et al, Control Clin Trials 2001) requiring 21 evaluable patients per arm; primary endpoint was response rate. The study was implemented at the student's home Institution with the support of the Alpe Adria Thoracic Oncology Multidisciplinary group (ATOM group). Fifty patients were enrolled at four Italian centers. It was a positive study: the level of activity for the combination docetaxel/oxaliplatin satisfied the pre-defined study primary endpoint, warranting further evaluation of this combination as second-line therapy for NSCLC. Final results have already been presented at International meetings and the manuscript is in preparation. This is only one of hundreds of trials designed during the Workshop since 1999. Without any doubt, the Flims Workshop is the best training opportunity to learn the essentials of clinical trials methodology: all young oncologists with a major interest in clinical cancer research should be encouraged to attend.

213 ECCO/EJC Young Investigators Award

The lessons that can be learned by studying the patterns of local recurrence after primary rectal cancer treatment

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Background: By determination of the subsite of locally recurrent rectal cancer on imaging and relating these to patient, treatment and tumor variables, the mechanisms of local relapse genesis can be reconstructed. The purpose of this study was to analyze the patterns of local recurrence