also promotes cell proliferation and oncogenic transformation [1]. The tumour suppressors RB and p53 keep tRNA expression under tight control by binding and directly repressing the pol III-specific transcription factor TFIIIB [2,3]. Inactivation of RB and/or p53 in cancer cells releases TFIIIB from restraint, allowing tRNA expression to rise. The situation is aggravated by several oncogene products that further stimulate tRNA production. For example, c-Myc binds directly to TFIIIB and raises pol III transcription [4]. Furthermore, mTOR associates with tRNA genes and stimulates their expression in response to signaling from Ras, Akt and PI3 kinases [5]. Combinations of these molecular events ensure elevated tRNA levels in most if not all tumours [6].

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15 INVITED

Small RNA Regulators of Gene Expression

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About fifty years ago the central role of RNA in life sciences was recognized with the discovery of t-RNA, ribosomal RNA and mRNA. A little over ten years ago with the discovery of RNA interference (RNAi), the generality of regulation of gene expression by RNA became apparent. Since then, the biology and biochemistry of small RNAs such as siRNAs, miRNAs and piRNAs have been investigated in many model systems. Over half of all mRNAs in mammalian cells are targets of miRNA and changes in miRNA activity are important in differentiation, growth and control of cell death. Although some miRNAs function as oncogenes where increases in expression promote tumour formation, it is much more common to find loss of miRNA activity as an important event in the development of a tumour. In this mode, miRNAs act as tumour suppressors. In fact, even though miRNAs are critical for normal development and differentiation, many cell lines are viable when the synthesis of all miRNAs is blocked by deletion of a gene essential for their production, Dicer. This puzzling finding suggests that miRNAs have a general role of providing robustness to systems so that transitions between cell states are balanced through the interactions of feed forward and feed backward systems. The small degree of change in gene expression upon loss of miRNAs indicates that they fine-tune protein expression in cells under steady state conditions. As part of the cellular system, the synthesis of miRNAs is regulated at levels of transcription, processing and stability. Developing an integrated concept of the roles of miRNAs will also require understanding their ability to buffer the response of cells to stress.
Deep sequencing of RNA from mammalian cells has revealed classes of small and large non-coding RNAs that are present at approximately one copy per cell. The functions of these RNAs in normal or disease states are not well established. However, there is growing confidence that RNAs can bridge between sequence-specific DNA recognition processes and regulator complexes. One example of this is the recognition of splicing signals in nascent RNA and control of elongation by RNA polymerase.

Scientific Symposium (Sat, 24 Sep, 11:15−13:15)
Molecular Imaging of Hypoxia

16 INVITED

Imaging of Hypoxia With PET Radiotracers, Including, Ca-IX Antibodies

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Hypoxia in tumours occurs when cell proliferation exceeds the rate of angiogenesis; tumour cells are then pushed beyond the boundaries for oxygen diffusion. In cancers, hypoxia is an indicator of poor prognosis, regardless of the treatment modality employed. It is probably one of the leading causes of radio- and chemotherapy failure. Hypoxia imaging with positron emission tomography (PET) is a non-invasive way of measuring regions of low partial oxygen pressure within the tumour tissue. A number of compounds are available for hypoxia imaging. In the past, most studies have used ^{18}F FMISO; other agents in clinical trials include ^{18}F EF5, $^{60/64}$Cu-ATSM, ^{18}F-FETNIM and ^{18}F-FAZA. The ideal hypoxia tracer should show high specific uptake and irreversible retention in hypoxic cells, low background activity in normoxic tissues, chemical stability against enzymatic cleavage in blood, rapid blood clearance enabling early imaging, and scan findings should be reproducible. None of the currently available agents meets all of these requirements. Current clinical trials are investigating the utility of hypoxia tracers for prognostication, radiotherapy target volume planning, and response prediction. For instance, a current multicenter trial with ^{64}Cu ATSM is investigating the prognostic value of hypoxia imaging in cervical cancer. Similar studies are ongoing with ^{18}F FMISO and ^{18}F FAZA in head and neck and rectal cancer. Ultimately, this should lead to changes in therapy regimens for hypoxic cancers (which are resistant to standard therapy). For instance, one clinical trial will investigate if therapy with the VEGF antibody bevacizumab can decrease tumour hypoxia in lung cancer and thus improve treatment response (as compared to chemotherapy alone) and patient outcome. Carbonic anhydrase IX (Ca-IX) is an enzyme that is overexpressed in many hypoxic tumours because it is a downstream target of HIF-1a; it is involved in pH regulation. Ca-IX expression can be imaged with the chimeric antibody cG250 labelled with ^{124}Iodine or ^{89}Zr. Whereas full antibodies may be suboptimal for clinical imaging (long blood circulation time; large size limits tissue penetration), smaller molecules including antibody fragments relying on the same principle, may be more suitable. In hypoxic tumour xenografts, the antibody fragment ^{89}Zr-cG250-F(ab')) showed good correlation with tissue expression of Ca-IX, thus providing the rationale for future clinical trials.

17 INVITED

Interest of Functional Imaging to Guide Stereotactic Radiotherapy

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Stereotactic Body Radiotherapy (SBRT) is used for the treatment of patients with early stage non-small cell lung cancer (T1-T2 N0M0), liver or prostate cancer.
The definition of tretament taget and the evaluation of the treatment's efficacy remains a challenge and, for follow up, it is often difficult to distinguish progression and therapeutic response.
As an example, the target definition for lung tumours is based on PET CT images, CT slices remaining the main informative tool. For liver and prostate, the role of MRI is increasing and the place of functionnal MRI starts to be crucial in case of partial tretaments (prostate boost).
Some issues are specific of SBRT and radiographic features after lung SBRT are significantly different from the images found after standard conformal three-dimensional radiation therapy, in both patterns and chronology. Early (lung injury) and late (lung fibrosis) toxicity must be known in order to differentiate progression from therapeutic response.
The role of functional imaging (PET and MRI) will be described in various clinical situations.

18 INVITED

Imaging of Hypoxia (HIF-1α) With Genetically Encoded Reporter Genes

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Hypoxia is an important factor involved in the progression of solid tumours, and alters tumour metabolism, angiogenesis and metastasis. Adaptation to hypoxia at the cellular or organism level is predominantly regulated by hypoxia inducible factors 1 and 2. HIF-1α, the most studied factor, is frequently activated by genetic alterations and by oncogenic pathway activation (e.g., cMYC, PI3K, MAPK, HSF1), in addition to physical hypoxia. HIF-2α expression has been linked to poor patient outcome in several tumour types, and was detected in tumour stem cells, but not in non-stem tumour cells or normal progenitor cells. The precise role of HIF-2α, in comparison to that of HIF-1α, in target gene activation and in tumour progression remains unclear.
The mechanisms of hypoxia-dependent stabilization of the HIF-1α and HIF-2α proteins and their mode of transcriptional activation are thought to be similar. The oxygen sensing mechanism controlling protein stability of the HIF-α subunits occurs through a post-translational modification within the oxygen-dependent degradation domain (ODDD), and is carried out by HIF-specific prolyl hydroxylase-domain proteins (PHDs). The PHDs hydroxylate two conserved proline residues; the prolyl hydroxylated HIF-α subunits are recognized by the von-Hippel Lindau (VHL) tumour suppressor protein which is part of a multiprotein E3 ubiquitin-ligase that polyubiquitylates and targets HIF-α for proteasomal degradation. The O_2-independent degradation of HIF-1α occurs by the competitive binding to either heat shock protein 90 (HSP90), which stabilizes the protein,

or by binding to the anchoring protein (RACK1), which leads to HIF-1α degradation.
Two HIF-1α chimeric reporter systems were developed that allowed us to investigate HIF-1α stabilization/degradation in different cell lines, both in culture and in xenografts. A comparison between HIF-1α/Fluc and HIF-1α(ΔODDD)/Fluc expression levels, as measured by bioluminescence imaging (BLI), demonstrate important differences between non-tumorigenic NIH3T3 and HEK293 reporter cells and tumorigenic PTEN-defective U87 cells. Non-tumorigenic NIH3T3 and HEK293 cells had low basal normoxic-levels of HIF-1α/FLuc expression that were readily detectable by BLI, but not by immunoblotting. In contrast, tumorigenic U87 reporter cells had high basal levels of HIF-1α/FLuc expression, and responded to hypoxia and hypoxia-mimetics as well. A significant reporter response was observed in animals bearing U87/HIF-1α/FLuc xenografts following an i.p. injection of $CoCl_2$, but not in animals bearing U87/HIF-1α(ΔODDD)/FLuc or native FLuc expressing (control) xenografts.
Immunofluorescence analysis of HIF-1α/FLuc subcellular localization and trafficking in reporter-transduced cell lines compared well with that of endogenous HIF-1α in wild-type cells. A bi-exponential BLI profile of HIF-1α/FLuc protein degradation was observed, indicating that both "rapid" and "slow" clearance mechanisms were operative. The half-time of the rapid clearance phase in these cells was ~3−6 min and consistent with the currently accepted half-life of HIF-1α (~5 min) under normal non-hypoxic conditions; a second slow clearance phase (~200 min) was newly identified. The immunofluorescence and kinetic profile analysis of HIF-1α/FLuc degradation suggests that the rapid and slow components of degradation are compartmentalized. Although the mechanism of HIF-1α shuttling between nucleus and cytoplasm is poorly understood, it is clear that HIF-1α subcellular distribution and degradation are regulated in a cell-specific manner, with significant differences between normal cells and cancer cells.

19 INVITED

Application of Hypoxia Imaging in Radiation Treatment Planning

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The appearance of tumour hypoxia is a common feature of solid tumours that is negatively correlated with prognosis and local control. Among other mechanisms, hypoxia-mediated radioresistance has been identified as a major obstacle for achieving permanent tumour control after radiotherapy. Derived from experimental studies, hypoxic mammalian cells are known to be up to 3 times less radiosensitive than euoxic cells. The elimination of tumour hypoxia has been a long standing therapeutic target. The PET tracers ^{18}F-fluoromisonidazole (F-MISO) and ^{18}F-fluoroazomycinarabinoside (FAZA) have been developed to provide a non-invasive tool for visualizing tumour hypoxia by positron emission tomography (PET). Accrdingly, tumour hypoxia assessed by PET has been found to be correlated with the risk of locoregional failure as well. Studies show F-MISO PET to be associated with a higher risk (HR 7) of locoregional failure after radiochemotherapy compared to non-hypoxic tumours [Rischin et al., JCO 2006]. One approach to target unfavorable tumour characteristics such as tumour hypoxia is the 'dose painting' concept. Thereby the radiation dose is selectively escalated within the most aggressive tumour areas. For hypoxia targeted radiation treatment hypoxic tumour subvolumes are derived from the hypoxia PET and treated with an increased radiation dose ('boost') using intensity modulated radiotherapy (IMRT) treatment planning. By selectively boosting hypoxic tumour cells the tumour control probability (TCP) is supposed to increase as shown by radiobiological considerations. Treatment planning may be based on baseline hypoxia as well as residual tumour hypoxia assessed at different timepoints during a timecourse of radiation treatment. However, radiation-induced tumour reoxygenation and dynamic changes in tumour oxygenation need to be considered. Data on the dynamics of tumour hypoxia during radiation treatment is scarce. Follow-up PET scans of tumour hypoxia during standard radiochemotherapy in locally advanced SCC of the head and neck will therefore be presented as well as examples and technical considerations for hypoxia besed treatment planning. While the role of hypoxia PET as a diagnostic tool is well established, the suitability and feasibility of a hypoxia based dose painting needs to be thoroughly discussed and addressed in clinical trials.

Scientific Symposium (Sat, 24 Sep, 11:15−13:15)
Long Term Follow Up in Childhood and Adolescent Cancer

20 INVITED

Pan-European Network for Care of Survivors After Childhood and Adolescent Cancer (PanCare)

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Background: Recognising the need for a voice in Europe for survivors with late complications of therapy for childhood and adolescent cancer, PanCare was founded to be the pan-European Network that addresses all aspects of childhood cancer survivorship.
Material and Methods: In March 2008, PanCare (Pan-European Network for Care of Survivors after Childhood and Adolescent Cancer) was founded by 26 doctors and scientists from 14 European countries at a meeting in Lund, Sweden. The" Erice statement" was adopted as backbone of PanCare vision and mission.
Results: Seven meetings of the Network have so far been held. At present, PanCare has members from 26 European countries plus Canada and Japan. Most are paediatric oncologists; second most common are epidemiologists; followed by radiation oncologists; survivors psychologists; parent representatives; paediatric neurologists; paediatric and adult endocrinologists; nurses (too few!), medical students and one each being a lawyer and representative of a funding body. The PanCareSurFup consortium, based in PanCare, is a 5-year FP7 Health2010 Collaborative Project focusing on epidemiological studies on mortality, secondary malignancies and cardiac disease after treatment for childhood cancer, and on guidelines for survivors and dissemination of results. In addition to this, working groups within PanCare are currently establishing research projects on fertility, quality of life and ototoxicity. PanCare is also a partner in an FP7 funded Network of Excellence led by SIOPE.
Conclusions: PanCare is a multidisciplinary pan-European network of professionals, survivors and their families that aims to reduce the frequency, severity and impact of late side-effects of the treatment of children and adolescents with cancer. PanCare is working to achieve equity of access to care for childhood cancer survivors across Europe, to perform collaborative research and to act as a resource of research based information concerning all late side-effects of cancer treatment. An important aim of PanCare is to work with the European Community and other stakeholders to increase awareness and research about childhood cancer survivors all over Europe. The long-term strategic aim of PanCare is to ensure that every European survivor of childhood and adolescent cancer receives optimal long-term care.

21 INVITED

The Epidemiology of Childhood Cancer Survivors

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Background: As a result of the ever-increasing success rates achieved in recent decades in pediatric oncology, an increasing number of children and adolescents have successfully overcome their cancer experience and have reached or are entering adulthood.