mucosal repopulation and differentiation during fractionated radiotherapy, *e.g.* Keratinocyte Growth Factor, or to protect endothelial cells from early apoptosis, *e.g.* basic Fibroblast Growth Factor. One obvious concern with this approach is that growth factors given during radiotherapy must not have a corresponding protective effect against tumor cell kill. The alternative approach is to give intervention therapy after the completion of treatment to block some of the aberrant cell signaling initiated by radiotherapy. Examples of this are Transforming Growth Factor-beta signaling, which is associated with fibrosis, and aberrant endothelial cell signaling, *e.g.* decreased production of ADPase and thrombomodulin and increased expression of Protease Activator Receptor 1, which creates a pro-inflammatory, pro-thrombotic environment associated with many types of late radiation injury. Examples of specific molecular modulation of normal tissue radiation injury will be discussed in relation to their potential to increase the therapeutic benefit of clinical radiotherapy.

Scientific Symposium
RNAi – the new tool in cancer

34 INVITED

RNA interference and cancer: will RNA interference help to cure cancer?

A. Harel-Bellan, I. Naguibneva, M. Ameyar, A. Polesskaya, S. Ait-Si-Ali, R. Groisman, M. Souidi. *Institut André Lwoff, UPR 9079 CNRS, Villejuif France*

As an introduction to the session, this talk will describe the basic principles of RNA interference (RNAi), and review how RNAi can be used to understand, and maybe cure, cancer.
The first part will describe the RNA interference process, and how it can be (and, in fact, has already been) used to explore all pathways involved in cell fate control and in oncogenesis. Special emphasis will be put on high-throughput or genome-wide assays. This part will be illustrated with a review of the literature.
The involvement of RNA interference pathways themselves in Cancer will also be discussed, with, in particular, the implication of microRNAs in the control of mammalian cell fate and in oncogenesis. This part will be illustrated by work from the author's lab as well as by work published by others.
The second part will address the potentiality of interfering RNAs as therapeutical tools to fight cancer, and will discuss essential questions that need to addressed before we can envision such an application.
In conclusion, a naive biologist's view of future cancer treatments will be proposed to open the discussion.

35 INVITED

Functional genetic screens identify oncogenic microRNAs

R. Agami, M. Voorhoeve, C. Le Sage, R. Nagel, E. Zlotorynsky, M. Schrier, J. van Duijse. *The Netherlands Cancer Institute, Department of Tumor Biology, Amsterdam, The Netherlands*

We used functional genetic screens to identify microRNAs (miRNAs) with oncogenic potential. miRNAs have emerged in recent years as exiting new effectors of gene regulation from nematodes to man and from stem cell biology to cancer development. The number of predicted (up to a thousand) and verified (around 300) human miRNAs is still expanding. However, very few of them have been functionally annotated, partly because of the lack of reliable and concurring in silico target prediction algorithms and partly because of a lack of proper genetic tools. To overcome this gap, we created a library of vectors expressing the majority of known human miRNAs by cloning a genomic region consisting of each miRNA precursor behind a CMV promoter in a retroviral vector. Both in transient transfections and retroviral transduction, these constructs were shown to express functional miRNAs. In addition, to facilitate the identification of miRNAs that confer cellular growth advantage or disadvantage, DNA fragments, corresponding to the miRNA expression constructs, were spotted on DNA-array slides. We used the library and DNA-arrays to identify miRNAs that can protect cells from oncogenic stress. We transduced primary human cells with the miRNA library and subsequently with a retrovirus encoding oncogenic Ras or a control virus. Primary human cells stop replicating following oncogenic signals, a response that depends on intact p53 pathway and termed premature senescence. After propagating the cells for three weeks, genomic DNA was isolated and the population of miRNA inserts was compared between stressed an unstressed cells. This way we identified three miRNAs that were enriched in the RASV12 expressing population. We confirmed their activity using various growth protocols and an acidic beta-gal staining (marker for senescent cells). Furthermore, the mechanism of action of these miRNAs and their contribution to cancer development in humans will be discussed and elucidated.
See also: *http://www.nki.nl/nkidep/agamil*

36 INVITED

New technologies for RNAi-based treatment optimization

O. Kallioniemi. *Medical Biotechnology, VTT Technical Research Centre of Finland and University of Turku, Turku, Finland*

Our aim is to identify new molecular targets and mechanisms for therapeutic intervention in cancer. To achieve this aim, we develop and apply multiple high-throughput technologies including molecular profiling, RNAi-based functional screening as well as rapid clinical validation tools. Data integration from these technology platforms is applied to facilitate interpretation and prioritization of the findings.
The molecular profiling of DNA-, RNA- or protein expression patterns in samples from cancer patients is not sufficient for implicating these molecules or molecular mechanisms as therapeutic targets. It is also necessary to generate functional information on such genes and pathways. Towards this aim, we have developed a high-throughput screening (HTS) system which is composed of a robotic, automated platform for the analysis of up to 20,000 functional experiments with living cells at a time using the 384-well microplate format. Cells are dispensed into culture wells, exposed to siRNAs or small molecule compounds, incubated for 1–3 days, washed, and stained with phenotype-specific markers for cell growth, cell cycle distribution or induction of apoptosis. The results are read by plate readers or cell cytometers.
Functional studies with large RNAi libraries (e.g. 1000–10,000 siRNAs) have implicated genes whose targeting by RNAi is lethal to specific cancer types, such as breast cancer. Integration of such functional RNAi data with gene expression and aCGH data has enabled us to identify genes that are targets of genetic alterations and whose expression is required for the maintenance of the malignant phenotype. Such genes represent attractive candidate drug targets. Furthermore, we are combining RNAi screening with drug and compound screening, to identify genes that are conferring resistance/sensitivity to an existing compound, or to identify novel compounds that are effective against cells that are lacking functions of specific tumor suppressor gene or other critical genes.
Taken together, these multiple RNAi strategies should facilitate development of novel therapeutic approaches for cancer.

37 INVITED

mRNA translational control of gene expression

B.G. Wouters. *University of Maastricht, Radiation Oncology, Maastricht, The Netherlands*

Cancer can be considered a disease that arises from a series of genetic changes that alter gene expression patterns within cells. This is supported by the fact that the primary function of many oncogenes and tumor suppressor genes is to regulate gene expression. In addition to cancer-associated genetic changes, the unique tumor microenvironment can elicit further variations in gene expression that influence patient prognosis. It is thus crucial to characterize the changes in gene expression that occur within tumors, as well as their underlying mechanistic basis. Although gene expression is controlled at many different levels, research has focused principally on transcriptional regulation. In recent years it has become clear that several additional mechanisms are also important contributors to gene expression under various conditions. These mechanisms include the recently discovered microRNA's that silence gene expression through mRNA degradation or through inhibition of mRNA translation. The regulation of mRNA translation is also emerging as an important mechanism for regulation of protein expression and is often deregulated in tumors. We have shown that tumor hypoxia causes a rapid and sustained inhibition of protein synthesis at the initiation step of mRNA translation. This inhibition is controlled by (at least) two different molecular mechanisms with different activation kinetics. The early phase of translation inhibition is mediated in large part by phosphorylation of the S51 residue of eukaryotic initiation factor eIF2α. Phosphorylation occurs as a result of the activation of an evolutionarily conserved pathway termed the unfolded protein response. Prolonged hypoxia independently activates a second pathway that leads to inhibition of the mRNA cap-binding complex eIF4F. eIF4F is necessary for cap-dependent translation, and its dissociation during hypoxia correlates with the dephosphorylation and activation of the negative regulator of eIF4F assembly, 4EBP1. Although each of these two distinct pathways inhibit overall mRNA translation, they also promote the translation of a subset of genes. We were able to identify a novel translational contribution to the expression of a number of hypoxia regulated genes. These included HIF-1 target genes like CAIX, transcriptional regulators like ATF-4 and CHOP as well as translational regulators like GADD34. As predicted, the kinetics

of translational repression and/or induction of the different genes varied widely. Global analysis using microarray technology indicated that as many as 5% of all genes may be differentially affected during hypoxia through regulation of mRNA translation.

Scientific Symposium
What is new in renal cancer

38 Abstract not received

39 Abstract not received

40 INVITED

Systemic therapy and novel targeted therapies in renal cancer

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Renal cell carcinoma has always been considered a chemo-resistant disease and data from the 1980s suggests that response rates are very low. There have however, only been limited data on the newer cytotoxic agents and more recently, non randomised trials have suggested that some patients may respond to combination treatments such as gemcitabine plus capecitabine. It is becoming increasingly recognised that renal cell carcinoma is not a single disease entity. Histology subtype and specific molecular abnormalities may not only define the behaviour of individual tumours but may also have therapeutic relevance. This is best exemplified in relation to targeted therapies. Hormone treatments have for many years been used as second-line treatment in patients who have failed first–line immunotherapy or as initial therapy in those unfit for immunotherapy. They are associated with low response rates and randomised trials suggest that at least, at first-line these treatments confer little or no benefit.

Standard therapy involves immunotherapy with either interferon or interleukin 2. There are randomised data that support the use of interferon and non-randomised data that suggest high dose bolus interleukin 2 is associated with durable complete remissions in a small percentage of patients. There is no evidence that combination immunotherapy is associated with an overall survival benefit. Case-controlled studies and a recent randomised trial from the French cooperative group show that immunotherapy is of no benefit to patients with intermediate or poor prognosis disease.

There is something of a revolution taking place in the treatment of renal cell carcinoma and a number of new targeted agents have shown activity in this disease. The most notable activity and best data produced so far involves Sutent and Sorafenib, the multi targeted tyrosine kinase inhibitors and Avastin, the monoclonal antibody directed against VEGF.

Sutent has shown response rates of nearly 40% in two consecutive phase 2 trials. These studies have involved 160 patients and this makes these data of great interest. Similarly, Sorafenib has shown significant activity in second-line with a doubling of progression-free survival. These are particularly impressive data as the trial was randomised; patients with stable or responding disease were randomised to continue on Sorafenib or placebo. Avastin has an overall response rate of 10% and at higher doses, a statistically significant prolongation of progression-free survival in a randomised trial against placebo. Other targeted agents that have shown activity include Temsirolimus and infliximab.

Trials in the first-line setting are currently underway with Sutent, Sorafenib and Temsirolimus being compared to interferon. Avastin has been combined with interferon and is being compared to single agent interferon. These agents and other targeted compounds are being combined and further data are awaited. Within the next 12–24 months we will have a clearer picture of the precise efficacy of these novel agents, particularly in comparison to interferon. Positive results from these studies will beg many questions: will these new agents replace interferon or will they be given in combination with it? Which targeted agents should be combined and will that be a better strategy than administering them sequentially? Do these compounds with their relatively good toxicity profile, open up therapeutic options for those patients with poor prognostic features who are currently considered unfit for active treatment? Can we now start developing maintenance strategies? We are entering a new therapeutic era in renal cell carcinoma and it is imperative that we now conduct a series of well-designed trials to precisely define how these new compounds can best be utilised.

41 Abstract not received

Scientific Symposium
Laparascopic surgery versus conventional surgery in colorectal cancer

42 INVITED

Laparoscopic versus open surgery for colon cancer: short-term outcomes of a randomised trial – COLOR Trial

H.J. Bonjer[1,2]. *On behalf of the COLOR Study Group.* [1]*Dalhousie University, Surgery, Halifax;* [2]*Capital Health, Surgery, Halifax, Canada*

Background: Oncological safety and short term benefits of laparoscopic colectomy for cancer remain under debate. To investigate these outcomes, a multicenter study randomizing patients with colonic cancer for either laparoscopic or open resection was performed.

Patients & Methods: Twenty-nine European hospitals participated in the COlon cancer Laparoscopic or Open Resection trial (COLOR trial). Patients with a solitary cancer of the right or left colon were randomly assigned to either laparoscopic or open surgery as curative treatment. Cancer free survival at three years after surgery was the primary outcome. Clinical characteristics, operative findings and postoperative outcome are presented.

Results: Of the 1248 patients randomly assigned to one of the two surgical procedures, 153 were excluded and 13 could not be analyzed due to missing data.

Blood loss was significantly less during laparoscopic than during open surgery ($p < 0.001$). Laparoscopic surgery took half an hour longer to perform than open surgery ($p < 0.001$). In 17% of the laparoscopic procedures conversion to open resection was necessary. Radicality of resection assessed by number of removed lymph nodes and length of resected oral and aboral bowel segments was similar after laparoscopic and open surgery. During the postoperative course, laparoscopic colectomy was associated with earlier recovery of bowel function ($p < 0.001$), fewer analgesics requirements ($p < 0.001$) and one day shorter hospital stay ($p < 0.001$). Rates of morbidity and mortality within 28 days after colectomy did not differ between arms.

Interpretation: Laparoscopic surgery allows safe and radical resection of colonic cancer of the right, left and sigmoid colon. Although laparoscopic colectomy requires more operating time, it is associated with less blood loss, earlier restoration of bowel function, fewer analgesic requirements and shorter hospital stay.

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43 INVITED

The CLASICC trial

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The CLASICC Trial is a randomised clinical trial of laparoscopic-assisted versus conventional surgery in colorectal cancer. Between 1996 and 2002 794 patients from 27 UK centres were allocated to undergo laparoscopic-assisted (n = 526) or open (n = 268) surgery for cancer of the colon (n = 413) or rectum (n = 381). All surgical resection specimens were treated identically and centrally reviewed for circumferential resection margins (CRM) positivity. In the lap-assisted group overall 29% underwent conversion to open surgery but this fell from 38% in year 1 to 16% in year 6 of the trial. Tumour stage was equivalent between the two arms of the trial and the proportions of Dukes stage C_2 tumours did not differ between the lap-assisted (7%) and open (6%) groups. Duration of operation was shorter in the open (135 [100–180] min) than in the lap-assisted (180 [135–220] min) group. Rates of CRM positivity were similar between groups except for those undergoing laparoscopic anterior resection for rectal cancer where CRM positivity was 12% ($^{16}/_{129}$) compared with 6% ($^{4}/_{64}$) in the group undergoing open anterior resection ($p = 0.19$). Lymph node yield was high in both arms (13.5 open, 12 lap-assisted). In the lap-assisted group average hospital stay was 2 days shorter than in the open group. Overall 30-day complication rates were identical in the two arms but in those who underwent conversion from laparoscopic to open surgery the complication rates were higher and this was reflected in a higher in-hospital mortality (open 5%, lap-assisted 1%, converted 9%, $p = 0.34$). Up to 3 months postoperatively quality of life scores (EORTC QLQ-C30, and QLQ-CR38) showed similar patterns between the two surgical groups. All patients have now been followed up for at least 3 years.

For cancer of the colon there seems to be little difference between laparoscopic-assisted and open resection and on the basis of the pathological data there is no reason to suspect that cancer-related outcomes will be different. Preliminary analysis of the 3-year overall and disease free survival bears this out. For rectal cancer the data might suggest that a higher local recurrence rate might be expected in those