of 2 disorders, pregerminal center cell derived CLL with poor prognosis and postgerminal center cell CLL with an excellent prognosis. Combined with single cell analysis, these techniques also proved that HL represents a monoclonal disease of germinal center B-cells.

Other investigators including our group, showed that many lymphomas contain chromosomal translocations activating oncogenes on the nonfunctional Ig allele. These include t(14;18) activating BCL2 in >90% of follicular lymphoma and 15-20% of diffuse large B cell lymphoma (DLBCL), t(11;14) activating cyclin D1 in >90% of mantle cell lymphoma and 10-15% of myeloma and t(8;14) or variants activating MYC in 100% of Burkitt lymphomas and 5-10% of DLBCL. These translocations bear diagnostic significance. At present the best way to detect them is interphase fluorescence in situ hybridization (FISH). Our group designed appropriate probe sets to detect these breakpoints including variant types and adapted the assays to routinely processed paraffin embedded tissue sections. Optimal probes were selected using DNA fiber FISH, a powerful adjunct to map probes and breakpoints. Other translocations involve BCL6 at 3q27 in 35% of DLBCL, the anti-apoptotic API2 on 11q21 involved in t(11;18) in 30% of gastric MALT lymphoma, and ALK on 2p23 in many CD30+ T cell lymphomas called ALCL. Detection of t(11;18) in gastric lymphoma predicts resistance to eradication of Helicobacter Pylori. Detection of ALK translocations in ALCL predicts a relatively excellent prognosis.

45

Novel molecular detection approaches in various cancers

D. Sidransky. *John Hopkins University, Department of Otolaryngology-Head and Neck Researc, Maryland, USA*

Cancers progress through a series of genetic and epigenetic changes. These alterations are rapidly being developed into novel molecular assays for cancer detection. Among the most promising, are microsatellite alterations, promoter hypermethylation and somatic mitochondrial mutations. Integrated viral DNA has also been identified as a specific marker for various viral associated malignancies. These alterations can be integrated into various molecular assays and targeted for the detection of a specific cancer type. Tested samples include bodily fluids that drain the organ of interest. Increasingly, plasma or serum DNA has become a fruitful target of new molecular assays to determine the absence or presence of cancer. In addition, the development of novel quantitative assays may allow us to accurately assess the burden of disease in addition to its presence or absence. Final clinical limitation will depend on further technological developments and the availability of large cohorts in which to test the candidate markers.

46

Tumour-targeted vectors for gene therapy

Abstract not received.

47

Retinoic acid and arsenic in acute premyelocytic leukemia

Abstract not received.

48

Genes that allow escape from senescence

Daniel Peeper, Avi Shvarts, Thijn Brummelkamp, Benjamin Rowland, Roderick Kortlever, Sirith Douma, René Bernards. *Division of Molecular Carcinogenesis, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands*

Senescence limits the proliferative capacity of primary cells in culture and contributes to resistance to transformation. In rodent cells, this process requires induction of the tumor suppressor genes *p19^{ARF}* and *p53* as mutation of either of these two genes allows escape from replicative senescence and causes immortalization. Expression of an active *RAS* oncogene in primary rodent cells accelerates this process of senescence and induces a state of premature senescence. Also in the case of RAS-induced premature senescence, the tumor suppressor genes *p19^{ARF}* and *p53* appear to be critical downstream targets in this response. To identify novel regulators of the p19^{ARF}-p53 pathway we performed functional genetic screens with retroviral cDNA expression libraries.

As a first approach, we generated a mouse fibroblast cell line conditionally immortalized by a temperature sensitive allele of SV40 Large T antigen. Upon shift to the non-permissive temperature these cells undergo rapid senescence. Introduction of a retroviral cDNA expression library prior to temperature shift led to the identification of several genes that allows escape from senescence. Interestingly, one these genes (*BCL6*) is frequently activated by translocation in human lymphoma.

As a second approach we introduced an activated *RAS^{V12}* oncogene in the conditionally immortalized mouse fibroblasts, together with cDNA expression libraries. Using this approach, we identified *hDRIL1* as a gene that allow escape of premature RAS-induced senescence. Interestingly, *myc* genes were not found in this screen even though full-length cDNAs of *myc* genes were abundantly present in these libraries.

Functional studies aimed at understanding how these novel genes interact with the p19^{ARF}-p53 pathway will be presented.

49

Cyclin-dependent kinase inhibitors: New compounds, selectivity, mechanisms of action and anti-proliferative activities

Laurent Meijer, Marie Knockaert. *C.N.R.S., Station Biologique, BP 74, 29682 Roscoff cedex, France*

Cyclin-dependent kinases (CDKs) directly regulate the cell division cycle phases, transcription, apoptosis and neuronal cells & thymocytes functions. Intensive screening has led in the last few years to the identification of several families of chemical inhibitors of CDKs. Some of these compounds display a high selectivity and efficiency (IC_{50} < 5 nM). Many have been co-crystallised with CDK2 and their atomic interactions with the kinase have been analysed in detail: all are located in the ATP-binding pocket of the enzyme. Some novel structures will be presented.

Despite high selectivity, most CDK inhibitors (except purines) are potent inhibitors of glycogen synthase kinase-3 (GSK-3). Whether this GSK-3 inhibitory property is favourable to the anti-mitotic properties of CDK inhibitors will be discussed. An overall method for the determination of selectivity of kinase inhibitors, based on the affinity chromatography purification of targets on immobilised inhibitor, will be presented. This method has led to the identification of unexpected targets.

CDK inhibitors display anti-proliferative properties, they arrest cells in G1 and in G2/M. Furthermore they facilitate or even trigger apoptosis in proliferating cells. In contrast, they protect neuronal cells and thymocytes from apoptosis. This suggest that CDKs may be involved both in triggering and inhibiting apoptosis. The consequences of this dual and conflicting effect need to be evaluated. The combination of CDK inhibitors with other anti-mitotic agents greatly enhances their anti-tumour activity. This will be illustrated by a few examples. The potential of CDK inhibitors is being extensively evaluated for cancer chemotherapy (clinical trials, phase I and II) and will be briefly reviewed.

50

Drugs targeting p53 regulatory mechanisms

S. Lain, D. Lane. *Ninewells Hospital & Medical School, Department of Surgery and Molecular Biology, Dundee, United Kingdom*

The p53 tumour suppressor is a short-lived protein that is maintained at low levels in normal cells. In response to stresses, p53 is transiently stabilised and its function as a transcriptional activator is increased. Activation of the p53 function causes cell cycle arrest and/or apoptosis, thus preventing the fixation of mutations in the following generations. The p53-mediated stress response is one of the most common mechanisms inactivated in tumour cells. More than 50% of all human cancers have mutations in the p53 gene. In many other cases, there are lesions that affect proteins that modulate the levels and activity of p53. This is the case of tumour cells in which the product of the Mdm2 gene is overexpressed, of tumours where there is a deficiency in the expression p14ARF tumour suppressor (the natural inhibitor of Mdm2) or of tumours associated with the expression of viral oncoproteins such as the HPV E6 protein.

The search for non-genotoxic ways to activate the p53 response is widely thought to be a promising approach in the development of new low risk strategies to treat cancers. There are many reports showing that Mdm2 is the major regulator of the p53 levels in the cell. This is due to its ability to inhibit the interaction of p53 with the transcriptional machinery and to its ubiquitin E3 ligase activity that promotes the ubiquitination and proteasome degradation of p53. Therefore, a direct and rational way to enhance the p53 response in a non-genotoxic way in tumours where the