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Molecular modelling of KIT and PDGRA mutant forms to predict sensitivity to new drugs in GISTs

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Background: Gastrointestinal stromal tumours (GISTs) are the most frequent mesenchymal tumours of the gastrointestinal tract. The molecular event determining GIST development is the activation of the KIT receptor or, alternatively, PDGFRA (hereafter RTKs), and their pathways. Imatinib-mesylate (Gleevec®, Novartis Pharmaceuticals) has been demonstrated to be able to block the activation of both the receptors and to inhibit GIST proliferation.
Evidence derived from clinical experience points out that GISTs respond well to this therapy and that different RTK mutation types correlate with a different response rate to Imatinib. However, an acquired resistance is often associated with the presence of secondary mutations affecting the ATP pocket of those RTKs. It is therefore necessary to develop second line therapies that could be greatly improved by a clearer understanding of the inhibition mechanisms eventually leading to the identification of new therapeutic strategies.
Material and Methods: Tumour specimens from different patients were analysed biochemically to determine KIT/PDGFRA activation and sequenced for the corresponding genes. Subsequently, an "in-silico" approach was developed by reproducing the 3D structure of the different RTK forms, while the affinity for ATP and the analysed drugs were calculated using the theoretical framework of the Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) method. Finally "in-vitro" cell lines expressing the different RTK forms were biochemically analysed to determine the RTK activation status.
Results: A good correlation between the "in silico" results and the biochemical analyses of the "in vitro" cell lines was observed in different instances and has also been observed "in vivo" in the treated patients.
Conclusions: This integrated multidisciplinary approach demonstrates that computer-based molecular simulation can be used as a reliable technique to estimate the affinity of binding between receptors and inhibitors. Accordingly, "in silico" approaches together with "in vitro" genetically engineered cell lines could be employed to predict the biological response to small molecules inhibiting RTKs. An analogous approach could then be utilized to screen new potentially therapeutic drugs in a rapid and efficient way.

114 EACR Young Cancer Researcher Award

Chromatin modulates DNA damage response activation in oncogene-expressing cells

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Early tumorigenesis is associated with the engagement of the DNA-damage checkpoint response (DDR). Cell proliferation and transformation induced by oncogene activation are restrained by cellular senescence. We have previously shown that retroviral-mediated expression of an activated oncogene in cultured normal human cells results in a permanent cell-cycle arrest caused by the activation of a robust DDR. Experimental inactivation of DDR abrogates senescence and promotes cell transformation. Oncogene-induced senescence is also associated with a global heterochromatinization of nuclear DNA. Senescence-associated heterochromatic foci (SAHFs) are enriched in heterochromatin markers and they have been proposed to enforce cellular senescence by suppressing the expression of proliferative genes.
Presently, DDR activation and heterochromatinization are considered the two main tumor suppressors mechanisms that control cellular senescence. However, whether these are independent pathways or whether there is an interplay and they modulate each other is unknown.
We have discovered that chromatin changes induced by oncogenes occur in a DNA replication-dependent manner and, differently from what had been previously proposed, that heterochromatin formation is not involved in the suppression of proliferative genes. Instead, our results indicate that cells expressing an oncogene induce chromatin changes to constrain DDR spreading and reduce signaling. Indeed, we have observed that heterochromatin reduction reproducibly leads to augmented DDR signaling and altered cell viability of oncogene-expressing senescent cells.
Our observations have potential important implications for the therapeutic treatment of cancers.

Keynote Lecture (Tue, 22 Sep, 11:15−12:00)
Targeting the cell cycle in cancer

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Targeting the cell cycle in cancer

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Unicellular organisms such as yeasts require a single cyclin-dependent kinase, Cdk1, to drive cell division. In contrast, mammalian cells are thought to require sequential activation of at least four different Cdks, Cdk2, Cdk3, Cdk4 and Cdk6, to drive cells through the different phases of the cell cycle interphase (G1, S and G2), as well as Cdk1 to proceed through mitosis (M phase). This model has been recently challenged by genetic evidence illustrating that mice survive in the absence of individual interphase Cdks and that most cell types proliferate in the absence of multiple interphase Cdks (Malumbres and Barbacid, Nat Rev Cancer, 9, 153, 2009). Indeed, we have recently shown that mouse embryos lacking all interphase Cdks (Cdk2, Cdk3, Cdk4 and Cdk6) undergo organogenesis and develop to midgestation, indicating that Cdk1 alone can efficiently drive the cell cycle even during one of the most active periods of cell proliferation (Santamaría et al., Nature 448, 811, 2007). Similar results have been recently obtained in adult mice. On the other hand, interphase Cdks are essential for proliferation of highly specialized cell types such as pancreatic beta cells, embryonic cardiomyocytes or hematopoietic precursors. Thus, suggesting that the generation of multiple interphase Cdks during eukaryotic evolution was required to drive the cell cycle in specialized cell types rather than to contribute to progression through the basic phases of the mammalian cell cycle.
Based on these observations, we have reasoned that individual Cdks might also be selectively required to drive the cell cycle of certain tumor cell types, depending on their tissue/cell type of origin and/or the driving oncogenic mutations. To test this hypothesis, we have used a well-characterized animal tumor model for non small cell lung carcinoma (NSCLC) driven by an endogenous K-RasG12V oncogene that can be activated at will in postnatal mice. In these mice, we have systematically ablated each of the interphase Cdks either in the germ line or in adult mice following a conditional strategy after tumors could be observed by CT analysis. Neither ablation of Cdk2 or Cdk6 had significant effects in the reduction of tumor burden or in survival. In contrast, ablation of Cdk4 resulted in a drastic reduction of tumor number as well as tumor size and grade. Mechanistic studies have revealed that lung alveolar cells selectively require Cdk4 to avoid entering senescence when they express a resident K-RasG12V oncogene. No induction of senescence could be observed in other cell types or upon ablation of other interphase Cdks such as Cdk2. These observations indicate that tumor cells also have specific requirements for selective interphase Cdks to drive their own cell cycle. Comparison of these results with pharmacological intervention using selective Cdk4 inhibitors should help us to establish a therapeutic strategic that may be eventually applicable to cancer patients.

Special Session (Tue, 22 Sep, 13:30−14:30)
National cancer plans: one size doesn't fit all

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The Netherlands cancer plan experience

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The experience in the Netherlands with national cancer plans has been rather mixed, so that lessons can be drawn: plans were/are only useful if they address the demographic and technological 'future' (adapting is already a great virtue) and need to be in line with 'the' Dutch Cancer Society (dominating the Dutch cancer control scene since the mid 50's), raising money for change (i.e. research and awareness), but also raising enough interest for (and satisfying!) the various – often autonomous – planning and programming agencies (around the Hague centre of polical power) and the more regionally oriented health insurance companies (going from 40 to <10) that fund the – now 8- Comprehensive Cancer Centres with their quality of care promoting role based on hosting the regional cancer registries. They are confronted with or driven by the aspirations of the various professionals (by definition renewing themselves in international perspective) and increasingly by the patients, who now command a strong national organisation, albeit being as diverse as there are cancers and becoming more voal with rising prevalence. Their motives are largely determined by their spokespersons who, besides willing to live, are mostly interested in quality of life and want to taken seriously.

The level of discussion has clearly raised over time, as each of the partners or interst groups has become more knowledgeable and aware by the pure fact of longer survival, media attention, internet and the availability since almost 20 years of a national, rather clinical, cancer registry, that increasingly publishes analyses on quality of oncological care. Current tendencies are increasing bureaucratic meddling (asking for full transparency), which is legitimised by the legal changes in health insurance in 2006, a sort of political 'big bang' aiming to unleash market forces; the current system supplies obligatory basis insurance to everybody – with about 1% of patients being uninsured – allows for more competition between hospitals (diminishing from 250 in 1980 to <100 now) and care supplyers, ever subspecialising and thus also adding to fragmentation in the care for increasing amounts of elderly patients.
Although it hardly works in oncology, the market ideology dictates that health insurance companies only buy low priced, high quality care for 'their' insured patients (who could run away every year which cancer patients do not tend to do) while getting maximal transparency.
Through all of these dynamic & chaotic movements there are the usual long term (10−20 years) developments in (wo)manpowerplanning taking on board subspecialization within surgical oncology, medical oncology and paediatric oncology, radiotherapy planning etc. Although drug development is also a long term affair, it seems to cause short term medical and budgetary shocks when they are allowed to the market, which especially plays a role in medical and hemato-oncology. The latter domain stands out because a long term planning was undertaken already more than 25 years ago as a result of which it not only became strong in manpower planning but also in strong emphasis of clinical trial organization (HOVON) with a high participation to clinical trials and which is now focussing on quality of care. It seems, 'everybody' is now focussed on quality of care, regardless of any plan.
The history of national cancer plans goes back to the 1970's (a start being made in the 1950's with failed cancer registration, except for the Eindhoven Cancer Registry and the Netherlands Cancer Institute and Daniel den Hoed registries), after the 'War against cancer' was unleashed and the Dutch Cancer Society retracted from its coordinating role in promoting quality of care. It resulted in the foundation of 9 (now 8, soon 5) regional comprehensive cancer centres, hosting also the cancer registries and various national initiatives (by the Health Council) for enlarging radiotherapy capacity, promoting home care and mass screening initiatives for cervical and breast cancer. This was facilitated much by scenario development during the 80's in a national committee of experts, followed by so called signalling committees since the early 90's, coming back under control of the Dutch Cancer Society in the late 90's. This resulted in a number of expert committees that reported on the role of prevention by dietary and physical exercise interventions, the need for colorectal cancer screening, the (again scenario-wise estimated) development of cancer prevalence and its implications for geriatric oncology and surveillance and the growing importance of imaging and molecular diagnostics and staging, soon te be followed by a report on quality of oncological care and the role of regionalization and subspecialization. Other major developments are in the domain of cervical cancer screening and HPV-vaccination, the incorporation of expensive drugs in the budgets and the need for cost-effectiveness. The diversity of topics makes it clear why a national cancer plan is so difficult to develop and remain actual. In fact, such efforts have been undertaken since 2003 by a coalition of functionaries from the Ministry of Health, health insurance companies, patient group and managers of comprehensive cancer centres aiming at more coordination at operational level, in fact the job of the CCC's. The greater role (or perspective) given to psycho-social care was of course not enough and so little professional involvement has resulted with just pleas for efficiency.
All in all, an optimal mix might still be available for a country of only (almost) 17 million people, whose culture and social structure is so affected by its neighbours from Scandinavia, the UK, Belgium/France and Germany and that thus tries to escape through its close liaisons with the USA.

Special Session (Tue, 22 Sep, 13:30−14:30)
Side-effects of treatment for early disease − which is the best?

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Radiotherapy

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Assessment of side-effects of treatment is not easy. Problems include the prevalence of bladder, bowel and sexual dysfunction in the normal ageing male population, the differences between patient-reported and physician-assessed toxicity, and the paucity of randomized controlled trials comparing the different treatments.
The recently published SPCG-7 trial, because it is a relatively large prospective trial that randomized patients to receive prostate radiotherapy or not, provides some of the best data available. SPCG-7 provides useful estimates of the risk of bladder and bowel toxicity from radiotherapy, although assessment of the impact of treatment on sexual function is more difficult because all patients received hormones. It should also be noted that the radiotherapy techniques used date back to the 1990's. It is likely that recent advances in IMRT and IGRT will reduce the risk of side-effects from prostate radiotherapy.
It is interesting to note that prostate radiotherapy can have beneficial side-effects as well as adverse side-effects. For example, there are good data that the frequency of nocturia is reduced after radiotherapy, in comparison with baseline levels.
Individual patient dosimetry and clinical characteristics, such as previous bowel surgery, influence the risk of radiotherapy toxicity. Taken together with a patient's values on the relative importance of treatment toxicity and efficacy, these factors could be used to individualise radiotherapy dose.
Which is the best? Although there are no randomised trials comparing radiotherapy with surgery in early prostate cancer, there are several good quality retrospective comparisons focusing on quality of life. The data from these studies should help patients to decide which treatment is best for them.

Special Session (Tue, 22 Sep, 13:30−14:30)
Very late normal tissue damage after radiotherapy

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Long-term risks after radiotherapy (RT) for testicular cancer (TC)

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Background: During the last five decades of the 20th century, RT has been the mainstay of treatment of TC, in particular of seminoma (S). Epidemiological studies have increasingly documented severe long-term health effects in testicular cancer survivors, related to their treatment.
Material and Methods: Review of the literature and institutional experience.
Results: Mediastinal RT (used before ca.1975) significantly increases the risk of long-term cardiac complications, and is no longer used routinely in the curative treatment of TC. After abdominal RT for TC the life time relative risk (RR) of second cancer is approximately doubled, the malignancy typically diagnosed with a latency of 10−15 years (1). Combination with chemotherapy increases this risk furthermore (RR $\approx$ 3). Most of the tumors are located in the G.I. tract or in the bladder. G.I. ulcera represent the most frequent benign long-term morbidity after abdominal RT, but in patients aged
After the introduction of cisplatin-based chemotherapy routine RT has been restricted to patients with S. The target dose has gradually been reduced from 36 to 20 Gy, applied to the paraaortic region. Testicular RT (20 Gy, due to cancer in situ) is highly effective in avoiding the development of invasive TC, but may lead to endocrine hypogonadism requiring testosterone substitution.
Conclusion: Though being an effective adjuvant and therapeutic treatment in S, the use of RT in TC should be minimized as much as possible due to its long-term risk of severe complications. In primary treatment there is usually no need to combine cisplatin-based chemotherapy with RT.

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