49 INVITED

What are the determinants of response to EGF-R signalling inhibitors in HNSCC?

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Therapeutic exploitation of molecular targets that drive oncogenesis and progression is required to improve the outcome of patients with advanced head and neck squamous cell carcinomas (HNSCC). The epidermal growth factor receptor (EGFR) is a validated target in HNSCC but predictive markers of response to receptor tyrosine kinase (RTK) inhibitors such as gefitinib and erlotinib have not been conclusively identified. Such markers are essential for appropriate patient selection and objective measurement of biological responses. We characterized a panel of 18 HNSCC cell lines for biomarkers of response to gefitinib previously reported in other cancers and correlated these with their intrinsic sensitivity. We also developed cell lines with acquired resistance by continued exposure to increasing concentrations of gefitinib. Sensitivity to gefitinib was determined using SRB cell proliferation assays, protein expression/activation was measured by western blot, flow cytometry and electrochemiluminescent immunoassay (MSD). EGFR gene amplification was demonstrated using fluorescence in situ hybridisation and EGFR TK mutations and intron 1 polymorphisms detected by PCR. Sensitivity to gefitinib varied 500-fold across the panel (GI50 0.04−20.2 μM); 7 cell lines were considered sensitive (GI50 < 1 μM). EGFR expression and activation was significantly greater in the most sensitive cells ($p = 0.0005$ and 0.0008, Spearman Rank). ERB B2 positive cells were more sensitive than ERB B2 negative cells with comparable EGFR expression. ERB B3 activation was only detected in the most sensitive cell lines. ERB B4 expression did not impact on intrinsic sensitivity. EGFR gene amplification was detected only in the most sensitive cell line and intron 1 polymorphisms in 3/5 of the high EGFR expressing, sensitive lines. MET and breast receptor kinase (BRK) were expressed, and amphiregulin was secreted at higher levels in sensitive cells. However levels of IGF-1R, E-cadherin, MAPK and AKT levels/activation did not correlate with sensitivity. In cells with acquired resistance, there were no EGFR mutations or upregulation of other major RTK detected. However, there were interesting differences in downstream signalling pathways that may contribute to induced resistance in HNSCC. In conclusion, biomarkers identified in other tumour types do not necessarily predict for intrinsic sensitivity to gefitinib in HNSCC, and acquired resistance may be due to different molecular mechanisms.

50 INVITED

Human papillomavirus infection in head and neck squamous cell carcinoma: another route to cancer

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High-risk human papillomavirus (HPV) type 16 has etiologically been linked to a subset (5 to 20%) of head and neck squamous cell carcinomas (HNSCC). HPV16-driven carcinogenesis is mediated by expression of the viral E6 and E7 oncoproteins, which cause deregulation of the cell cycle by inactivating p53 and pRb, respectively. We tested the hypothesis that two routes to HNSCC exist: one determined by HPV16 and one by environmental carcinogens (tobacco and alcohol). To define the critical events in these routes, we performed a detailed genetic analysis of 12 HNSCC with and 39 without HPV16 involvement. Criterion for HPV16-positivity were presence of HPV DNA with mRNA expression of E6 and/or E7 in the tumor. We analyzed the mutation status of TP53, allelic loss of 28 microsatellite markers at chromosome arms 3p, 6q, 8p, 9p, 13q, 17p, and 18q, including markers located in regions of chromosome arms 9p and 17p that harbor genes involved the p53 and pRb pathways and performed high resolution micro-array comparative genomic hybridization (ma-CGH). The results showed that none of the HPV16–positive tumors had TP53 gene mutations, whereas 75% HPV16 DNA–negative tumors had such mutations. The HPV16-positive HNSCCs had statistically significantly lower levels of allelic loss for 13 of the 15 markers on 3p, 9p, and 17p. Regarding ma-CGH, HPV-positive cancers showed generally less alterations, and in particular significantly less alterations at 3p, 5q and 9p (losses) and 11q (gains/amplifications). Shared gains were found at 3q, 5p, 8p, 9q and 20p and shared losses at 11q and 13q. A subset of HNSCC positive for HPV16 DNA, but negative for E6 and/or E7 mRNA showed a genetic pattern similar to that of the HPV16-negative tumors, indicating that presence of HPV16 DNA alone is of limited biological importance. Analysis of a group of HPV-negative HNSCC without TP53 mutation showed that the observed differences were HPV- and not TP53 related. Conclusions: Our data indicate that two genetic routes to HNSCC exist, one induced by life-style related carcinogens and one by an HPV16 infection. The distinct patterns of specific genetic alterations suggest that HPV16 infection is an early event in carcinogenesis. Some alterations are shared in both tumor groups and can be considered crucial in the later stages of HNSCC progression. Setting criteria to define HPV-positivity is an important issue in HNSCC and should not rely on DNA PCR assays only.

Symposium (Mon, 24 Sep, 14:45−16:45)
Rehabilitation and children's survivorship

51 INVITED

Long-term functional results and outcomes after bone tumour resections in children

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Background: The combination of chemotherapy and surgery nowadays cures over 60% of both children and adults with primary bone tumours. Over 85% of this group will have had an initial limb salvage surgical procedure. The aim of this paper is to review the outcomes experienced by the survivors.
Methods: There are several tools used to assess functional outcome the most popular of which are the MSTS (Musculo Skeletal Tumour Society) score and the TESS (Toronto Extremity Salvage Score), both of which can be measured as a percentage (with 100% being normal). Longitudinal measurement of functional scores in a cohort of patients allows comparison of different reconstructive procedures.
Results: Patients who undergo a primary amputation will have functional disability very much related to the level of amputation. The more distal the less the disability, but high thigh or through hip amputation, along with any upper limb amputation leave permanent significant disability. Although the aim of limb salvage is to restore and maintain function this does not always succeed, particularly if complications develop which may lead to infection, stiffness, pain or even the need for amputation.
The risk of infection following limb salvage is around 10% in most forms of reconstruction and approximately 70% can be cured by further surgery. The risk of amputation is also about 10% - due either to infection or local recurrence. Overall functional outcomes following limb salvage are best around the knee and shoulder (TESS 77%, MSTS 80%) but slightly worse around the hip (TESS 69%, MSTS 75%). The functional scores deteriorate as complications develop but successful management of the complication usually results in a return of function.
Conclusion: Functional outcomes give a useful method of comparing different types of reconstruction at a point in time, whilst longitudinal scores give a much better idea of the total amount of disability that an individual may suffer following treatment of a bone tumour.

52 INVITED

Childhood ALL – the costs of cure

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Acute lymphoblastic leukemia (ALL) is the most common cancer in children below 15 years of age, the second childhood cancer death cause, and approximately one in 2500 young adult will be a survivor of childhood ALL. The improvement in cure for childhood ALL over the latest decades has been achieved by intensification of treatment, including high-dose chemotherapy followed by allogeneic stem cell transplantation. Although the mere existence of late effects among childhood cancer patients reflects the success of therapy, they affect patient survival, quality of life, and socioeconomic status. Their standard mortality ratio is significantly increased with the most frequent premature deaths being caused by the leukemia itself and second malignant neoplasms, and more rarely organ dysfunction including cardiac disease. Children with ALL are at risk of impaired intellectual and psychosocial functioning, neuroendocrine abnormalities, impaired reproductive capacity including early ovarian failure, cardiotoxicity, and second malignant neoplasms. The known burden of these side effects logically limits the intensity of the first line-treatment offered to low-risk patients, and the vast majority of these patients are expected to have few long-term sequelae. For the more intensively treated patients the scenario is less optimistic. Unfortunately, the interindividual variability in the tolerance to such treatment modalities as topo-II inhibitors, CNS irradiation, and stem cell transplantation have been difficult to quantify prior to the therapy, and except for the infants with ALL, the prognostic risk factors related to the leukemia are in general the only determinant of the treatment intensity. Not least the irradiated patients have an

increased risk of second cancer, and endocrinological, neuropsychological, and psychosocial side-effects. The risk of second cancer have been related to intensive epipodophyllotoxin schedules, G-CSF therapy, and low activity of the enzyme thiopurine methyltransferase (the primary determinant of 6-mercaptopurine). Use of organ protectors during therapy, cryopreservation of semen and ovary tissue, and screening for endocrine and other side-effects that can be counteracted by interventions are some of the approaches applied to improve the quality of life for the long-term survivors. In addition, there is promising development in methods (including monitoring of in-vitro chemosensitivity, pharmacogenetics, and minimal residual disease) that can differentiate between clinically chemoresistant and -sensitive patients to allow for more individualised therapy, and thus avoid overtreatment. Future titration of treatment intensity should optimally reflect both the severity of the disease and the CTx-tolerance of the normal tissue to secure not only cure, but also a normal life for the survivors.

53 INVITED

Comparative assessment of long-term functional outcome in patients with soft tissue sarcoma of the extremities

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Background: Limb-saving therapy has become the treatment of choice in soft tissue sarcomas of the extremities during the past two decades. Reconstructive procedures as part of a multimodal treatment concept have abolished primary amputation without compromising survival and local recurrence-free survival. Beyond survival, there are few data evaluating physical impairment and disability in the context of quality of life, an aspect becoming increasingly important in the growing proportion of long-term survivors. When the complex medical treatment is concluded, patients must often accept physical disability after resection of soft tissue sarcomas of the extremities, which can lead to long-term limitations in the personal and social sphere.
Methods: The assessment of a treatment outcome can be made at the most varied levels (physical, mental, cosmetic, social etc.). The complex concept of disability was initially defined by the WHO with the "International Classification of Impairments, Disabilities and Handicaps" (ICIDH). The anatomical damage (impairment) was distinguished from functional limitation (disability) and social disadvantage (handicap). Following this, it is apparent that the MSTS score reflects mainly "impairment", the TESS (Toronto Extremity Salvage Score) "disability" and the RNL index (Reintegration to Normal Living index) "handicap". More than 200 patients with soft tissue sarcoma of the extremities were included in this retrospective analysis. The three scoring systems were analyzed according to gender, tumor site, age, type of surgery and self-assessment of disability.
Results: All of the patients with objective anatomical deficits were well integrated socially and the function of the affected limb was also assessed as good. No gender-specific differences were found in the three scores. This finding can most probably be explained by the equal social status. The age grouping related to the occupational situation. Children and adolescents rated themselves better in the TESS and RNL index compared to their assessment in the MSTS score. This result could be attributed to the fact that young patients demonstrate a better adaptation to a changed physical situation and learn to live with their disability. Older patients were assessed worse in the MSTS score on the one hand, and also rated themselves more poorly in the TESS compared to the RNL index. The patients' self-assessment (TESS, RNL index) yielded better results through all subgroups than the evaluation by a physician's clinical examination (MSTS score). Radiation therapy and isolated limb perfusion showed a higher rate of long-term complications related to specific treatment modalities.
Conclusion: By using different standardized tools, it could be demonstrated that physical disability is perceived to only a small degree by the patients, despite the anatomical impairments after multimodal therapy of soft tissue sarcoma of the extremities. Parallel recording of these scores allows a much better evaluation of the quality of life after multimodal therapy, taking into account tumor site and patient age. Without the use of a patient self-rating scale, "objective" measurements by the physician tend to overestimate the impact of anatomical impairment. Combining different tools for outcome assessment provides an improved understanding of the often complex post therapeutic situation of our patients.

54 INVITED

Long-term cancer survivorship issues: Is there a role for CAM?

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Background: Complementary and Alternative Medicine (CAM) use in cancer survivors has been increasing, especially in the area of mind-body medicine. We recently conducted a study comprehensively assessing health and musculoskeletal function in survivors of pediatric sarcomas. Results will be summarized and implications for the study of the effects of mind-body interventions in cancer survivors will be discussed.
Patients and Methods: Thirty-two individuals treated for Ewing sarcoma family of tumors (ESFT), rhabdomyosarcoma (RMS) or non-rhabdomyosarcoma soft tissue sarcomas (NR-STS) with multimodality therapy were enrolled on this cross-sectional study. Participants underwent assessments of musculoskeletal functioning, cardiac function, metabolic and lipid analyses, renal and gonadal function and psychological evaluation.
Results: We previously reported that this cohort of sarcoma survivors shows decreased musculoskeletal functioning, elevated body fat index, hyperlipidemia and chronic psychological distress. Recent analyses demonstrated a higher prevalence of the metabolic syndrome (OR 4.29 95% CI: [1.50, 11.21]), defined as 3 or more metabolic syndrome traits (MST) in subjects aged 20−39 years. Analysis of individual MST demonstrated higher prevalence of hypertension (OR 2.61 95% CI: [1.20, 5.59]), hypertriglyceridemia (OR 3.63 95% CI: [1.75, 7.60]) and male visceral abdominal obesity (20−39yo OR 4.63 95% CI: [0.91, 21.63], 40−59yo OR infinity). In male subjects, total testosterone declined ($p = 0.0027$) as the number of MST increased. Average ($p = 0.014$) and maximum ($p = 0.021$) physical activity levels decreased as the number of MST increased.
Conclusion: An increased number of MST especially in younger sarcoma survivors, combined with decreased physical functioning, dyslipidemia and psychological stress suggest an increased risk for cardiovascular disease, even many years after completion of sarcoma therapy. A recently initiated study will compare the effect of a Mind-body intervention (Tai Chi Chuan) and aerobic exercise on stress and physical fitness in cancer survivors.

Award Lecture (Mon, 24 Sep, 17:00−17:45)
Pezcoller Foundation/FECS recognition for Contribution to Oncology

55 Pezcoller/FECS Award

Perspectives on virus–host interactions and tumour development

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It is estimated that approximately 20 percent of human cancers are linked to infectious agents. Beginning with the identification of Epstein-Barr virus in Burkitt lymphoma cells in 1964, intensive research into the connections between viral infections and cancer have led to the identification of 6 human tumour viruses, and continued research is likely to uncover new agents. Dissection of the interactions between tumour viruses and host cells have yielded significant advances in our understanding of how these oncogenic infectious agents elude the immune system, persist in their host, and promote the step-wise chain of events that results in neoplastic transformation.
While tumour viruses are quite ubiquitous in many geografic areas, the development of the associated neoplastic diseases represents a rare event. Furthermore, a prolonged incubation period (years or decades) from the initial virus infection is usually needed before the appearance of clinically detectable associated tumour. Clearly, multiple co-factors following the primary exposure to the infectious agent must be at work to cause cancer. Some experimental and clinical findings, focused mainly on Human T-cell Leukemia virus, type 1 and Kaposi's sarcoma-associated human herpes virus-8, will be discussed to illustrate the complexity of the virus–host interplay in oncogenesis.