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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.

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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

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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.

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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