to harbor secondary mutations in EGFR which may predict both primary and secondary resistance. The best studied of these is the T790M mutation which is seen in nearly half of patients with acquired resistance to drug. Furthermore mutation testing allows clinicians to base treatment based on the type of mutation. It is highly likely that not all mutations will be equivalent in predicting response. Deletions in exon 19 are likely to be more predictive of response to TKI treatment than point mutations in exon 21. Furthermore there is emerging evidence that mutations in exon 20 might be predictive of primary resistance to EGFR treatment.

Selection of agents based on mutation testing is under review in the setting of secondary resistance to EGFR-TKI treatment. A phase II study of dual kinase, irreversible inhibitor HKI-272 is underway in North America and Europe. In this trial patients who have progressed after 12 weeks of TKI treatment are treated with HKI-272 in cohorts based on mutation status. It is highly likely that several new agents in development will be entered into similarly designed studies.

EGFR is not the only target where molecular staging will be of use. Ras is an important target in lung cancer, particularly smoker's lung cancer. Mutations in K-ras are seen in nearly 30% of NSCLC patients. While the Ras farnesyl transferase inhibitors have not proven to be of clinical benefit there are several other agents that have preclinical activity and will be examined in patients with ras-mutations. Determination of ras-mutation status has been proposed by some to be helpful in predicting resistance to EGFR-TKI so this might have even more relevance in lung cancer treatment.

Finally gene expression arrays are under intensive investigation as potentially useful molecular staging tests. Studies are underway to determine the utility of expression profiling as a means of determining prognosis in lung cancer. While this technique is currently limited to fresh tissue if it proves useful it may become a routine test performed on resected lung cancers to help prognosticate outcome and to select patients for potential adjuvant trials.

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S56. siRNA-TECHNOLOGY ON THE ROAD TO MOLECULAR THERAPY

Steven Dooley, Patrico Godoy. *Center of Molecular Alcohol Research in Gastroenterology, II. Medical Clinic, University Hospital Mannheim, University of Heidelberg, Germany.*

The possibility to specifically knock down gene expression in a cellular system is a powerful way to study its function. RNA interference (RNAi) as molecular mechanism for gene silencing in mammalian cells has allowed the discovery of several new and exciting regulatory mechanisms in apoptosis, cell cycle and cancer. The technology was recently improved by new delivery methods allowing both enhanced and long lasting silencing of target genes, that is, more efficient transfection reagents, and generation of plasmid and virus based vectors for siRNA delivery. The possibility of RNAi-scale up for high-throughput (HT) analyses was used in several genome-wide screens. Thus, HT knock down for mammalian kinases revealed important new features in the control of critical cellular steps of endocytosis. The power of these approaches can be harnessed to delineate complex signal transduction pathways leading to disease. TGF-β is a major cytokine in liver physiology. It regulates both cell proliferation and apoptosis of hepatocytes, and is the main drive of fibrosis, a typical complication of chronic liver diseases. Our goals are to use high throughput siRNA technologies to discover new and pathobiochemically relevant regulatory components of the TGF-β pathway in liver cells with potential as targets for therapeutic intervention.

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S57. INTEGRATION SITE MONITORING IN CLINICAL GENE THERAPY

Christof von Kalle. *National Center of Tumor Diseases (NCT) Heidelberg, Germany.*

Retroviral gene therapy has been coming of age, reaching unprecedented levels of therapeutic efficacy. While the monoallelic integration of replication-defective vectors was thought to be without considerable consequences on the host genome, recent reports on vector integration related genotoxicity in preclinical animal models have raised concerns on the biosafety of clinically applied vectors. Possible side effects of vector integration ranged from immortalization of transduced cells, clonal dominance to insertional oncogenesis. A vector related genotoxicity has occurred in leukemogenesis in 3 SCID-X1 patients, with remarkable consistency by vector-induced LMO2 oncogene activation, putatively enhanced by a synergetic effect of the constitutively expressed vector transgene IL2RG. In the first successful gene therapy trial of chronic granulomatous disease, we have now observed even more intensive but thus far non-toxic insertional side effects resulting in activation of MDS1/EVI1, PRDM16 or SETBP1 5 months after therapy that produced a 3- to 4-fold expansion of gene-corrected long term myelopoiesis in both treated patients, notably without signs of leukaemia to date. Prospective monitoring of vector integration sites in clinical gene therapy studies is feasible, can detect possible side effects of gene therapy in real-time and may gain new insights in basic monogenic mechanisms leading to specific clonal behaviour in vivo.

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S58. TARGETING THE EGF RECEPTOR: EXPERIENCE AND LESSONS

John Mendelsohn. *The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030, USA. E-mail address: jmendelsohn@mdanderson.org*

In 1981 we hypothesized that blockade of the binding site for EGF and TGF-alpha on EGFR with an antireceptor monoclonal antibody (mAb) might be an effective anti-cancer therapy, by inhibiting activation of the receptor tyrosine kinase. Murine mAb 225 inhibited EGFR tyrosine kinase activity, and inhibited tumor cell growth in cultures and in nude mouse xenografts. C225 (cetuxi-

mab) is the human:murine chimeric version of mAb 225. Pharmaceutical companies subsequently have developed a number of oral, low molecular-weight inhibitors which act on the ATP binding site of EGFR, also blocking receptor activation, and additional mAbs have been produced. C225 and many of the low molecular-weight agents are in clinical trials with a variety of cancers. The mechanisms of tumor inhibition include growth inhibition mediated by upregulation of $p27^{Kip1}$, enhancement of apoptosis, and inhibition of angiogenesis and metastasis. In addition, these agents enhance the cytotoxicity of chemotherapy and radiotherapy in experimental systems. In the case of mAb C225, immune mechanisms may contribute to the antitumor activity. Three agents – gefitinib, cetuximab and erlotinib – have been approved by the FDA. The series of clinical studies leading to approval of C225 will be reviewed in detail. Predictions of efficacy from animal models were not always helpful when anti-EGF receptor agents were tested in the clinic. Recent research has uncovered potential markers that may identify patients more likely to respond to some of these agents, but definitive conclusions cannot be made. Clinical trials are pursuing mechanisms of action, identifications of markers predicting efficacy, pharmacodynamic analyses to verify adequate dosing, and continuing exploration of EGF receptor inhibition in combination with other therapies. More research will be required to optimize the use of this class of anti-cancer therapy.

FURTHER READING

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