suggesting a similar overall survival benefit for the two drugs. Furthermore survival subset analyses in BR21 were consistent with ISEL, with the largest survival benefits for erlotinib also seen in the never-smoking and Asian subgroups. The objective response rates were comparable for gefitinib and erlotinib in these two studies (8% vs. 9%, respectively).[1,2]

As the ISEL result was surprising, a number of comparisons have been made.[3]

In ISEL 45% of patients had progressed and only 18% responded on the most recent chemotherapy, whereas for BR21 28% progressed and 38% had responded, the more refractory patients may have had less chance of benefiting. Erlotinib has a greater affinity for the receptor and was used at the MTD (150 mg) the similar dose for gefitinib would be 700 mg not the 250 mg used in ISEL. Further work investigating patient characteristics e.g. smoking status, identification of more sensitive populations and molecular markers will be important.

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S54. PROTEIN LYSATE ARRAY ASSESSMENT OF THERAPEUTIC TARGETS IN SARCOMA

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Small molecule inhibitors have brought new hope for cancers with dire prognoses. These molecular medicines turn off specific signaling intermediaries within cells, leaving others unaffected. Their efficacy has been demonstrated clinically with medicines such as imatinib for CML and GI stromal tumor and erlotinib for EGFR-dependent head and neck, lung and breast cancer. More small molecules are being developed. To rationally apply this development to more diseases, a rapid screening tool is required to identify expression and activity of protein targets in an individual patient's tumor. The technical challenges for this tool are significant: assessing dozens, if not hundreds, of potential targets accurately using the small amount of tissue available through core needle biopsies. We have begun applying a novel technology – protein lysate array analysis – to address this problem in sarcoma. Tumor lysates are arrayed on nitrocellulose matrix using a modified DNA arrayer, creating 100+ duplicate slides using as little as one microgram total protein. Individual slides are assayed with monospecific antibodies and comparisons made between phospho- and total protein levels, identifying the activation state of dozens of potential therapeutic targets. We have used this technique preclinically to test the downstream effects of erlotinib in osteosarcoma and Ewing sarcoma, identifying changes in MAPK, mTOR, AKT and JNK pathway signaling. We will use it in a clinical trial of an anti-ERBB medicine to assess the correlation between disease response and changes in signaling, using paired samples of pre- and post-treatment tissue. We envision prospective testing of tumor tissue, allowing the clinician to choose those small molecule(s) able to inhibit the specific pathway(s) active in an individual's tumor.

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S55. MOLECULAR STAGING OF NSCLC: 2006

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The treatment of lung cancer has undergone a remarkable transformation over the past five years. Previously histology and anatomic stage were the primary determinants of treatment. While these still have an important role, the future of treating this disease will be based on molecular staging strategies. This will allow us to select more effective and less toxic treatments in the initial treatment of metastatic disease. It will also permit informed selection of patients for adjuvant treatment. Finally aggressive molecular staging will hopefully uncover new targets that will result in new drugs that may one day transform lung cancer into a chronic disease with long-term survival the rule and not the exception.

Agents that target the epidermal growth factor receptor (EGFR) tyrosine kinase are among the most important new drugs in use to treat non-small cell lung cancer. Both gefitinib and erlotinib are capable of producing remarkable tumor responses as single agents that are durable. These dramatic responses are often associated with mutations in the EGFR tyrosine kinase domain. In addition when used in second and third line treatment of lung cancer erlotinib has been shown to prolong survival in this setting. This clinical benefit is best predicted by increased EGFR gene copy number as measured by FISH.

The use of EGFR-TKI provides an exceptional opportunity for molecular staging. EGFR mutation testing is being used to select patients for first line treatment with both gefitinib and erlotinib. Trials in the United States, Japan and Europe are employing this strategy and early results should be reported by the end of 2006. The potential to identify a population of patients who might be able to be treated with EGFR-TKI monotherapy as first line would potentially deliver equivalent anti-tumor activity with fewer side effects than combination chemotherapy.

Measurement of gene copy number by FISH is being used to select patients for treatment with EGFR-TKI treatment in several clinical scenarios. Patients who are FISH positive are being entered onto trials of erlotinib plus chemotherapy as first line treatment. Adjuvant studies of chemotherapy plus erlotinib given as sequential therapy are under review. Finally there is some controversy as to the relative value of EGFR protein expression as measured by immunoperoxidase staining. Some thoughtful investigators feel that the best way to select adjuvant and first line metastatic patients for TKI treatment is by using a combination of FISH and immunoperoxidase staining.

While FISH and immunoperoxidase may be important modalities in the molecular staging of lung cancer, mutation testing offers a potential benefit not available with those methods. Patients who are resistant to EGFR-TKI treatment have been found

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

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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

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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

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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-