preproGRP transcripts were found in 28% of the blood samples. The detection of a hematogenic tumor cell spread with preproGRP in MTC correlated significantly with advanced tumor categories. CK20 transcripts were detected in 75% of the blood samples of patients with thyroid carcinoma and distant metastases. Moreover disseminated tumor cells were detected in 21% of the bone marrow samples with CK20-PCR and 13% with preproGRP (only for MTC).

**Conclusion:** Both assays are sensitive enough to detect disseminated thyroid carcinoma cells in blood and bone marrow samples. However, the prognostic relevance of these disseminated tumor cells is not completely understood and has to be addressed in further investigations.

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## S52. Pdcd4 TARGETS eIF4A TO INHIBIT TRANSLATION, TRANSCRIPTION, TUMORIGENESIS, AND INVASION

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Despite its name, Programmed Cell Death 4 (Pdcd4) may or may not induce apoptosis. Pdcd4 was discovered as a highly expressed gene in mouse JB6 cells resistant to transformation. Pdcd4 inhibits transformation and tumorigenesis, in part by specifically inhibiting AP-1 dependent transcription. The binding partners of Pdcd4 are not Jun or Fos proteins but are translation initiation factors eIF4A and eIF4G. Pdcd4 inhibits translation initiation by directly binding to translation initiation factor eIF4A and inhibiting its helicase activity. The helicase activity of eIF4A is important for unwinding 5′UTR structured mRNAs prior to scanning to the translational start site. Pdcd4 also interferes with scaffold eIF4G function. Pdcd4 must interact with eIF4A and inhibit translation in order to inhibit AP-1 transactivation, as Pdcd4 mutants inactivated for eIF4A binding fail to inhibit AP-1. Recent findings with K14-driven Pdcd4 expression in mice have established that Pdcd4 inhibits translation of a 5′UTR-structured mRNA as well as expression of "translationally repressed" proteins. Pdcd4 inhibits AP-1 dependent transcription and acts to attenuate papillomagenesis and papilloma to carcinoma conversion. Moreover Pdcd4 expression (a) is downregulated with progression in several human cancer sites, (b) confers sensitivity to certain therapeutic drugs, and (c) suppresses invasion and motility in human cancer cell lines. Pdcd4 suppresses cancer cell invasion by targeting the expression of MAP4K1, an upstream regulator of Jun N-terminal Kinase signaling, with consequent inhibition of AP-1 dependent transcription. Thus, activating or mimicking the expression of Pdcd4 might be an attractive preventive or therapeutic strategy. Enhancing the interaction of Pdcd4 with eIF4A or targeting downstream translational targets may produce the "desired" but not the "undesired" outcomes achieved with mTOR inhibitors. mTOR inhibitors repress translation by enhancing the interaction of 4E-BP with cap binding protein eIF4E but are also immunosuppressive. Pdcd4 appears not to show immunosuppressive activity. Although we and others have identified translationally repressed candidates, the functionally significant translational targets of Pdcd4 are still unknown. Knowing these Pdcd4 targets is important for designing prevention strategies. In summary, Pdcd4 is the first suppressor of tumorigenesis and invasion known to directly inhibit translation initiation. Translation initiation thus appears to be a promising molecular target for cancer prevention and intervention.

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## S53. THE ISEL AND BR21 TRIALS – OUTCOMES SIMILAR OR DIFFERENT?

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The survival effects of EGFR-TKI therapy have been evaluated in two Phase III placebo-controlled studies in refractory NSCLC: ISEL (IRESSA Survival Evaluation in Lung cancer no. = 1692) and BR21 (erlotinib no. = 731).[1,2] Gefitinib (Iressa) showed some improvement in survival compared with placebo, but the difference did not reach statistical significance on the prespecified stratified log rank test required for registration in either the overall population (HR 0.89; $p = 0.087$; median 5.6 vs. 5.1 months) or in patients with adenocarcinoma (HR 0.84; $p = 0.089$; median 6.3 vs. 5.4 months). However, preplanned subgroup analyses showed that gefitinib significantly prolonged survival in patients of Asian ethnicity and in patients who had never smoked. The erlotinib BR21 study had a similar design to ISEL, but demonstrated a statistically significant overall survival benefit for erlotinib HR = 0.7 $p < 0.001$ median 6.7 vs. 4.7 months.[2] However the 95% confidence intervals for the HRs overlap ISEL 0.77–1.02 and 0.58–0.85 for BR21

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