SP 125
Functional activity versus clinical predictivity

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Advanced imaging methodologies play a pivotal role in cancer care, by providing the ability to detect tumors early and to guide therapy as well as in subsequent disease monitoring and surveillance. Advantages inherent in the imaging (in vivo) assays include the ability to obtain spatially localized information over large volumes of tissue or the entire body, compared to the limited sampling required for in vitro assays and its inherent drawbacks. In addition, in vivo imaging assays have the ability to provide multiple evaluations of a molecular target or tumor metabolism over time − allowing for adaptive therapy without invasive procedures. Continued progress in research and development of imaging agents, methodologies and technologies holds promise for better cancer care, for example, with improved tumor detection and characterization. These new agents and approaches exploit various pathophysiologic and anatomic characteristics of tumors with evaluations of phenomena such as metabolism, proliferation, hypoxia, angiogenesis, essential signal pathway blockage(s) and other tumor microenvironment modifications. In addition, the use of validated molecular imaging agents is critical to the NCI drug discovery and development process as well as the ongoing NCI commitment to further our understanding of cancer biology. However, incorporation of biomarker and imaging endpoints into early phase clinical trials is not straightforward, requiring appropriately designed studies to enable the use of the results to direct further trials. This presentation will discuss the activities at NCI to evaluate which of the promising bio-markers will provide clinically meaningful information − for the development of novel therapeutics, as well as clinical care.

SP 110
Epigenetics and drug resistance

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Minimal residual disease or fractional tumor cell killing is the sine qua non of acquired drug resistance and is one of the major causes of relapse in cancer patients. While the mechanisms by which cancer cells evade a therapeutic in the patient are hard to investigate, the observation of a similar phenomenon in vitro suggested that the problem may perhaps be tractable.
A variety of oncogene addicted human tumor derived cell lines were treated with anti-cancer agents directed against the addicting oncogenes. While most cells die from such treatments, in all cases we consistently detected a small sub-population of drug-refractory/reversibly drug resistant cells (which we refer to as "drug-tolerant persisters" [DTPs]). Over time, DTPs evolve into cells with acquired drug resistance. We therefore studied the DTPs to better understand their reversible drug refractory nature.
DTPs, which comprised about 0.2 to 2% of the cancer cell population, demonstrated several 100-fold reduced drug sensitivity compared to the bulk of the tumor cells and were highly reminiscent of minimal residual disease seen in patients. The high frequency of these cells together with their reversible drug resistance, strongly suggested a non-genetic/epigenetic basis of drug tolerance. Molecular analysis revealed that DTPs maintain viability, at least in part, via engagement of altered signaling pathways and an altered chromatin state. Intriguingly, the drug-tolerant phenotype was transiently acquired and relinquished at low frequency by individual cells within the population, implicating the dynamic regulation of phenotypic heterogeneity in drug tolerance. DTPs could be selectively ablated by treatment with specific signal transduction inhibitors or chromatin-modifying agents. Chromatin modifying agents were found to prevent acquired drug resistance in a variety of human cancer derived cell lines.
These findings suggest that cancer cell populations employ a dynamic survival strategy in which individual cells transiently assume a reversibly drug-tolerant state to protect the population from eradication by potentially lethal exposures. Better understanding of this drug tolerant state could lead to strategies to greatly delay/prevent acquired resistance to most commonly used cancer therapeutics and provide more effective strategies for the deployment of these agents in the clinic.

SP 123
Overcoming drug resistance in melanoma

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Over the past 2 years, molecularly based therapy has become central to the treatment of metastatic melanoma through the ability to effectively target the BRAF V600 mutation present in 50% of melanomas. BRAF inhibitors such as vemurafenib and dabrafenib have demonstrated clinical responses in over 50% of patients (V600E/K) characterized by rapid tumor reduction and improvement in symptoms within days. Recently, a phase III trial of vemurafenib in metastatic BRAF V600 mutant melanoma demonstrated striking improvement in overall survival (OS) compared to standard chemotherapy with a 63% reduction in death. This dramatic improvement in OS was due to the BRAF inhibitor's ability to control rapidly growing melanoma that otherwise would lead to patients death within a few months. However, the median duration of response and progression-free period are just less than 7 months. Though a few remissions last over 2 years, ultimately, all patients will relapse; The mechanism of this acquired resistance in under intense investigation. The significance of the findings requires their validation in tumors from resistant melanoma patients treated with BRAF inhibitors. Several mechanisms have already been described; most frequently the presence of a mutation upstream in the NRAS molecule that can reactivate the MAPkinase pathway even in the presence of the BRAF inhibitor. Also, mutations in the downstream MEK1 molecule leading to its activation and the overexpression and activation of COT, a MAP3K8 that can independently activate MEK have been reported. In other reports, MEK1 mutations have also been seen prior to therapy and may not be functionally significant in acquired resistance. Others have seen activation of receptor tyrosine kinases (PDGFRβ and IGFR1) or loss of PTEN that then drive resistance through alternate pathways (i.e. PI3K/Akt). Finally, an alternate mechanism is the result of splice variants of BRAF V600 mRNA that code for proteins that allow signaling through RAF dimerization and MAPKinase pathway reactivation. Among the diverse number of mechanisms of resistance, not reported is secondary (second site) mutations in the BRAF V600E molecule that act as gatekeeper mutations or the loss of BRAFV600E mutation in resistant melanoma samples. There will likely be other mechanisms that have not been identified at this time. These efforts are crucial in developing strategies to treat BRAF inhibitor resistant tumors and more importantly in preventing the development of resistance. Combination trials are already underway and in the future these approaches may be better targeted to the patients individual melanoma's actual mechanism of resistance.

SP 111
miRNA and lung cancer: early detection in high-risk subjects

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Lung cancer remains the major cause of cancer mortality in the world. In addition to primary prevention, earlier detection and more targeted treatments, tailored on the biological characteristics of the tumor and its microenvironment, could significantly reduce morbidity and mortality for this disease. The real efficacy of lung cancer screening by spiral-computed tomography (CT) in heavy smokers is still to be defined since, in spite of a proved capacity to detect small asymptomatic nodules, the frequency of false positive CTs as well as unnecessary treatments is very high if compared to the limited mortality reductions. The development of biomarkers able to identify tumors in a pre-clinical phase and to track the different aggressiveness of lung tumors is of paramount importance. MicroRNAs (miRNAs) represent a recently identified class of regulatory molecules and several studies showed that miRNA are involved in lung tumor development and progression and also circulate in plasma and serum of lung cancer patients.
We analysed miRNA profiles of lung tumors, normal lung tissues and plasma samples from cases with variable prognosis identified in two independent spiral-CT screening trials where multiple plasma samples, collected from one to four years before radiological detection of the disease were available.
We found miRNA expression profiles associated with aggressiveness of the disease and poor survival in tumors and also in normal lung tissues of the patients, thus proving the critical influence of a smoking-related lung microenvironment on tumor progression. Specific microRNA signatures were identified in plasma samples collected up to two years before spiral-CT detection of the disease, thus able to catch the earlier biological phases of disease development. We also defined a plasma signature that discriminates subjects according to aggressiveness of their future tumors, and in particular the occurrence of early metastatic but spiral-CT invisible lung tumors or small spiral-CT detected lesions with aggressive potential. Of interest miRNas involved in the signatures of lung cancer risk and of aggressive disease more closely reflected miRNAs expressed in the normal lung rather than those characterizing the tumor samples of patients, supporting the concept that normal lung microenvironment has a critical influence on tumor development and aggressiveness.
These results open up the prospective of using miRNA as non invasive lung cancer biomarkers.