the PD efficacy in the 3 different schedules. Plasma EMD72000 concentrations are determined at sequential time-points by a validated ELISA assay. PD studies include determination of EGFR expression in archived tumor material, saturation of EGFR in skin after treatment, inhibition of signaling pathways in skin and tumor before and after treatment (total and activated EGFR and MAPK; Ki67 and p27 expression; pAkt expression and TUNEL apoptosis assay). The skin and tumor tissue samples were obtained on day 0 (before treatment) and on day 43. A total of 13 p with EGFR+ tumors have been included: colon (7), gastric (3), ovarian (2) and renal (1), with a median Karnofsky index of 90% (range: 80%-100%), a median age of 57 (range: 39-75), and receiving a median of 3 prior chemotherapy regimens (range: 1-11). Toxicity has been limited to grade 1-2 nausea, vomiting, rash, pruritus, headache and diarrhea with no DLTs observed. In 9 p evaluable for response there have been 2 not confirmed PRs in p with colon cancer and 2 stable disease (>15 weeks) in a renal cell and in a colon cancer p. These responses were observed among the three different schedules. EMD 72000 treatment demonstrated an almost complete inhibition of the activated EGFR and MAPK with a decrease in the Ki67 expression and an increase in the p27 expression in skin samples in all the three different schedules. Moreover, the typical pattern of epidermal changes observed after EGFR blockade have been observed in the three cohorts of p. Complete PK and skin and tumor PD profiles will be presented. In summary, the preliminary efficacy and PD data suggest that more convenient every 2-3 weeks administration schedule may be feasible.

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Sustained activation of JNK cascade and apoptosis induced by rapamycin is suppressed by p53

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Rapamycin analogues CCI-779 and RAD001 are currently in early clinical trials for treatment of malignant disease. However, there is emerging data to support selective activity of these agents based upon genetic changes that occur during neoplastic transformation. In response to rapamycin, cells lacking functional p53 undergo apoptosis, whereas cells expressing wild-type p53 or p21Cip1 arrest in G1 and remain viable. Our studies have focused on understanding the mechanism by which rapamycins induce cell death. In rhabdomyosarcoma cells and murine embryo fibroblasts rapamycin induces rapid, sustained activation of JNK only in cells lacking functional p53, but only transient activation in cells expressing wild-type p53 or p21Cip1. Overexpression of dominant negative c-Jun (TAM67) or ASK1 (K709M) prevents rapamycin-induced phosphorylation of c-Jun, and apoptosis. Furthermore, cytoplasmic p21Cip1 interacts with ASK1, negatively regulating JNK cascade. Overexpression of p21Cip1 or p21Cip1 lacking the nuclear localization signal (DNLS-p21) also protects cells from death. The results suggest that rapamycin induces apoptosis by prolonged activation of JNK cascade, and p53 and p21Cip1 prevent cell death through inactivation of ASK1 by p21Cip1, and not through enforcing G1 arrest. These results indicate that the response to inhibition of the rapamycin target (mammalian TOR) is determined at least in part by p53 functional status, and reveals a rational basis for developing tumor-selective therapy.

Thursday 21 November

PLENARY SESSION 7

Transcription factors and related pathways

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Cooperating mutations as targets for therapy in leukemia

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AML, like other human cancers, is caused by more than one mutation. Epidemiologic evidence, and data from murine models, indicates that two broad classes of mutation cooperate to cause acute leukemia. One class of mutation provides a proliferative and/or survival signal to leukemic cells, as exemplified by activation mutations in tyrosine kinases, such as FLT3, c-KIT, BCR/ABL, or TEL/PDGFβR. A second broad class of mutations impairs hematopoietic differentiation. These include chromosomal translocations or point mutations that result in aberrant function, or loss of function, of hematopoietic transcription factors required for normal hematopoietic development. Examples include translocations involving core binding factor; the retinoic acid receptor a; HOX family members; and point mutations in AML1 and C/EBPa. Together, these two classes of mutations result in the acute leukemia phenotype of uncontrolled proliferation and impaired differentiation. Epidemiologic data supports the hypothesis that two classes of mutation cooperate to cause leukemia. For example, progression of CML to blast crisis may be associated with acquisition of a NUP98/HOXA9 fusion as a consequence of t(7;11); and FLT3 activating mutations occur in 40% of APL patients with the PML/RARa gene rearrangement. We have also developed murine models of acute leukemia to support this hypothesis, including models of cooperativity between BCR/ABL and NUP98/HOXA9; and between FLT3-ITD mutations and PML/RARalpha. Inhibition of the respective tyrosine kinase, both in humans and in murine models, has therapeutic efficacy against leukemia blasts that harbor more than one mutation. That is, agents that target the proliferative signal in acute leukemia blasts may have therapeutic activity. Furthermore, as mutations in FLT3, c-KIT and RAS account collectively for no more than 50% of leukemias, it is likely that additional as yet unidentified kinases or their effectors are mutant in AML, and may represent novel therapeutic targets. It may also be possible to achieve synergistic therapeutic effects by using agents that target the respective kinase with agents that override the block in differentiation, as in the case of ATRA therapy of APL. For example, combinations of FLT3 inhibitors and ATRA may have therapeutic benefit in a select group of APL patients. The future holds great promise for further development of molecular therapies that target these two respective classes of mutation.

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Targeting C/EBP alpha in leukemia and lung cancer

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The primary defect in acute myeloid leukemia (AML), like many cancers, is a block in differentiation. Development of new drugs which specifically target the differentiation block, such as ATRA in t(15;17) APL, are likely to be more effective and less toxic than less specific chemotherapy. Recent data from our laboratory have demonstrated that the C/EBP alpha transcription factor (1) is the critical factor for myeloid differentiation; (2) is mutated or down-regulated in many AML subtypes; and (3) restoration of expression relieves differentiation block of leukemic cells lines. Therefore, we have been working on methods to identify and characterize drugs which augment C/EBP alpha expression and/or function in order to develop novel specific therapies for AML. To date we have generated stable myeloid cell lines to serve as indicator cells for high throughput drug screening for drugs activating C/EBPa function, including the human myeloid U937 and murine myeloid progenitor 416B lines. Prior to the drug screening, all lines were tested for responsiveness to a known inducer of granulocytic differentiation of these cells, all-trans retinoic acid (ATRA). We then collaborated with Robert Shoemaker and Ed Sausville at the National Cancer Institute's Developmental Therapeutics Program. One U937 line was used for initial pilot drug screens in which 1990 compounds were tested (the NCI "Diversity Set", which was selected by computer algorithm in an attempt to represent the greater chemical diversity of the total NCI repository of almost 140,000 compounds). Subsequently 135,640 compounds were screened at a single, 1 mM concentration. 70 of the most active compounds were subsequently analyzed in 20-concentration titration experiments. After completion of the primary (single-dose) drug screen, reaching a total of 140,000 compounds, the first set of active compounds identified thus far will be subjected to additional testing in our leukemic cell line models. In addition, recent data from our laboratory has indicated that C/EBP alpha is downregulated in a significant number of patients with non-small cell lung cancer, suggesting that drugs identified in our screen using leukemic cell lines might also have usefulness in other types of cancer as well.