in determination of the OBD for such therapies in clinical trials. We are currently measuring EGFR ligands in the plasma of cancer patients undergoing treatment with Cetuximab and preliminary results will be available at the time of the meeting.

218 POSTER

Phase I/II study of CNTO 95, a fully human monoclonal antibody (mAb) to alpha-v integrins, in patients with metastatic melanoma

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Background: CNTO 95 is a fully human anti-α_v integrin antibody that inhibits the growth of human melanoma xenografts in nude mice and rats by ~80% and >99%, respectively. The objective of this study is to assess the safety and preliminary efficacy of CNTO 95, alone or in combination with dacarbazine (DTIC), in patients with advanced melanoma.
Material and Methods: CNTO 95 alone (3, 5 and 10 mg/kg) or in combination (5 and 10 mg/kg) with DTIC (1000 mg/m^2) was infused on day 1 of three-week treatment cycles. Safety data from the first cycle were used for the evaluation of acute toxicity. Tumor assessments were performed every two cycles.
Results: Fifteen patients were enrolled in phase 1 at 3 (n = 3), 5 (n = 3) and 10 mg/kg (n = 3) of CNTO 95 alone and 5 (n = 3) and 10 mg/kg (n = 3) of CNTO 95 + 1000 mg/m^2 DTIC. No dose-limiting toxicities were observed. The maximum tolerated doses of either CNTO 95 alone or its combination with DTIC were not reached. CNTO 95 exposure (AUC) increased in a greater than dose proportional manner and might be attributed to a tissue binding effect. Mean terminal half-life at 10 mg/kg dose level is 5.3 days. The pharmacokinetics of CNTO 95 was unaffected in the presence of DTIC (preliminary data).
One subject achieved a complete response (CR) and three subjects had stable disease (SD). One subject [3 mg/kg CNTO 95] developed bilateral Grade 2 asymptomatic uveitis following the first administration of CNTO 95, which was treated and resolved. The subject continued in the study without recurrence and no additional cases have been reported. Another subject [3 mg/kg CNTO 95] experienced a seizure-like event 39 days after study agent discontinuation. Neither event was considered dose limiting.
One subject with mediastinal metastases [5 mg/kg CNTO 95] had a confirmed CR after 2 cycles of CNTO 95; this subject has received 14 cycles of treatment. One subject [5 mg/kg CNTO 95] had SD for 6 months, experienced progressive disease, and is now being escalated to 10 mg/kg CNTO 95. One subject [10 mg/kg CNTO 95] had SD for 6 months and underwent complete surgical resection. One subject [10 mg/kg CNTO 95 + 1000 mg/m^2 DTIC] has SD after 7 cycles; treatment is ongoing.
Conclusion: CNTO 95, a fully human mAb to α_v integrins, is well tolerated and demonstrates activity alone or in combination with DTIC in subjects with advanced melanoma. Additional data is being accumulated to further characterize the safety and efficacy of CNTO 95.

Structure–activity relationships

219 POSTER

Equilibrium on hold. A computational rationale for the role of kit juxtamembrane mutations in controlling receptor autophosphorylation

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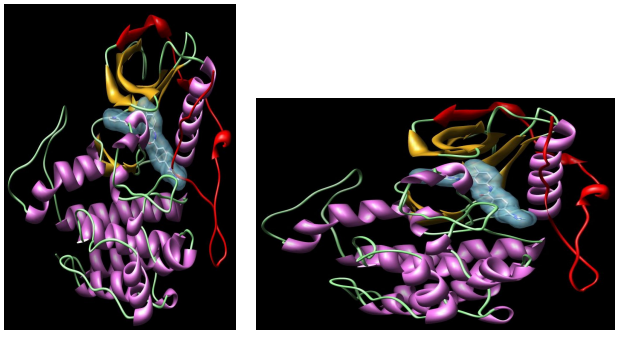
Background: Mutations in the Kit receptor tyrosine kinase (RTK), which result in ligand-independent activation of the kinase, are associated with cancers such as gastrointestinal stromal tumors (GISTs) and mastocytosis. Kit mutations in GISTs most frequently occur in the noncatalytic Kit juxtamembrane (JXM) region, suggesting that this domain is crucial in regulation of kinase activity. Moreover, genetic and crystallographic studies have implicated the cytosolic JXM region of the Kit RTK as an autoinhibitory regulatory domain. In this study we propose a computational rationale for the role of wild-type and clinically relevant mutant Kit JXMs in controlling receptor autophosphorylation and its response to imatinib.
Materials and Methods: We have used advanced molecular simulation techniques, based on the so-called self-guided molecular dynamics (SGMD) and molecular mechanics/Poisson-Boltzmann free energy calculations (MM/PBSA), to investigate the behavior of isolated wild-type and mutant Kit fragments formed by the JXM residues that fold into a -hairpin folding of the Kit wild-type and several mutant JXM domains was directly simulated in explicit water at native folding conditions in three 300-ns SGMD simulations. Through structural and energetic analysis of the folding events, we answered some basic questions about the folding of these domains in water.
Results: The wild-type sequence folded into a series of β-hairpin structures in our simulations, the major cluster of which agrees well with the X-ray experimental observation. On the contrary, altered structures were obtained, as function of the different type of mutation considered (i.e., missense and deletions). Different intrapeptide interactions drive the JXM to misfolded conformations, and the solvation/entropic effects, which resist folding, are also shown to prevent the mutant sequences peptide from folding into wild-type like structures. These structures then act differently in keeping the Kit in its autoinhibited conformation. Finally, simulations of the entire protein with wild-type and mutant JXMs allowed to calculate the free energy of binding (and hence the IC_{50} value) of these RTK and Imatinib.



Conclusions: Our simulations contributed for the first time to highlight the possible effects exerted by the presence of Kit JXM mutations on the active/inactive structure of Kit and on its affinity towards Imatinib.

220 POSTER

Identification of elongation factor-2 kinase as a regulator of autophagy in cancer cells: implications to cancer therapy

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Elongation factor-2 kinase (eEF-2 kinase), also known as Ca++/calmodulin-dependent kinase III, is a structurally and functionally unique protein kinase that regulates protein synthesis by controlling the rate of peptide chain elongation. The activity/expression of eEF-2 kinase is increased in glioblastoma and other malignancies, yet its role in neoplasia remains uncertain. Activation of eEF-2K transiently inhibits protein synthesis by phosphorylation of Thr-56 of eEF-2, thereby disrupting peptide elongation. In the presence of adequate nutrients and growth factors, eEF-2K is inhibited (and protein translation promoted) by activated mTOR and S6 kinase, which phosphorylate Ser-78 and Ser-366, respectively. In the absence of nutrients and growth factors the activity of eEF-2 kinase is increased (and protein translation inhibited) due to decreased activity of mTOR and S6 kinase as well as increased activity of 5′AMP kinase, which directly inhibits eEF-2 kinase by phosphorylation of Ser-398. Since protein elongation accounts for a major use of cellular energy, we sought to determine the role of eEF-2 kinase in the regulation of cell survival during times of nutrient and growth factor depletion. Autophagy is a conserved response to nutrient deprivation through 1). self-digestion of cytoplasm and organelles and the recycling of amino acids for energy utilization and involves formation of a double-membrane vesicle (“autophagosome”) in the cytosol that engulfs organelles and cytoplasm, then fuses with the lysosome where the contents are degraded and recycled. This form of self-digestion can lead to self-preservation in times of nutrient deprivation. However, if left unchecked autophagy has the potential of producing terminal self-consumption. Recent evidence suggests that autophagy plays an important role in oncogenesis and that this can be regulated by mTOR. Since eEF-2 kinase lies downstream of mTOR, we studied the role of eEF-2 kinase in autophagy using human glioblastoma cell lines. We found that knockdown of eEF-2 kinase by RNA interference inhibited autophagy in glioblastoma cell lines, as measured by LC3-II formation, acidic vesicular organelle staining, and electron microscopy. In contrast, overexpression of eEF-2 kinase increased autophagy. Furthermore, inhibition of autophagy markedly decreased the viability of glioblastoma cells grown under conditions of nutrient depletion. Nutrient deprivation increased eEF-2

kinase activity and decreased the activity of S6 kinase, suggesting an involvement of mTOR pathway in the eEF-2 kinase regulation of autophagy. These results suggest that: (1) eEF-2 kinase plays a regulatory role in the autophagic process in tumor cells; (2) eEF-2 kinase is a downstream member of the mTOR signaling; (3) eEF-2 kinase may promote cancer cell survival under conditions of nutrient deprivation through regulating autophagy. Therefore, eEF-2 kinase may be a part of a survival mechanism in glioblastoma, and targeting this kinase may represent a novel approach to cancer treatment.

Supportive care agents

221 POSTER

Leteprinim attenuates cisplatin-induced neuropathy

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Sensory peripheral neuropathy is a dose-limiting toxicity of cisplatin chemotherapy. Cisplatin-induced neuropathy is generally associated with a reduction in the signal amplitude and velocity of sensory nerve, reflecting nerve fiber dysfunction. This dysfunction can be revealed in a rat model by behavioral sensory test such as hot plate test and by electrophysiological measures.
The aim of our study was to determine whether leteprinim (SPI-205) could improve changes and dysfunctions associated with cisplatin-induced neuropathy. In the present study, the neuroprotective effect of different formulations of SPI-205 was evaluated in a rat model of cisplatin-induced neuropathy.
Ten week-old female Dark Agouti rats were randomly distributed in 5 experimental groups: (a) a control group (n = 17), receiving sc treatment with the Placebo of SPI-205 suspension; (b) a control group (n = 17), receiving sc treatment with the Placebo of SPI-205 salt (0.9% NaCl); (c) a cisplatin-intoxicated group (n = 17), (d) a cisplatin-intoxicated group (n = 17) receiving sc treatment with SPI-205 suspension (50 mg/kg/d); (e) a cisplatin-intoxicated group (n = 17) receiving sc treatment with SPI-205 soluble salt solution (50 mg/kg/d). Cisplatin was given iv at 2 mg/kg biweekly during 4 weeks; SPI 205 was given at 50 mg/kg daily for 7 weeks. Body weight and survival rate were recorded daily. Animals were evaluated functionally by hot plate and EMG testing once a week for 7 weeks. Sciatic nerves were harvested from 5 animals per group at week 5 for histological analysis.
Results showed that treatment with SPI-205 markedly attenuates cisplatin-induced nerve dysfunction and accelerates the recovery from this disorder. These improvements were evident in most of studied parameters (H-wave amplitude and latency, SNCV and axonal degeneration) and seemed to be in good correlation with the improvement observed in the hot plate test. The results were similar with the two SPI-205 formulation
Histological results showed that the axonal diameter of cisplatin group is slightly increased. This might represent axonal degeneration, a phenomenon observed as a consequence of cisplatin intoxication in developing rat brain (Rzeski et al, 2004). SPI-205 treatment seemed to completely prevent this axonal swelling.
In summary, the present study showed that daily treatment with 50 mg/kg SPI-205 injected subcutaneously can improve cisplatin-related sensory neuropathy in rats.

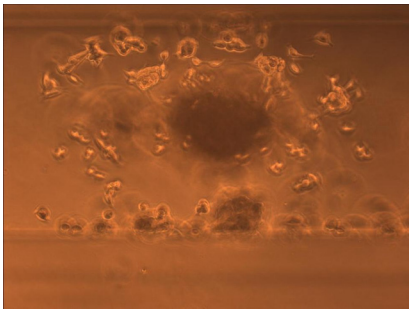
Toxicology methods and models

222 POSTER

Study of in vitro tumor invasion and metastasis: the application of an innovative three dimensional tumor model

H. Liu, W. Yang. *Min Hang Hospital, Rui Jing Hospital Group, Shanghai, China*

Background: As the biological characteristics of malignant tumors, invasion and metastasis are the most dangerous situations during the process of tumor growth and progression. About 60% of cancer patients are detected with metastasis at the time of first diagnosis, and 80% of them actually die from tumor invasion and metastasis. Since it is difficult to observe the process of cancer invasion and metastasis in a patient, and the spontaneous tumor in animal models rarely metastasizes in a short term, there is a pressing need to develop an in vitro three dimensional (3D) tumor model with the features that mimic the characteristics of in vivo solid tumor for the study of tumor invasion and metastasis.
Materials and Methods: Several tumor cell lines such as liver/colon/ovary/lung/breast/stomach cancer, and insulinoma were obtained from ATCC. These cells were seeded and cultured in an invented 3Dtissue culture device. Then the in vitro invasion and metastasis of the tumor were observed after the "primary" tumors were reestablished from these cell lines in this culture device.
Results: The biological characteristics of tumor invasion and metastasis were observed. For example, the rapid growth of tumor cells, the stationary and translocative motility, cellular structure of microvillus, lamellipodia and pilipodia, spread and adhesion of the tumor cells, the penetrative invasion, the dislodge and/or moving away of the tumor cells from the parent tumor, etc. For liver and lung cancers, many tumor cells were actively spread and moved to the surrounding and distant areas. These cells adhered and demonstrated a colonial dominance growth pattern and formed multiple metastasis tumors in the distant areas. The onset time, the frequency and the degree of tumor invasion and metastasis were different among different types of tumors. Two different types of liver cancers behaved quite differently in the biological characteristics of tumor invasion and metastasis. The process of tumor invasion and metastasis could be dynamically followed up for several months without destroying the specimens.



Conclusions: To our best knowledge, this is the first report of direct investigation of tumor invasion and metastasis in vitro after the 3D tumor models are rebuilt from tumor cell lines. Our results indicate that this innovative 3D tumor model can be used as an extremely valuable tool for the in vitro study of tumor invasion and metastasis, for the selection of subpopulations of the tumor cell with different potential of invasion and metastasis, and for the evaluation of potential tumor invasion and metastasis in individual cancer patient for the selection of proper treatment and the prediction of prognosis. Apply this 3D tumor models as an in vitro assay for the molecular targeted medicine study may help the discovery of therapeutic strategy specifically designed for the prevention and treatment of tumor invasion and metastasis.

223 POSTER

An innovative three dimensional tumor model for in vitro study of tumor biology

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Background: Malignant tumors in patients have different biological characteristics based on their intrinsic genetic diversity and the development of heterogeneous sub-clones with divergent phenotypes. Identifying the tumor malignancy behavior such as the proliferative ability and metastatic potential in vitro is critical to choice the appropriate treatment regimens and to evaluation the prognosis. Here we report an in vitro investigation of tumor biology by using an innovative three dimensional (3D) tumor model.
Materials and Methods: Various types of tumor cell lines such as liver, colon, ovary, lung, breast, stomach cancer and insulinoma were obtained from ATCC. These tumor cell lines were seeded and cultured in an innovative three dimensional tissue culture device. We observed the biological characteristics of each tumor such as the tumor morphology, the proliferative ability, in vitro invasion and metastasis, and apoptosis. For the comparison, normal stomach cells, hepatocytes and pancreas islet cells were cultured under same condition as control.
Results: The tumors rebuilt in vitro demonstrated the characteristics associated with solid tumor in vivo. Such as unlimited rapid growth of the cells and structurally arranged containing a necrotic core surrounded by an outer shell of proliferating viable cells.
Different types of tumors exhibited their unique morphology. For example, a round global shape for small cell type lung cancer; irregular nodular and cauliflower shapes for colon cancer. Some of the tumors expressed tumor associated antigen. For example, liver cancer expressed AFP, colon cancer secreted CEA and ovary cancer was associated with CA-125.
The biological characteristics of tumor invasion and metastasis were also observed: the stationary and translocative motility, cellular structure of microvillus, lamellipodia and pilipodia, spread and adhesion of the tumor cells, the penetrative invasion, dislodge and/or moving away of the tumor