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.

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Phase III study of CNT05, a fully human monoclonal antibody (mAb) to alpha+ integrin, in patients with metastatic melanoma

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Background: CNT05 is a fully human anti-α1, integrin antibody that inhibits the growth of human melanoma xenografts in nude mice and rats by 80% and 95% respectively. The objective of this study is to assess the safety and preliminary efficacy of CNT05, alone or in combination with dacarbazine (DTIC), in patients with advanced melanoma.

Material and Methods: CNT05 alone (3, 5, and 10 mg/kg) or in combination (5 and 10 mg/kg with DTIC 1000 mg/m²) 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 I at 3 (n = 3), 5 (n = 3) and 10 mg/kg (n = 3) of CNT05 and 5 (n = 3) and 10 mg/kg (n = 3) of CNT05 + 1000 mg/m² DTIC. No dose-limiting toxicities were observed. The maximum tolerated dose of either CNT05 alone or its combination with DTIC was not reached. CNT05 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 CNT05 were 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 CNT05 alone) developed bilateral grade 2 asymptomatic uveitis following the first administration of CNT05, 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 CNT05) experienced a seizure-like event 39 days after study agent discontinuation. Neither event was considered dose limiting.

Conclusion: CNT05 is a fully human mAb to α1, integrin, is well tolerated and demonstrates activity alone or in combination with DTIC in patients with advanced melanoma. Additional data is needed to determine the safety and efficacy of CNT05.

Structure-activity relationships

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

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Background: Mutations in the K+ receptor tyrosine kinase (RTK), which result in ligand-independent activation of the kinase, are associated with cancers such as gastrointestinal stromal tumors (GISTs) and melanocytosis. RTK mutations in GISTs most frequently occur in the noncatalytic K+ juxtamembrane (JXM) region, suggesting that this domain is crucial in regulation of kinase activity. Moreover, genetic and crystallographic studies have implicated the c-tailand JXM region of the K+RTK as an autoinhibitory regulatory domain. In this study we propose a computational rationale for the role of wild-type and clinically relevant mutant K+RTKs 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 (SMGD) and molecular mechanics/Poisson-Boltzmann/soap energy calculator (MM/PBSA), to investigate the behavior of isolated wild-type and mutant K+RTK fragments formed by the JXM residues that fold into a hairpin motif of the K+ wild-type and several mutant JXM domans was directly simulated in explicit water at native folding conditions in three 300-ns SMGD 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-pleated structures, the major one of which agreed 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 interproteptide interactions drive the wild-type to misfolded conformations, and the solvatochromic effects, which mask folding, are also shown to prevent the mutant sequences peptide fold into wild-type like Structures. These structures then get differently keeping the K+ in its autoinhibited conformation. Finally, simulations of the whole protein with wild-type and mutant JXMs allowed to calculate the free energy of binding (and hence the IC50 value) of these RTKs and imatinib.

Conclusions: Our simulations compared for the first time to highlight the possible effects exerted by the presence of K+ JXM mutations on the active inaduct structure of K+ and its affinity to imatinib.

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POSTER

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


Elongation factor-2 kinase (eEF-2 kinase), also known as Ca+2/calmodulin-dependent kinase III, is a structurally and functionally unique protein kinase that regulates protein synthesis by controlling the rate of polypeptide chain elongation. The activity of eEF-2 kinase is increased in glioblastoma and other malignancies, yet its role in malignancy remains uncertain. Activation of eEF-2 kinase transiently inhibits protein synthesis by phosphorylation of the 56kD of eEF-2, thereby disrupting peptide elongation. In the presence of adequate nutrients and growth factors, eEF-2 kinase is inhibited (protein translation promoted) by activated mTOR and 88 kinase, which phosphorylates Ser-75 and Ser-286, respectively. In the absence of nutrients and growth factors, the activity of eEF-2 kinase increases (protein translation inhibited) due to decreased activity of mTOR and 88 kinase as well as increased activity of AMP kinase (AMPK) which directly inhibits eEF-2 kinase by phosphorylation at Ser-358. Sirt 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 periods of nutrient and growth factor restriction. Autophagy is a conserved response to nutrient deprivation through (). self-degradation of cytosol and organelles and the recycling of amino acids for energy utilization an involves formation of a double-membrane vesicle ("autophagosome") in the cytosol that engulf s organelles and cytoplasm, then fuses with the lysosome where the contents are degraded and recycled. This form of self-degradation can lead to self-preservation in times of nutrient deprivation. However, if left unchecked autophagy has the potential of producing self-destruction. Recent evidence suggests that autophagy play an important role in oncogenesis and that this can be regulated by mTOR. Since eEF-2 kinase is downstream of mTOR, we studied the role of eEF-2 kinase in autophagy using human glioblastoma cell lines. We found the knockdown of eEF-2 kinase by RNA interference inhibited autophagy in glioblastoma cell lines, as measured by LC3-II formation, acidic vesicle formation, and electron microscopy. In contrast, overexpression eEF-2 kinase increased autophagy. Furthermore, inhibition of autophagy markedly decreased the viability of glioblastoma cells grown under conditions of nutrient sequestration. Nutrient deprivation increased eEF-2 kinase.
in determination of the GSD for such therapies in clinical trials. We are currently investigating EGFR ligands in the plasma of cancer patients undergoing treatment with gefitinib and preliminary results will be available at the time of the meeting.

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inhibitor Kit segments 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 using folding simulations in three 300-ns-long 5-fold 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 -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 solvent/entropic effects, which resist folding, are also shown to prevent the mutant sequences from folding into wild-type like structures. These structures then differently keep 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 IC50 value) of these RTK and matribin.

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

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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 II, is a structurally and functionally unique protein kinase that regulates protein synthesis by controlling the role of peptide chain elongation. The activity of eEF-2 kinase is increased in glioblastoma and other malignant tumors, yet its role in neoplasia remains unclear. Activation of eEF-2 kinase 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-2 kinase is inhibited (and protein translation promoted) by activated mTOR and S6 kinase, which phosphorylate Ser78 and Ser36, 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 Ser398. 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 lysosomes where the content is degraded and recycled. This form of self-digestion can lead to self-preservation in times of nutrient deprivation. However, it left unchecked autophagy has the potential of producing terminal self-destruction. 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 the human glioblastoma cell lines under conditions of nutrient deprivation. Nutrient deprivation increased eEF-2

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Study of autophagy as an innovative therapeutic approach for lung cancer

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Background: In the invasion and metastasis process of cancer cells, there are detectable alterations in them that can be used to observe the spread of cancer cells. This study aims to study the novel and innovative method of imaging tumor angiogenesis factors (3G) formation in solid tumors that can be used to monitor tumor progression and metastasis.

Materials and Methods: Using the method of immunohistochemical staining for tumor angiogenesis factors (3G), we detected the expression of tumor angiogenesis factors (3G) in solid tumors and measured the density and distribution range of (3G) in solid tumors. We used a commercial software to analyze the expression of tumor angiogenesis factors (3G) in solid tumors and the expression of tumor angiogenesis factors (3G) in different stages of solid tumors. Finally, we used a statistical software to analyze the correlation between the expression of tumor angiogenesis factors (3G) and the tumor stage, grade, and metastasis.