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 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

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

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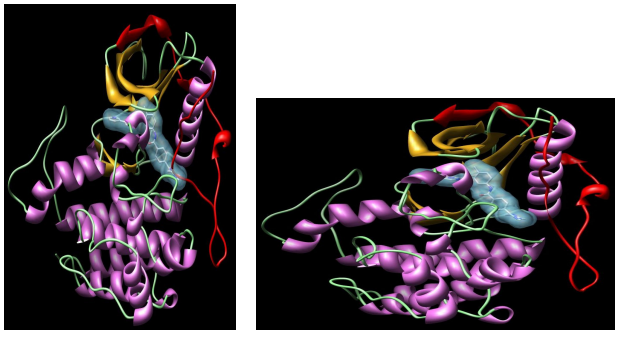
Background: Mutations in the Kit receptor tyrosine kinase (RTK), which result in ligand-independent activation of the kinase, are associated with cancers such as gastrointestinal stromal tumors (GISTs) and mastocytosis. Kit mutations in GISTs most frequently occur in the noncatalytic Kit juxtamembrane (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.

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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