activity. One of the lead antibodies termed R1507 potently inhibits IGF-1R signaling. R1507 binds with low nanomolar affinity to the human IGF-1R as measured by BIAcore. It does not show detectable binding to the closely related human Insulin Receptor (IR). In cell-based assays the huMab competes both with IGF-1 and IGF-2 for receptor binding and inhibits IGF-1R autophosphorylation and subsequent signal transduction. Furthermore, it inhibits ligand-induced proliferation of human tumor cells in vitro. Incubation with huMAb R1507 leads to a rapid downregulation of IGF-1R from the cell surface. In vivo testing of the antibody in a recombinant model (3T3 murine fibroblasts transfected with the huIGF-1R) and in several human xenograft models (including NCI-H322M and Colo205) demonstrated dose-dependent single agent activity against established tumors which was correlated with the downmodulation of IGF-1R in the tumor tissue. Taken together, the in vitro and in vivo data make R1507 a promising molecule for further evaluation in clinical trials and development of a novel immunotherapeutic approach for treatment of IGF-1R expressing cancers.

215 POSTER

HER2/Neu-Herceptin biomarker development for theranostic management of breast cancer patients

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Background: Approximately 25% of breast cancers over-express the HER2/Neu gene, measured by Immunohistochemistry (IHC) staining or FISH probe counts. A new antibody-based therapy (Herceptin™) is highly effective in these cases. Current methods of HER2/Neu evaluation are neither cost-effective nor highly accurate; therefore it is desirable to find more practical and effective predictive biomarkers.
Materials and Methods: We developed techniques for automated objective analysis of IHC-labeled tissue sections and microarrays utilizing laser scanning cytometry. Standardized CAP survey arrays stained for HER2/Neu were evaluated and compared with results of traditional methods. Excellent correlation between the automated results, the pathologist's evaluation and FISH probe spot counts was achieved.
Herceptin is humanized hybrid antibody containing human Fc fragment and mouse variable region. It would be reasonable to assume that the actual therapeutic Ab Herceptin may be a better primary antibody in theranostic IHC tests for Herceptin therapy patient selection. We developed a novel method of staining breast tissue with Herceptin, overcoming a major challenge of human Fc fragment IHC staining.
Results: Serial breast tumor TMA sections were stained for HER2/Neu and Herceptin. Most HER2/Neu-positive core elements also showed Herceptin expression. Conversely, 9% of tissue core elements were labeled with polyclonal anti-HER2/Neu but not with Herceptin. The discordance suggests that binding specificity of Herceptin differs from that of the xeno-antibodies. In 3% of cases, tissue was reactive to Herceptin but not to polyclonal anti-HER2/Neu. The latter result could not be explained by existing knowledge of polyclonal and monoclonal antibodies specificity.
We performed double staining of IHC tissue sections and TMAs (HER2/Neu and Herceptin (DAB and BCIP/NBT)) to clarify this phenomenon. Some tissues exhibit mosaic staining patterns, with various cells positive for both markers and neighboring cells positive for only one marker. Automated analysis methods allowed objective evaluation of the degree of mosaicism in the tumor tissues. Demonstrated mosaic staining of tumor tissues may identify additional candidate patients for use of anti-HER2/Neu therapeutic antibodies different from Herceptin-target peptide areas. Current studies are underway to investigate mosaicism in tissues from patients undergoing Herceptin therapy.
Conclusions: We demonstrated that Herceptin can effectively replace xeno-antibodies for IHC-based patient selection for breast cancer therapy. Our data suggests that Herceptin binds to a different epitope than traditional HER2/Neu Abs, perhaps resulting in a more relevant specificity. Automated laser scanning cytometry analysis was proven to be an invaluable tool in objective tissue characterization.

216 POSTER

The cytotoxicity of antibody-drug conjugates to bystander cells

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One approach to limit toxic effects of a chemotherapeutic drug to tumor tissues is to target the drug to the tumor with the help of a tumor-selective monoclonal antibody. Several antibody-drug conjugates have been developed that specifically target and kill antigen-positive cells. However, solid tumors often contain both antigen-positive and antigen-negative cells. Therapeutic agents that kill not only antigen-presenting cancer cells, but also the adjacent antigen-negative cancer cells, may be more effective in eradicating such tumors. We have previously reported that a conjugate of the microtubule polymerization inhibitor, maytansine, attached via a disulfide linker to the anti-CanAg antibody, huC242, is cytotoxic to both the target cells and proximally located antigen-negative cells in culture and in mouse xenograft models (1). The maytansinoid species produced by the target cells following their exposure to the conjugate were also identified (2). We performed similar studies with antibody-drug conjugates containing various cytotoxic drugs, including several new maytansinoids, taxol analogues and analogues of the DNA alkylator, CC1065, and found that these conjugates can generate the bystander effect. The disulfide linkage between the antibody and the drug was a prerequisite for the bystander cytotoxicity of the antibody-drug conjugates. To further investigate the role of the linker cleavage in the bystander cytotoxicity, a series of huC242-maytansinoid conjugates with various disulfide-containing linkers were constructed. These linkers differed in the degree of hindrance around the disulfide bond, and in the rate of cleavage by cytoplasmic thiols within the target cells. The bystander potencies of these conjugates correlated with the cleavage rates of their disulfide linkers; faster disulfide bond cleavage resulted in stronger bystander cytotoxicity. Analysis of the metabolism of these conjugates in CanAg-positive target cells revealed that the nature of the linker affected the composition of metabolites. The accumulation of a stable metabolite, *S*-methyl-maytansinoid, correlated with the bystander potency of a conjugate. This metabolite is a hydrophobic molecule that can diffuse out of the antigen-positive target cells and kill proximally located dividing cells. This study defines requirements for effective antibody-drug conjugates.

References

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217 POSTER

Circulating levels of ligand as a potential biomarker for optimal dosing of targeted antibody drugs to the epidermal growth factor receptor

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Background: A lack of biomarkers that are predictive of the optimal biologic dose (OBD) is a major hurdle for the successful clinical translation of many targeted therapies. We have recently reported such a marker for a targeted anti-angiogenic drug called DC101 – a monoclonal antibody which blocks the mouse endothelial cell receptor tyrosine kinase for vascular endothelial growth factor (VEGF), known as VEGFR-2 (flk-1 in mice, KDR in humans; *Bocci et al Cancer Research 2004; 64:6616–6625*). Administration of DC101 to both normal and tumor-bearing mice leads to a rapid and remarkable increase in the plasma levels of circulating VEGF, which parallels anti-tumor activity. In contrast, small molecule VEGFR-2 antagonists did not cause a similar effect in normal mice. This discovery has lead to the examination of antibodies against human EGFR to determine whether this surrogate marker paradigm may extend to this biological system.
Materials and Methods: Human tumor cell lines that co-express EGFR and one or more of its ligands were grown in culture in the presence of Cetuximab (Erbitux), Nimotuzumab (TheraCIM/h-R3), Erlotinib (Tarceva), or Gefitinib (Iressa). TGFa and EGF were subsequently measured in conditioned media using ELISA. Similar experiments were conducted *in vivo* utilizing the HT29 cell line injected into the peritoneum of nude mice and treated with Cetuximab.
Results: *In vitro* experiments utilizing numerous human cancer cell lines showed a rapid elevation in human TGFa in the conditioned media (within 15 minutes) after treatment with antibody drugs that target the external ligand-binding domain of EGFR, but not when small molecule inhibitors were used. The elevation showed a dose-response effect and plateau at higher drug concentrations. Factors that appear to influence the nature of this result include antibody affinity, EGFR expression level, and endogenous ligand production. Human TGFa/EGF elevations were also demonstrated in the ascites fluid of mice injected with HT29 after a single Cetuximab injection.
Conclusions: These results suggest that the ligand elevations observed with DC101 and mouse VEGF extend to antibodies aimed against human EGFR, and should be explored as potential biomarkers to aid

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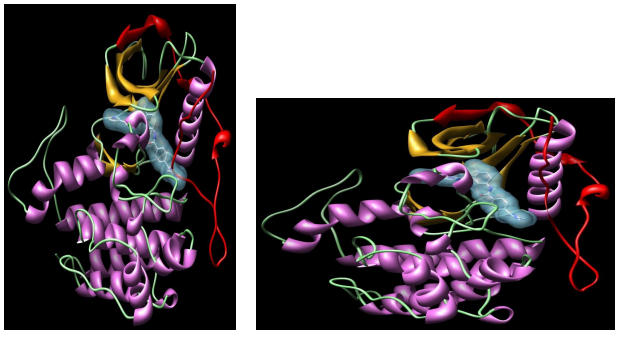
Background: Mutations in the Kit receptor tyrosine kinase (RTK), which result in ligand-independent activation of the kinase, are associated with cancers such as gastrointestinal stromal tumors (GISTs) and mastocytosis. Kit mutations in GISTs most frequently occur in the noncatalytic Kit juxtamembrane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

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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