higher IC50s in the range of 5–200 nM. Three out of the four candidates inhibited Her3 phosphorylation with IC50s of 0.03, 5.3, and 12 nM. The most potent candidate showed similar IC50s (~0.03 nM) for Her2 and Her3 phosphorylation. Moreover, it inhibited both the Akt and Erk phosphorylation with IC50s at ~0.1 nM. The inhibition of Her3-PI3K complex was also detected with IC50 at 64 nM. This compound likely possesses functional efficacy in inhibiting cell proliferation and possibly tumor growth in appropriate human xenograft models provided that it has favorable pharmacokinetic properties. Another compound showed 2 logs higher IC50s for Her3 (5.3 nM), Akt (3.5 nM), and Erk (1.9 nM) phosphorylation compared to Her2. It therefore may not be as potent as compound 4 in inhibiting cellular growth. The other 2 compounds had IC50s of 0.097 nM and 0.086 nM, respectively, for Her2 phosphorylation.
In summary, we demonstrated that multiplexed eTag Assay system for receptor dimerization, phosphorylation, and signaling pathways could provide a unique robust tool for the screening of cancer drug candidates in a fast, reliable, and efficient manner as compared to other currently existing methods.

597 POSTER

Differential Her family receptor dimerization and downstream signaling in cancer cell lines

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Her receptors are validated targets for cancer therapies in solid tumors. The efficacies of targeted therapeutics vary in different types of cancer as well as from patient to patient. The downstream signaling mechanisms for these receptors have been well characterized. However, the cross-talking between the receptor signaling pathways warrants comprehensive analysis of downstream signaling in each cancer cell line or clinical sample to determine the activation status that leads to perpetual cell proliferation and survival. We developed multiplexed proximity-based eTag assays for Her family receptor dimerization and signaling phosphorylation to streamline analysis of in vitro or in vivo models of cancer.
Cancer cell lines with different expression levels of Her1, 2 and 3 receptors were analyzed for receptor dimerization and downstream signaling pathway activation. These cell lines were MCF7, SKBR3, MDA-MB-468, 22RV1, A431, BT474, Clau6, MDA-MB-231, A549, ZR-75–1 and BT-20. They were stimulated for 10 min with different doses (0–100 nM) of HRG or EGF, followed by immediate lysis. The lysates were analyzed with four proximity-based multiplexed eTag assays as follow: Multiplex I for Akt, Erk, JNK, P38; Mutiplex II for FAK, MEK, Stat3, BAD, and RSK; Multiplex III for Her3/2, Her3/1, Her3-PI3K, Her3-SHC, Her3 phosphorylation; and Multiplex IV for Her2/3, Her1/2, Her2-PI3K, Her2-SHC, and Her2 phosphorylation
The results showed that the MAP kinase pathway was activated in all cell lines stimulated with EGF or HRG. We detected the Her2-SHC and Her3-SHC complex formation concurrent with Mek, Erk, Rsk, and BAD phosphorylation, a linear cascade of MAP kinase activation. The induction levels vary from 2 to10 folds. Her3-PI3K and Akt activation varied in different cell lines; they were detected in MCF7 treated with HRG. The activation of Her3-PI3K pathway in HRG-stimulated MCF7 was related to Her2/3 heterodimerization. The Her2-PI3K complex detected in HRG-stimulated MCF-7 cells likely represents an indirect interaction via Her3 in the HRG-induced Her2/3 heterodimer. FAK is strongly down regulated by EGF in BT-20 and A431 cells that express high levels of Her1. Stat3 was strongly phosphorylated following EGF stimulation in cell lines expressing high levels of Her1, such as MDA-MB-468, A431, and BT-20.
In summary, we validated the multiplexed eTag assay system as unique robust tool for the receptor dimerization and signaling pathway profiling. The current analysis demonstrated unique signaling patterns that are related to different receptor expression of EGFR, Her2 and Her3 in various cancer cell lines.

Regulatory affairs

598 POSTER

The cancer therapy evaluation program, National Cancer Institute initiative to enhance combination investigational agent clinical trials

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The Cancer Therapy Evaluation Program (CTEP) of the Division of Cancer Treatment and Diagnosis, National Cancer Institute is committed to facilitating preclinical and clinical studies involving the combinations of anticancer investigational agents originating from more than one pharmaceutical collaborator. CTEP has 150 active Investigational New Drug applications (INDs); this puts CTEP in a unique position to facilitate combinations of biologics and drugs for multiple therapeutic target types. All of the collaborative clinical agreements between CTEP and pharmaceutical or biotechnology collaborators contain provisions to allow for mutually agreeable combination studies, both preclinical and clinical, sponsored by the NCI without additional agreements between the collaborators or CTEP. To expedite the initiation of such studies, a modification of the Intellectual Property Option to Collaborator (the Option) has been finalized which provides all collaborators contributing an agent for a combination study with a non-exclusive royalty free license to any invention that might arise using the combination. Furthermore, this same option applies to preclinical studies designed to provide data in support of a clinical trial. The provisions for the sharing of data between collaborators have also been updated. Thus, the need for collaborators to negotiate cumbersome intellectual property or data sharing agreements prior to approving such studies has been eliminated. Such arrangements have led to the initiation of approximately two dozen investigational agent combination protocols and there are currently an equal number in preparation or in review. More detailed information on this and on other initiatives to enhance the initiation of clinical trials will be presented.

Drug delivery

599 POSTER

Phase I and pharmacokinetic (PK) study of OSI-7904L in combination with Cisplatin (CDDP) in patients with advanced solid tumors

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OSI-7904L is a liposomal formulation of a potent non-competitive thymidylate synthase inhibitor (TSI) that does not require polyglutamation for activity; the parent drug was previously tested as 1843U89. This formulation increases plasma residence and offers superior preclinical antitumor activity compared to parent drug or 5-FU. The minimally overlapping toxicity profiles of OSI-7904L and CDDP and the additive antitumor activity seen when platinum analogues were combined with OSI-7904L in xenograft studies provided the rationale for this phase I study. This evaluation is designed to determine the maximum tolerated dose (MTD), dose limiting toxicities (DLT) and PK profile of the combination. The order of dosing is based on a suggestion of sequence-dependent efficacy observed in xenograft models. CDDP is administered via 2 h IV infusion followed by OSI-7904L given IV over 30 minutes; both given every 21 days. To date, 11 pts have been treated (6M/5F), median age 53 (range 39–84) and tumor types: cholangial (3), colorectal (2), pancreas (2), renal, head & neck, breast and mesothelioma (1 each). All except one pt received prior chemotherapy with a median of 2 regimens (range 0–6). OSI-7904L/CDDP doses in mg/m^2 (no. pts/cohort) were: 6/60(4), 9/60(3), 12/60(4). A total of 30 cycles have been given, median 3/pt (range 1–6). Mild to moderate toxicity was observed up to 9/60 mg/m^2 including fatigue, nausea, vomiting, anorexia, diarrhea, mucositis and rash. DLT was observed in 2 of 4 pts at 12/60 mg/m^2. One pt experienced grade (G) 3 rash, G3 hyperbilirubinemia, G3 anemia, G3 thrombocytopenia, G4 febrile neutropenia and G4 mucositis which proved to be fatal; the other reported G3 nausea and vomiting despite adequate treatment and G3 ileus. The MTD has been exceeded. Therefore, the 9/60 mg/m^2 cohort is being expanded. A PR has been confirmed in a pt in the 6/60 mg/m^2 cohort with refractory breast cancer who had prior TSI exposure (5-FU and capecitabine). One pt with cholangiocarcinoma has ongoing stable disease after 16 weeks. PK data indicate biphasic plasma clearance of total OSI-7904L with a median terminal half-life of 77.8 h. Cmax values increased linearly with dose. Substantial inter-patient variability was observed in AUC for each dose group. There was no apparent increase in the free platinum AUC with increasing OSI-7904L dose. 2′-dU levels as well as baseline homocysteine and TS genotype samples are being analysed. In conclusion, this schedule has a toxicity profile similar to other TSI/CDDP combinations. Accrual continues with further analyses of PK/PD information.

600 POSTER

Endothelin-1 antagonist selectively modulates tumor blood flow and potentiates responses to both chemotherapy and radiotherapy

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Although derived from the host tissue, the tumor vasculature is under the influence of the tumor microenvironment and needs to adapt to

the resistance to blood flow inherent to the dynamics of tumor growth. Such vascular remodeling can offer selective targets to pharmacologically modulate tumor perfusion and thereby improve the efficacy of conventional anti-cancer treatments. Radiotherapy and chemotherapy can, indeed, take advantage of a better tumor oxygenation and drug delivery, respectively, both partly dependent on the tumor blood supply.

Here, we showed that isolated tumor arterioles mounted in a pressure myograph have the ability, contrary to size-matched healthy arterioles, to contract in response to a transluminal pressure increase. This myogenic tone was exquisitely dependent on the endothelin-1 pathway since it was completely abolished by the selective ET_A antagonist BQ123. This selectivity was further supported by the large increase in endothelin-1 abundance in tumors (5 to 15-fold according to tumor models) and the higher density of the ET_A receptors in tumor vessels. We also documented by using laser doppler microprobes and imaging that administration of the ET_A antagonist led to a significant increase in tumor blood flow whereas the perfusion in control healthy tissue was not altered. Finally, we provided evidence that acute administration of BQ123 could significantly increase cyclophosphamide delivery to the tumor. The tumor response to low-dose, clinically-relevant fractionated radiotherapy was also significantly improved by the concomitant administration of the ET_A antagonist as anticipated by the observed increase in tumor oxygenation, as determined by EPR oximetry. The dose-dependency of the ET_A antagonist effects and the consistency of these findings in various mouse tumor models further emphasized the relevance of our data.

Thus, blocking the tumor-selective increase in the vascular endothelin-1/ET_A pathway unravels an important reserve of vasorelaxation which can be exploited to selectively increase tumor response to chemotherapy and radiotherapy.

601 POSTER

Increased transport of nanoparticle albumin-bound paclitaxel (ABI-007) by endothelial gp60-mediated caveolar transcytosis: a pathway inhibited by Taxol

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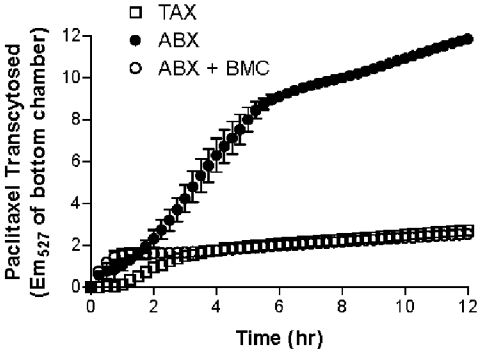
Background: Paclitaxel (P) albumin nanoparticles (Abraxane, ABX or ABI-007) demonstrated improved response rate over Taxol (TAX) in a phase 3 metastatic breast cancer trial (33% vs 19%, p<0.0001) (SABCS, O'Shaughnessy, 2003). Cremophor in TAX entraps P in micelles in plasma, reducing the P available for cellular partitioning (Sparreboom, Cancer Res 1999;59:1454). Studies in athymic mice have shown 30–40% higher intratumor P concentrations with ABX compared to equal doses of TAX (SABCS, Desai, 2003). Albumin is transported across endothelial cells (EC) by specific receptor (gp60)-mediated caveolar transport (John, Am J Physiol 2001;284:L187). Albumin-bound P in ABX may be transported across tumor microvessel EC by gp60, and this mechanism may be particularly active for ABX as compared to TAX. A series of studies were performed to evaluate binding and transport of P by human umbilical vein endothelial cells (HUVEC) and human lung microvessel endothelial cells (HLMVEC) for ABX and TAX.

Methods: ABX and TAX were formulated with fluorescent (FL) P. EC monolayers were grown to confluence on 96-well plates or transwell chambers. Binding was measured after incubation (1 hr @ 37ºC) with FL ABX or TAX in 96 well plates. For transport, FL ABX or TAX was added to the top chamber. P crossing the EC to the lower chamber was monitored for 12 hrs using a fluorometer in the presence or absence of selective inhibitors of transport.



Transcytosis of paclitaxel across EC monolayers.

Results: Binding of P to HUVEC was 10X higher for ABX than TAX. The transport of P from ABX across EC monolayers was enhanced 2–3 fold and 2–4 fold for HUVEC and HMVEC, respectively, as compared to TAX. Transport was dependent on albumin. Transport of P from ABX was inhibited by anti-SPARC antibody, known to bind gp60, the receptor required for caveolar albumin transcytosis. Known inhibitors of calveolar transcytosis, NEM and β-methyl cyclodextrin (BMC), also inhibited the transport of P from ABX across the endothelial monolayers (Figure). Inhibition of caveolar transport decreased ABX transport to the level of TAX.

Conclusion: P from ABX was actively transported across EC by gp60-mediated caveolar transcytosis, a process that was inhibited by TAX. P from ABX was transported at a 2–4 fold higher rate than TAX, which relied on a non-caveolar mechanism, presumed to be paracellular. Utilization of this pathway by albumin-bound paclitaxel may be responsible in part for increased intratumoral concentrations of P seen for ABX relative to TAX.

602 POSTER

SMA-pirarubicin micelles: highly efficient tumor targeting and therapeutic effects without apparent toxicity

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Material and Methods: Present SMA-micelles were prepared through multiple steps: (a) hydrolysis of styrene-co-maleic anhydride copolymer to generate poly (bicarboxylic acid), SMA, (b) mixing of SMA and pirarubicin in water, and shifting pH to low (~5), and then high (~10), and neutralization, (c) followed by purification through ultrafiltration using an exclusion size of 10kDa. Analysis was carried out by UV and fluorescence spectroscopy, TLC, molecular sieve chromatography (Sephadex G-50 and G-150) and elemental analyses. In vitro biological activity was tested with use of human breast cancer MCF-7, human colon adenocarcinoma SW480, HeLa and human epidermal carcinoma KB cells. For in vivo activity and safety studies, ddY and C57BL/6 mice bearing sarcoma S-180, adenocarcinoma Colon 38 tumor models were used respectively.

Results: Present micelle was found to bind to plasma albumin and showed an apparent MW of 94 kDa. In vitro cytotoxic activity of SMA-pirarubicin micelle measured by MTT assay showed 85–100% activity of equivalent molar concentrations of pirarubicin. The micelle at a total dose of 20-mg/kg pirarubicin equivalent, given over four aliquots (5mgx 4/kg) i.v., showed 100% tumor regression of S-180 tumor in all of tested animals, and 80% regression of colon 38 tumor bearing mice. The drug was safe up to 100mg/kg (given 25 mgx4/kg) body weight of pirarubicin equivalent in ddY mice and rats (15 fold more than LD_{50} of pirarubicin). Treated animal showed extended survival for more than 2 years of follow-up without tumor recurrence.

Conclusion: SMA–pirarubicin micelle appears potentially very interesting polymeric drug, exhibiting remarkably excellent antitumor effect and very high safety margin that warrants clinical evaluation.

603 POSTER

Effect of membrane transport proteins on the disposition of vincristine in brain-tumor bearing mice

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Vincristine [VCR] is a natural product microtubule inhibitor that is used to treat a variety of CNS tumors. Recently, we reported that P-glycoprotein [Pgp] influenced the penetration of paclitaxel into brain tumors [*Cancer Res*63:5114, 2003]. However, the impact of Pgp on brain tumor penetration of other agents, and the influence of other pumps on this process, is unknown. In the present study we use a mouse genetic approach to investigate how brain tumor concentrations of VCR, a substrate of both Pgp and multidrug resistance protein-1 [MRP1], is affected by these two pumps which are known to be expressed at the blood-brain barrier (Pgp) or the choroid plexus (Pgp and MRP1). Pharmacokinetic investigations were conducted in wild-type [wt], Pgp knockout [KO, mdr1a$^{-/-}$/1b$^{-/-}$], and MRP1 KO [mrp1$^{-/-}$] mice bearing intracerebral tumors derived from B16 melanoma cells. Mice had implanted jugular vein cannulas for drug administrations and carotid artery cannulas for serial collection of blood samples. First, pharmacokinetic [PK] parameters (total drug clearance [CL], volume of distribution at steady-state [Vss] and elimination half-life [t1/2]) were determined for each strain (4 mg/kg intravenous bolus). Based on the CL and Vss, a second series of studies were performed under steady-state VCR plasma concentrations to assess drug distribution, enumerated as tissue/plasma concentration ratios. VCR was measured in plasma, normal brain, brain tumor and bone marrow [BM] by a LC/MS technique. The Table below summarizes the PK data for VCR. These preliminary data show: i) Pgp KO mice exhibited a significant reduction in total clearance, consistent with the known contribution of Pgp to hepatobiliary elimination; ii) there is a large and unexpected increase in Vss in MRP1 KO mice, which may be