Src family (nearly ubiquitous in the tumor spectrum), all of which have been documented and validated as therapeutic targets. MP371 has proven itself to be a potent inhibitor of these kinases, with IC_{50} values in the low nanomolar range. MP371 has activity against a broad spectrum of human tumor cell lines, causing both growth inhibition and apoptosis. Tumor cell lines with mutations in the c-Kit kinase are especially sensitive, which is expected from the selectivity of MP371 for mutant c-Kit. The anti-tumor activity of MP371 has been evaluated in a number of human tumor xenograft models and has shown effectiveness with minimal toxicity. Further biopharmaceutics property profiling was performed with MP371, and the results from these studies are very favorable, demonstrating good cell permeability as well as stability in the presence of liver enzymes. Meanwhile, MP371 remains highly selective; kinase profiling has revealed that only the kinases listed above have significant sensitivity to MP371, such that it will not abrogate all kinases with impunity. The spectrum of kinase activity of MP371, in concert with its desirable drug-like properties, therefore make it a promising next step in targeted therapeutics.

Telomerase- targeting agents

623 POSTER

GRN163L, a telomerase inhibitor under development for cancer treatment: data guiding clinical trial design

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Background: GRN163L is a lipidated thiophosphoramidate oligonucleotide which binds with high affinity to the template region of human telomerase RNA, causing direct enzyme inhibition. IC50 values range from 0.8 to 6.5 mcg/mL among 13 tumor lines and GRN163L inhibits tumor growth in multiple human tumor xenograft studies. Due to the high affinity and slow off-rate of binding, telomerase inhibition is long-lasting following exposure to the drug (Oncogene, 24, 5262, 2005). Polyanionic oligonucleotides can generally cause reversible inhibition of the intrinsic coagulation pathway and complement activation at high concentrations. Data reported here confirm that GRN163L can attain inhibitory concentrations in vivo at plasma concentrations below the threshold for these potential toxicities.
Methods: Preclinical PK studies have been conducted in cynomolgus monkeys, using a validated hybridization-ELISA.
Results: Studies in cynomolgus monkeys demonstrated that GRN163L at a dose of 5 mg/kg infused for 6 hours (h) attained maximal plasma concentrations of ~30−60 mcg/mL. At 10 mg/kg over 6 h, concentrations ranged from ~90–115 mcg/mL and were well tolerated, with <2-fold increases in APTT and no significant complement activation. These data, combined with the ~5 h plasma T1/2alpha; in cynomolgus monkeys predict that at such doses the plasma concentration will remain above 10 mcg/mL (~2 microM) for >12 h, consistent with the target concentration of GRN163L necessary to attain 50 to 80% telomerase inhibition in tissue. In PD experiments in mice, target inhibition from single doses was long-lasting (>7 days).
Conclusions: A safe and practical pharmacodynamic window exists for weekly delivery of GRN163L at concentrations sufficient to inhibit telomerase. Based upon these findings, two clinical trials have been activated: a Phase I/II study in CLL with i.v. infusion durations of 6h, and a Phase I study in solid tumor malignancies with 2–6 h infusion durations, both on weekly × 8 schedules. GRN163L is the first specific telomerase modulating agent to enter clinical trials in man. Initial PK and PD results from these trials are consistent with those from the monkey studies, and support the hypothesis that active levels can be achieved at well tolerated doses.

624 POSTER

Rapid induction of telomeric DNA damage response and reduction of clonogenic tumor cell growth by the telomere targeting agent RHPS4

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Telomerase and telomeres are attractive targets for anticancer therapy. However, interest in developing telomerase inhibitors was recently renewed when evidence emerged that overexpression of the human telomerase catalytic subunit hTERT is a critical step in converting normal into tumor stem cells. The pentacyclic acridine RHPS4 is a G-quadruplex ligand that was designed to induce the 3′ single-stranded guanosine-rich telomeric overhang to fold into a G-qaudruplex structure. This is incompatible with an attachment of telomerase to the telomere ends and thus, we could show that RHPS4 can effectively inhibit both, the catalytic and capping functions of telomerase. To further study mechanisms underlying telomere uncapping by RHPS4 and rapid induction of cell death, we have evaluated the effects of RHPS4 on telomeric DNA damage response. We used MCF-7 breast cancer cells as a model system and compared the extent and onset of DNA damage caused by the RHPS4 to that seen in untreated MCF-7 and MCF-7 cells expressing mutant hTERT. The latter have low telomerase activity and short telomeres (1.9 kb) owing to gradual erosion after over 200 population doublings (PDs). In addition, we compared the clonogenicity of MCF-7, *mt* hTERT MCF-7 and MCF-7 cells treated with RHPS4 in the human tumor stem cell assay. Induction of DNA damage was assessed by measuring, phosphorylation of histone variant H2AX, γ-H2AX. We found that treatment of MCF-7 cells with RHPS4 at 1 μM for 24 hours caused marked γ-H2AX phosphorylation that was similar to that seen in *mt* hTERT MCF-7 cells after 200 PDs. Consequently, mitotic abnormalities such as anaphase bridges, dicentric and ring chromosomes were observed. In the clonogenic assay, MCF-7 cells expressing *mt* hTERT formed 5-times less colonies than parental MCF-7 cells. MCF-7 cells treated with RHPS4 showed a similar behavior and had an IC50 (= 0.05 μM) which was 50 times lower than the IC50 of RHPS4 (= 2.5 μM) in a whole cell population. Our data indicate that RHPS4 can produce effects which are similar to genetic inhibition of hTERT in MCF-7 cells, but that RHPS4 effects occur more rapidly. Moreover, the potent activity of RHPS4 in the clonogenic assay suggests that telomere targeting agents should be exploited as tumor stem cell treatments.

625 POSTER

Progress in the preclinical development of RHPS4, a telomere signalling targeted agent

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Targeting telomeric integrity is a promising strategy for cancer treatment. A novel G-quadruplex-stabilising telomere targeted pentacyclic salt, RHPS4 (3,11-difluoro-6,8,13-trimethyl-8*H*-quino[4,3,2-*kl*]acridinium methosulfate, Mol. Wt. 458.5), inhibits telomerase and causes shortening and uncapping of telomeres and subsequent growth arrest – to effectively inhibit the growth of tumours *in vivo*. Against the short-telomere UXF 1138LX xenograft, serially passaged into new mice to achieve prolonged tumour exposure to the drug, tumour growth (median relative tumour volume) was inhibited by ~40 % compared to control after 28 days. Biopsies taken at passage 3 showed clear effects on the telomere/telomerase complex: telomere shortening (~1 Kb), reduction in clonogenicity (~50%), reduced hTERT expression (immunocytostaining) and ~3-fold increase in anaphase bridges. The combination of RHPS4 with paclitaxel was synergistic and led to complete tumour remission.
RHPS4 is currently in preclinical development. Two synthetic routes to RHPS4 are being evaluated to source material for clinical trial: one route is a two-step process which has potential scale-up problems; the second six-step route may be appropriate for large-scale synthesis. RHPS4 is soluble in water, stable, largely untransformed *in vitro* by a panel of cytochrome P450 enzymes and, despite its cationic character, readily accesses the nuclei of cells where the molecular target is located. To accompany ongoing preclinical efficacy studies with RHPS4 and to aid our understanding of the mechanism of action of this compound (and some of its analogues) we are adopting a systems biology approach to rationalize our observations of the phenotypic changes that occur in response to RHPS4. The resulting model, which incorporates data on senescence induction by RHPS4, and changes in growth rate and cell cycle distribution, shows where the cell cycle phase transitions are disturbed and to what extent, depending on the time and dose schedule used, highlighting possible novel PD markers.

626 POSTER

The activity of CKD601, telomerase inhibitor, against gastric cancer cell lines and resistance mechanism, which is associated with hTERt expression and ALT

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Background: CKD601, a newly developed telomerase inhibitor, shows an anti-cancer effect through its inhibitory effect on telomerase, by intercalation

of the drug into the structures of the G-quadruplex. No study has been conducted to assess the anti-cancer effects of CKD601 in regards to gastric cancer. We attempted to confirm the anti-cancer effect of CKD601 in the gastric cancer cell line, and to investigate the mechanisms of the anti-cancer effect and resistance in some cell lines.
Methods: After long-term drug exposure, we performed Southern analysis, TRAP, and β-Gal staining about the extracted DNA, RNA, and protein from the gastric cancer cell lines and the U2OS cell line to confirm the anti-cancer effect of CKD601. We attempted to investigate the change in the hTERT expression of cancer cells as a result of exposure to CKD601 by RT-PCR and real-time PCR, and to confirm the presence of the ALT (alternate lengthening of telomere) mechanism by metaphase telomere FISH and IF.
Results: The anticancer effect of CKD601, including the shortening of telomere, inhibition of telomerase activity, cellular aging, and decreased growth rates, was observed in some gastric cancer cell lines (SNU-1, 5 and SNU-601). SNU-484 and SNU-668 cell lines showed no anti-cancer effect of CKD601. The resistance mechanism of SNU-484 was the significant overexpression of hTERT following exposure to CKD601. ALT, another mechanism that functions in the maintenance of telomere length, was detected in SNU-668 following exposure to CKD601 by IF (colocalization of PML and TRF1) and metaphase telomere FISH (telomere length heterogeneity), and it is the resistance mechanism against CKD601.
Conclusions: CKD601 is active in gastric cancer by the inhibition of telomerase activity. The resistance mechanisms of gastric cancer cell lines against CKD601 are the induction of the overexpression of hTERT and the ALT mechanism.

627 POSTER

New screening technology for development of effective anti-cancer drugs targeting telomere G-tail

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Telomeres are special structures at the ends of chromosomes that are essential for chromosome maintenance. The 3′ single-stranded telomeric overhang (G-tail) exists at the very end of telomere DNA. G-tail length is essential for generating t-loops that can form by inserting the G-tail into duplex telomeric DNA to prevent genomic instability. Recent studies showed that G-tail reduction by G-quadruplex interacting agents have strong anti-cancer activity. In addition, our recent study (Oncogene, 25, p1955–1966, 2006) showed that rapid induction of apoptosis accompanied by G-tail reduction by G-quadruplex interacting agent, telomestatin, is specific for cancer cells but not for normal cells. These G-tail reductions are associated with TRF2 dissociation from telomere DNA, and induce anaphase bridge formation. These results showed that TRF2 protein also possible target to induce apoptosis accompanied by loss of the G-tails. Importantly, reductions of total telomere length are not essential for induction of cell death. These findings suggest that agents that induce G-tail reduction are rapidly and effectively kill cancer cells. While G-tails is a good target for anticancer drug, no methods are reported to measure G-tail length rapidly and quantitatively. To explore this problem, we developed new technique, G-tail telomere HPA, to measure G-tail length. This method has the advantage of being simple to perform, accurate and highly sensitive for G-tail as short as 20 nucleotides. In addition, this assay is specific and quantitative for G-tail, and can be used for large-scale high throughput screening. Furthermore, this method can be used to assay cell lysates as well as genomic DNA. Our finding suggests that G-tail telomere HPA have practical benefits for screening of anti-cancer drugs that induce reduction of G-tail length.

628 POSTER

Single agent and combination treatment studies with the telomerase inhibitor GRN163L in ovarian cancer and non-small cell lung carcinoma (NSCLC) xenograft models

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Telomerase, an enzyme essential for telomere length maintenance and cell immortality, is present in 80−90% human cancers and plays a key role in tumor progression. Normal adult cells and tissues typically have low or undetectable levels of telomerase activity and long telomere lengths, in contrast to tumor cells which have much shorter telomere lengths and high levels of telomerase activity. These differences in telomerase levels and telomere lengths make targeting telomerase a promising approach for the treatment of cancer. GRN163L, a potent specific inhibitor of telomerase, has single agent growth inhibitory activity in lung, ovarian, hepatocellular, myeloma, as well as antimetastatic activity in lung, myeloma and orthotopic breast cancer xenograft models. GRN163L is currently in Phase I and Phase I/II clinical trials for solid tumor and hematological malignancies, respectively. Here we present new single agent and combination treatment efficacy data in subcutaneous ovarian and NSCLC xenograft tumor models. In the SKOV-3 ovarian tumor model, cisplatin (CDDP) and GRN163L used in combination had significant antitumor activity (ANOVA $p < 0.001$), while neither CDDP nor GRN163L alone was effective. The CDDP and GRN163L combination was well tolerated as monitored by animals' body weight changes. In the A549 NSCLC model, tumor inoculated animals received treatment with GRN163L, mismatch control, gemcitabine or GRN163L+Gemcitabine. Dose-dependent tumor growth inhibition (TGI) was noted in animals treated with GRN163L alone (38% and 72% at 15 and 36 mg/kg GRN163L on day 49, respectively), compared to mismatch control or saline treated groups, (ANOVA $p < 0.001$ for both groups). The GRN163L+gemcitabine combination was well tolerated, but did not show additivity compared to gemcitabine alone (63% and 64% TGI, respectively). The lack of additivity may be due to the use of Gemcitabine near its MTD, thereby eliciting the maximal level of inhibition for this agent, and lower doses may be necessary to detect an additive effect. Follow-on studies with lower doses of gemcitabine combined with GRN163L are underway. emcitabine with GRN163L was well tolerated. Our data shows that GRN163L has anti-tumor properties in a broad range of tumor types, and has promising activity when used in combination with select therapeutics. Follow-on dose optimization, combination with cytotoxics with different mode of actions and combination treatment in different tumor models are ongoing.

Tubulin- interacting agents

629 POSTER

Hypoxia, TUBB3 expression and tissue selectivity. Does a tubulin code exist?

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TUBB3 has been reported as a major factor of resistance to paclitaxel (PTX) in lung and ovarian cancer. In order to understand biological mechanism(s) underlying TUBB3 overexpression in cancer cells, we tested hypoxia as a potential inducer of overexpression. As a cellular model, we used human cancer cells from three tissues, namely A2780, A549 and HeLa cells, derived from ovarian, lung and cervical cancer, respectively. All the three cancer cell lines were treated with desferroxamine (DFO) as a model for hypoxia or actually cultured in hypoxia. TUBB3 was analyzed using real time PCR and western blots. While in A2780 and A549 cells treatments were able to increase up to tenfold the expression of TUBB3 at the mRNA and protein level, in HeLa cells this phenomenon was not detectable. In order to assess if DFO-treated cells are resistant to paclitaxel-induced microtubule polymerization, A2780 cells were treated with PTX after 72 h of DFO exposure and the fractions of soluble and polymerized tubulin were quantified. DFO-treated cells with overexpression of TUBB3 were resistant to PTX. To understand the mechanism of regulation, the flanking regions of the gene were analyzed. Three putative HIF-1 α binding sites were identified, two at the 5′ plus strand (396 and 1362 upstream of the transcription start) and one on both strands at the 3′UTR (+161 downstream the stop). Through chromatin immunoprecipitation we were able to demonstrate that after DFO treatment only the 3′ site is engaged by HIF-1 α, while the other two sites are not active. In order to assess the 3′ site, EMSA was performed, using a specific probe containing the HIF-1-specific sequence at +161. In both A2780 and HeLa cells treatments were able to increase nuclear expression of HIF-1 α. In A2780 cells EMSA revealed an enhancement of binding to the 3′ site, while in HeLa no increase was noticeable. Altogether these findings demonstrate that hypoxia and HIF-1 α are able to modulate the expression of TUBB3, thereby inducing drug resistance to PTX. In cervical cancer the 3′ enhancing did not respond to HIF-1 α, thereby explaining why in these cells hypoxia is unable to raise levels of TUBB3. This finding is in line with our recent observation that TUBB3 does not act as a prognostic marker in cervical cancer. Therefore, a tubulin survival code is activated upon hypoxia in lung and ovary, but not in cervical cancer. This finding will be useful for TUBB3 targeted therapies currently in development.