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