Telomerase-targeting agents

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Pharmacodynamics (PD) of GRN163L in a Phase I study in refractory, solid tumors

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Background: GRN163L is a 13-mer oligonucleotide inhibitor of telomerase activity being evaluated in a dose-escalation Phase I trial in refractory solid tumors. Gradual or delayed platelet (plt) decreases have been noted in some patients in this study (ASCO '08). This effect has been most apparent in the highest dose cohorts (3.2–4.8 mg/kg), but with significant interpatient variability at each dose level. An on-target effect upon telomerase in bone marrow cells is a hypothesized mechanism for the effect of this drug on plt counts.
Methods/Materials: To better understand this effect, we constructed models to assess contributions of multiple clinical, pharmacokinetic (PK) and biomarker factors. Percent change from baseline in platelet values at 4 weeks (wks) was modeled in a univariate and multivariate fashion using age, sex, number of prior cytotoxic regimens, baseline peripheral blood granulocyte telomere length (TL), Cmax, dose level, and baseline platelet values. TL was measured by the Flow-FISH method. Granulocyte TL has been used in studies as a biomarker reflecting bone marrow progenitor TL, including those of the megakaryocytic/erythroid lineages.
Results: As of May 12, 2008, 28 patients have been enrolled in this study. This includes 20 with TL measurements who were evaluable for this analysis. Shorter TL ($p = 0.02$) and higher dose level ($p = 0.03$) were the only significant predictors of plt decline at 4 wks: %change in plt at 4 wks = $-43 - 7*\text{Dose} + 8*\text{TL}$ ($p = 0.02$). No dose by TL interaction was found. Of note, there was a significant correlation between TL and number of prior cytotoxic regimens, $r = -0.52$, $p = 0.01$.
Conclusions: The PD model incorporating both dose and baseline TL is considered hypothesis-generating, but is consistent with changes in plt count secondary to inhibition of telomerase by GRN163L. Additional patient accrual and model development are ongoing.

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The cytotoxic activity of the telomere binding agent KML001 in non-small cell lung cancer cells is dependent on telomere length and p53 status and is enhanced by cisplatin

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KML001 (sodium metaarsenite) is an orally bioavailable, trivalent arsenic compound that is currently undergoing phase II clinical studies in Europe. We have previously found that KML001 binds to telomeric sequences at a ratio of one per three TTAGGG repeats. This results in telomere-associated DNA damage and telomere attrition. Similarly, cisplatin is known to bind to the guanine-rich telomeric sequences at a ratio of one molecule per telomeric repeat and to activate p53.
Tumor cells with short telomeres are particularly responsive to telomere targeting agents such as KML001 and cisplatin. In this study we investigated telomere length and the functional status of the DNA damage response regulator p53 in a panel of 12 non-small cell lung cancer cell lines (A549, H460, H23, H183, H59, H177, H182, H292, H358, H522, Calu-1, and patient-derived primary cells) and compared them to KML001 antiproliferative activity. We further assessed whether a combination of the two telomere binding agents KML001 and cisplatin would be synergistic. Inhibitory concentrations 50% (IC50) for KML001 and cisplatin in lung cancer cells were determined by using the methyltetrazolium proliferation assay. Combination studies were performed at a fixed IC50 ratio according to the method developed by Chou and Talalay and combination indices (CI) were calculated. Telomere length was determined by Southern blotting and employing the teloTAGGG-telomere length assay. To assess p53 mutational status PCR-based exon specific mutation analyses were performed.
KML001 IC50s ranged between 2 and 20 μM, concentrations that have been reached in plasma of patients in phase I studies. Mean telomere lengths varied from very short (2.1 kb in Calu-1) to very long (13 kb in H183). While there was no overall correlation between mean telomere length of a cell line and its IC50 for KML001, we found that lung cancer cell lines with dysfunctional or impaired p53 (H23 transcriptionally defective, H522 mt, and Calu-1 null) and short telomeres were sensitive to KML001 (Spearman r2=0.87). Cell lines with wt p53 (H460 and A549) had a 3−4 fold higher IC50 than the p53 defective lines. When we combined KML001 and cisplatin in lung cancer cell lines with short telomeres, we obtained synergism (CI < 1) in A549, H460 and Calu-1, or additivity for H522 (CI ~1), indicating that enhancement of KML001 cytotoxic activity by cisplatin is p53 independent.
Our data suggest that KML001 effectively inhibits tumor cell growth in non-small cell lung cancers with short telomeres and p53 functional defects, and that the single agent or its combination with cisplatin should be exploited clinically.

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Integrative biological studies of antitumour agents

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Background: We are developing a systems biology approach to analyse and integrate biological data from investigations of the activity of 3,11-difluoro-6,8,13-trimethyl-8H-quino[4,3,2-kl] acridinium methosulphate (RHPS4). Initial studies of RHPS4 suggested the mechanism of action involves telomerase inhibition at the sub micromolar level, possibly linked to its selectivity for G-quadruplex DNA. Recent studies, however, suggest a more complex mechanism of action. Data has shown RHPS4 has a time and dose-dependant effect on growth rate, cell cycle distribution, and movement into senescence and/or death.
Methods: A mathematical model of adequate complexity yet sufficient simplicity to yield predictive information and biological insights has been developed to reproduce results from the laboratory and model the relationship between cell cycle distribution, growth rates and senescence. The model has 5 compartments; where X represents G1/G0, Y represents S, Z represents G2/M, σ represents senescence and A represents apoptosis with rate and movement between the compartments denoted by the parameters kxy, kyz, kzx, kxσ and kσA. Parameterisation of this model requires robust data from the well-characterised HCT116 cell line, which displayed appropriate growth characteristics, good sensitivity to RHPS4, tractability in biological assays and clinical relevance in previous experiments. Methods employed to analyse HCT116 cells in response to RHPS4 include flow cytometry (combining propidium iodide, BrdU, Ki-67 and Histone H3 immunofluorescence), MTT assays and β-galactosidase staining.
Results: Data regarding the effects of RHPS4 on cell cycle distribution, growth rate and senescence on HCT116 cells is easily derived in control cells and in response to anti-tumour agents, however, this gives no direct information about the underlying molecular processes that are being perturbed by drug treatment, namely the kinetics of the cell cycle. Data was fitted to the model and revealed aspects of RHPS4 action that would be difficult to appreciate without it. The model suggests RHPS4 increases the rate of cells moving into a senescent state (increase in kxσ), however, inhibits apoptosis (reduction in kσA) in a dose-dependant manner.
Conclusion: The approach is general and is being applied to other antitumour agents allowing us to relate the changes observed to potential underlying molecular targets and gain a better understanding of the complex mechanism of action of RHPS4.

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The G-quadruplex ligand RHPS4 potentiates the antitumor activity of camptothecins in preclinical models of solid tumors

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Background: New anti-telomere strategies represent important goals for development of selective cancer therapies because most human cancers acquire the ability to activate telomerase and possess altered telomeres as compared to normal somatic cells. From this point of view, G-quadruplex ligands are promising compounds because they both inhibit telomerase activity, limiting long term proliferation of cancer cell, and directly target components of the protective cap of telomere, leading to immediate effects on cancer cell proliferation. We recently showed that one of them, the pentacyclic acridinium salt RHPS4, has an antitumoral effect by targeting