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New biological assay to evaluate the pharmacodynamic effects of rapamycin in cancer patients

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Rapamycin is a natural macrolide antibiotic with anti-tumor properties which inhibits the kinase activity of the mammalian target of rapamycin (mTOR) resulting in inhibition of the translational regulators p70S6 kinase and 4E-BP1. The clinical development of rapamycin and analog agents have been optimize by incorporating biological correlative endpoints to guide dose selection and, eventually, to predict patients outcome. Previous studies have shown that inhibition of p70S6 kinase in PBMCs can be used to determine the pharmacodynamic effects of rapamycin, and to establish a relationship between dose and plasma concentration of the agent that can be used as a predictor of treatment efficacy. In this study we have developed a new biological assay to determine the biological effects of rapamycin in patient's plasma.
Standard curve samples were prepared by diluting rapamycin in cell culture medium, and a linear dose related inhibition of the phosphorylation of the ribosomal p70S6 kinase by the agent was detected at a range between 0 to 700 pM, in presence of 1% normal human plasma, in the flasks containing Raji's B lymphoma cell culture. Patient plasma samples were equally diluted in cell culture media to maintain 1% plasma/flask. Protein extracts from cells were immunoprecipitated with p70S6 kinase antibody, 32P-labeled kinase assay was performed to the immunoprecipitated fractions, and kinase activity measurements of the samples were made by scintillation counting. We also performed a western blot analysis for the dose-response effect of rapamycin on Raji's protein extracts and looking for both phospho and total p70 S6 kinase to validate the accuracy of our bioassay.
In conclusion, we have developed a biological assay to measure biological effects of rapamycin in plasma that can be used to predict treatment efficacy in clinical trials.

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Dielectrophoresis (DEP) as sorting technology for microdiagnosis in breast cancer

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Background: Recent studies have demonstrated the existence of breast cancer subtypes as identified by gene expression profiling (Perou et al, Nature 2000) and postulated the existence of a breast cancer stem cell population (Al Hajj et al, PNAS, 2003). These data suggested a critical role for enrichment techniques that would allow for a detailed identification and analysis of these various breast cancer cell populations. Dielectrophoretic (DEP) forces are characteristic cell properties that occur when a cell is exposed to a non-uniform electrical field. We compared two different techniques, dielectrophoresis fluid-flow-fractionation (DEP-FFF) and superparamagnetic beads assay used to enrich breast cancer cells from specimens collected with a fine-needle aspiration biopsy (FNAB) in an *in vivo* model of breast cancer.
Methods: Three sets of animals (5 mice for each set) were evaluated in a total of eight experiments. Breast cancer cells (MDA-435) were grown, subsequently injected above the rear flank of female athymic nude mice and, approximately 3 weeks after the implant an FNAB of the implanted tumor was performed. A pathologist evaluated the cellularity of the specimen and if considered adequate two paired samples were distributed for a) DEP-FFF; b) Superparamagnetic beads assay. *DEP-FFF methods*: Cells were counted and then suspended in electrosmear buffer suspension at a known concentration. Between 0.5 to 1.2×10^6 total cells were loaded into the electrosmear chamber at the low frequency end and were allowed to relax for 5 min. Using a syringe pump, cells were then flowed over the electrosmear slide from the low frequency (20KHz) end at a flow rate of 100 μl/min towards the high frequency end (1 MHz).
Results: Eight different FNA samples were obtained and electrosmear and superparamagnetic experiments were run as soon as the samples arrived. Time between harvest and electrosmear analysis varied from 2 to 8 hours. Microscopic examination of the samples following electrosmear preparation and staining revealed that all samples comprised mixtures of tumor cells and RBC. A separation between cancer cells and RBCs was observed in all specimens processed with DEP/FFF (100%). Positive selection with Anti-HEA antibodies with superparamagnetic assay was observed in only 1 (12.5%) of the total cases.
Conclusions: The study demonstrated that DEP/FFF is superior to the superparamagnetic sorting technique when applied to microscopic specimens collected by FNAB. DEP/FFF represents an innovative technology with the potential of sorting and enriching human samples collected with minimally invasive techniques (e.g. FNAB and ductal lavage).

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Correlation between effects of PTK787/ZK 222584 on the interstitial fluid pressure of rat mammary tumours and their vasculature measured by contrast-enhanced MRI

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PTK787/ZK 222584 (PTK/ZK) is a novel oral angiogenesis inhibitor that targets all VEGFR tyrosine kinases (VEGF-R1, -R2, -R3) which contribute to tumour angiogenesis and metastasisis*. Anti-angiogenic activity has been confirmed in the clinic by dynamic contrast enhanced MRI (DCE-MRI). Compared to normal tissues, solid tumours have a raised interstitial fluid pressure (IFP) due to low lymphatic drainage, high vessel permeability and poor perfusion. We compared the effects of PTK/ZK on IFP and DCE-MRI measured parameters.
BN472 rat mammary carcinomas were grown orthotopically in syngeneic rats. Tumours of >0.5 g were studied by insertion of a needle (WIN method) to measure IFP and non-invasively by DCE-MRI using GdDOTA and Endorem to measure tumour vascular permeability (VP), interstitial leakage space (LS), blood inflow (BFI) and relative blood volume (rBV). Data are summarised as mean±SEM with significance set at $p<0.05$.
Daily treatment (30–200 mg/kg po) for 7 days inhibited tumour growth (max T/C=37%) and decreased the IFP (max: 37±12%) dose-dependently; these effects were significantly correlated (r=0.62, p=0.002). One dose of PTK/ZK (100 mg/kg po, daily) was used to compare changes in IFP with those in the DCE-MRI measured vascular parameters after 3 days treatment. PTK/ZK significantly inhibited tumour growth (T/C=53%), and decreased LS compared to baseline (22±4%, $p<0.001$). There was a trend for the rBV to decrease (11%), and this was significantly different to the change in rBV for vehicle-treated animals (+8%). Other DCE-MRI parameters were not affected. Vehicle treatment had no significant effects compared to baseline for DCE-MRI or IFP. The resting (pre-treatment) IFP correlated significantly with rBV (r=0.44) and BFI (r=0.46). After PTK/ZK treatment, the decrease in IFP (17±4%, p=0.006) showed a significant positive correlation with the decrease in rBV (r=0.71), but BFI and rBV were significantly *negatively* correlated ($r = -0.78$). VP and LS did not correlate significantly with IFP either pre- or post-treatment.
An IFP decrease may be a biomarker for tumour response to PTK/ZK. The decrease in IFP can be detected as a decrease in rBV which may, paradoxically, reflect improved tumour perfusion. Thus, an early effect of anti-angiogeneic therapy can be 'normalisation' of tumour vasculature which could aid combination with cytotoxic therapy by increasing drug uptake.
*PTK/ZK is co-developed by Novartis and Schering AG, Berlin.

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IC50 determination for receptor-targeted compounds and downstream signaling

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Receptor tyrosin kinase inhibitors (TKI) have been widely used for targeted therapy in cancer. These drugs inhibit the phosphorylation of tyrosin kinases at the receptor level as well as downstream signaling pathways such as MAP Kinase and PI3K-Akt pathways, therefore inhibiting cell proliferation and tumor growth. Further development of TKIs targeting the EGFR family is ongoing. We herein describe a streamlined approach to identify TKI by analyzing multiple signaling pathway components in multiplexed assays.
We used MCF7 cell line as a model to study the effect of 12 lead tyrosine kinase inhibitor candidates (A1-A12) on MAP Kinase and PI3K-Akt pathways. MCF-7 cells were treated with different doses (0.1 nM – 10 μM) of the drug for 1 hour followed by HRG stimulation for 5 min. Cells were immediately lysed and assayed for Her2 and Her3 phosphorylation, Her3-PI3K, Her3-Shc, Akt and Erk phosphorylation in multiplexed proximity-based eTag assays.
Among the 12 TKI candidates tested, four compounds exhibited IC50s for Her2 Phosphorylation in sub-nanomolar range, while others show