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Design of a screening procedure to select patients with leukemia for treatment with F 14512, a novel targeted cytotoxic agent

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The Polyamine Transport System (PTS) is an energy-dependent machinery generally hyper-active in cancer cells with a high demand for polyamines. This system can be viewed as a suitable molecular gate to deliver selectively polyamine-based molecules into cancer cells. We exploited this strategy to target to PTS-positive cancer cells F14512, a novel polyamine-epipodophyllotoxin conjugate, that exhibits significant anti-tumor activity and has been selected recently for clinical development.
This study was undertaken to investigate the potential of the fluorescent polyamine probe F96982 designed to select patients with PTS-positive leukemic cells. For this purpose, the uptake of this fluorescent molecule was first measured by flow cytometry in a panel of 12 human leukemia cell lines. Results showed that a high level of fluorescence was detected in F14512-sensitive cancer cell lines whereas leukemia cells responding poorly to F14512 generally exhibited very low levels of PTS. A flow cytometry procedure was then adapted and optimized to measure F96982-induced fluorescence in blood samples from healthy donors. We demonstrated that human leukocytes incorporate F96982 in a time, concentration and temperature dependent manner, confirming the active transport of polyamines in these cells. Using a CD45 gating, the incorporation in lymphocytes was found low and with a weak inter-individual variation. This technical validation then provided the basis to investigate a panel of human leukemia samples which revealed a larger inter-individual variation and a preferential uptake of the probe in tumor cells. Indeed, most interestingly, we observed that the incorporation of F96982 was higher in leukemia blasts than in lymphocytes. Altogether, the data suggest that this specifically designed fluorescent probe will be useful to guide patients selection for future enrollment in clinical trials with F14512.

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A clinical chemistry approach for identifying biomarkers as surrogate endpoints in therapy directed against chemokine mediated metastasis

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Introduction: CTCE-9908 (Chemokine Therapeutics) is an antagonist of CXCR4 and has also shown antiangiogenic properties. SDF-1 (ligand of CXCR4) and VEGF have been reported to act synergistically to promote neoangiogenesis in human cancers. Tumors are able to downregulate adhesion molecules on the vascular endothelium by exposure of endothelial cells to angiogenic growth factors produced by the tumor (Griffioen A. Cancer Immunol Immunother, 2008). Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are involved in trafficking of leucocytes through endothelial and epithelial barriers, in metastasis formation and in the immune surveillance of tumor. With their known function in cancer progression, cytokines, growth factors, and proteins involved in adhesion and movement are ideal biomarkers to measure when assessing cancer treatment. We measured 17 biomarkers in nine patients enrolled in an open label, Phase I/II study of CTCE-9908 a novel compound that targets SDF-1/CXCR4 mediated metastasis.
Methods: After receiving research ethics approval, EDTA plasma was collected prior to study drug administration on day 1 (baseline), and days 5, 12, 19, 25. The blood was centrifuged according to standard laboratory procedures and rapidly separated into aliquots. All aliquots were frozen below −70ºC until biochip array analysis. One aliquot was thawed for each time point and analysis was performed using the Cytokine & Growth Factor and Adhesion Molecules arrays using the evidence investigator biochip platform from Randox Laboratories Ltd. Between-group comparisons of central tendency were based on the t-test for parametric analyses with significance set at $p < 0.05$ (analyses via GraphPad Prism).
Results: Of the 9 subjects enrolled in the study, only 7 subjects had sufficient volume on days 1, 5, 19, and 25 for analysis on both arrays. Of the 17 biomarkers analysed; IL-2, IL-1α, IL-1β, and IFN-γ had a majority of measurements below the limit of detection of the assays, leaving 13 biomarkers (IL-4, IL-6, IL-8, IL-10, VEGF, TNF-α, MCP-1, EGF, VCAM-1, ICAM-1, ESel, PSel, LSel) available for evaluation. Two subjects had stable disease (SD) and 5 had progressive disease (PD) on day 25. Only VCAM-1 and ICAM-1 were different between the 2 groups at each of the time points (table).

Table 1

Day	PD VCAM (mean)	SD VCAM (mean)	p-value	PD ICAM (mean)	SD ICAM (mean)	p-value
1	590.3	1502.0	0.03	444.3	1588.5	0.04
5	678.1	1487.7	0.04	468.6	1518.2	0.05
19	483.7	1360.2	0.05	412.5	1327.2	0.03
25	756.6	1347.5	0.12	534.3	1373.4	0.02

Conclusions: This pilot study indicates that VCAM and ICAM are elevated in the SD group as compared to PD group. Previous reports indicate that soluble VCAM and ICAM enhance SDF-1 function. Thus higher levels of these cell adhesion molecules may represent metastatic growth that is more dependent on the SDF-1 pathway, and may offer a possible rationale as to why CTCE-9908 may be able to cause disease stabilization. Further studies are warranted to validate these initial findings.

Chemoprevention

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The RhoA kinase (ROCK) inhibitor Y27632 and specific novel structural analogues of this compound cause irreversible elimination of transformed NIH3T3 cells from cultures

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Background: NIH3T3 cells will readily transform in vitro with accompanying loss of cell polarity and contact inhibited growth. As a consequence, these cells have been used extensively as a model system for the study of cellular transformation.
Materials and Methods: Stable transfection of NIH3T3 cells with the novel guanidine exchange factor (GEF) 16 caused the formation of multilayered non-contact inhibited transformed colonies. GEF16 transfected cells were treated with either the ROCK inhibitor Y27632 or one of 64 different structural analogues of this compound. Their ability to effect, cell growth, transformed colony formation and persistence following drug withdrawal was then evaluated. In vitro kinase inhibitory activity assays were carried out with selected compounds against X40 recombinant kinases.
Results: At 10 μM concentration, Y27632 and three other structural analogues prevented both the formation and irreversibly eliminated transformed colony forming cells from GEF16 transfected NIH3T3 cells. Unlike Y27632 one of our compounds also removed pre-formed colonies from these cultures yet none of them showed any adverse toxicity against sub-confluent cells in logarithmic growth. Most surprising was the observation that our compounds did not kill transformed cells but rather caused them to irreversibly loose their transformed properties. Interestingly all three novel Y27632 analogues had minimal ROCK inhibitory activity-showing instead maximum activity against the kinases Aurora A, p38 (MAPK14) and Hgk (MAP4K4).
Conclusions: Our data are the first to indicate that blockade of different yet specific kinases can cause transformed NIH3T3 cells to loose their transformed properties whilst having no discernible effect against non transformed cells. Since this effect was not produced by cell killing and was more pronounced in mixed cultures of transformed and non transformed cells, this indicates that our compounds may produce this effect by promoting intercellular communication. These observations imply that chemical modulation of selected kinases could form the basis of novel strategies for cancer chemoprevention by facilitating the ability of non transformed cells to re-establish growth control over transformed foci.