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DNA damage induced by camptothecins is stabilized by G-quadruplex ligands

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Background: We previously reported that the combination of the G-quadruplex (G4) ligand RHPS4 with standard camptothecins has a strong synergistic interaction *in vitro* and produced a marked antitumor activity on xenografts. The present study aims at investigating the mechanisms involved in the specific interaction between G4 ligands and camptothecins by using well-established G4 ligands and novel camptothecins.
Material and Methods: Chromatin immunoprecipitation (ChIP) assay was performed to evaluate the presence at the telomeres of the different topisomerase isoforms after treatment with G4 ligand compounds. Combination index test based on clonogenic assay was used to study synergism, additivity or antagonism of the different G-quadruplex/camptothecin combination. Co-immunofluorescence experiments were performed to measure the formation of damaged foci and FACS analysis to evaluate cell cycle perturbation/apoptosis. *In vivo* experiments were carried-out to evaluate the therapeutic efficacy of novel camptothecins/RHPS4 combination.
Results: We found that the G4 ligand RHPS4, Coron and Pip-piper increased Topo I at telomeres, while no change of TopoIIα was observed in treated compared to untreated cells. We therefore studied the effect of G4 compounds in combination with either the TopoI inhibitor SN-38 or the topoisomerase II poison doxorubicin. A marked reduction of clonogenic ability and a strong synergism effect was observed in the G4/camptothecin combination, while the combination with doxorubicin showed only a slight decrease of cell survival. Of note, the sequence of administrating the two drugs is critical in determining the chemo-sensitizer activity of the G4 ligands to SN-38, since the opposite sequence of treatment did not produce an increase in camptothecin cytotoxicity. Analysis of damage foci revealed that SN-38 produced a strong phosphorylation of γH2AX both at the telomeric (TIFs) and non telomeric regions but cells recovered the damage as it is evident by the decrease of γH2AX foci at 48 hrs after the treatment. Interestingly, DNA damage was highly increased in cells treated with SN-38/RHPS4 combination and the percentage of γH2AX-positive cells and the number of TIFs were stabilized by the G4 ligands. In addition, the synergistic effect of SN-38/G4 ligands was confirmed with the novel ST1481, IDN5174 and ST1968 camptothecins in combination with RHPS4. Finally, treatment of mice with ST1481 and RHPS4 was able to inhibit tumor weight, delay tumor regrowth and increase survival of mice bearing colon cancer xenografts.
Conclusions: Our data demonstrate the high therapeutic efficacy of TopoI inhibitors/G4 ligands combination and suggest that the stabilization of DNA damage by G4 ligands can account for the antitumor effect of this therapeutic strategy both *in vitro* and in xenografts.

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The mTOR kinase inhibitor AZD8055 induces cell death in Her2+ tumours partially or intrinsically resistant to ErbB2 inhibitors

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The PI3K-Akt-mTOR pathway is one of the most frequently activated pathways in human tumours. Oncogenic drives such as PI3K or KRas mutations, PTEN loss of function or ErbB2 amplification produce high dependency on the PI3K/mTOR pathway for growth and survival. mTOR is part of 2 complexes mTORC1 and mTORC2. mTORC1 activates S6K and 4EBP1, both involved in cap-dependent translation. mTORC2 directly activates AKT on Ser 473. Clinically, mTOR is the only validated target in the PI3K pathway. However, allosteric inhibitors of mTOR such as rapamycin inhibit mTORC1 only, leading to AKT hyperactivation, limiting their clinical activity.
AZD8055 is a mTOR kinase inhibitor, targeting mTORC1 and mTORC2 inducing greater growth inhibition and cell death than rapamycin. The aim of this study was to establish the activity and biomarkers profile of AZD8055 in Her2-overexpressing models both as single agent and in combination *in vitro* and *in vivo*.
A gene expression and copy number analysis selected a series of Her2+ cell lines with differential PI3K activation. AZD8055 induced significant growth inhibition and cell death in this cell line panel. A subset of cell lines were established as xenografts and tested *in vivo*. AZD8055 induced growth inhibition or regression in BT474, MDA-MB-453 and H1954 xenograft models at a dose of 20 mg/kg daily. In BT474 cells, AZD8055 increased the growth inhibitory effect of herceptin in vitro. In vivo, BT474 xenografts were hypersensitive to herceptin and the added beneficial effect of AZD8055 was limited. In MDA-MB-453 moderately sensitive to lapatinib *in vitro*, the combination of AZD8055 and lapatinib induced cell death while only growth inhibition was observed with either agent. Finally, AZD8055 induced regression in a primary explants model of Her2-amplified breast cancer xenografts resistant to herceptin. In MDA-MB-453, the combination effect was associated with greater inhibition of mTORC1 and mTORC2 markers, induction of apoptosis as well as abrogation of pHer3 feedback activation. Taken together this study demonstrates that AZD8055 enhances the efficacy of Her2 targeted agents in sensitive models. AZD8055 is also active in tumour models partially or intrinsically resistant to ErbB2 inhibitors, suggesting that it may have broad activity in ErbB2+ tumours.

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3D tumour models for the assessment of tumour micro-environment targeted therapies

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Background: It is becoming increasingly apparent that the interaction between the tumour and the surrounding stromal cells is critical to tumour growth, invasion and metastasis. As a consequence, tumour-microenvironment targeted therapies and therapies which disrupt the paracrine signalling pathways that support tumour growth are under development. Current pre-clinical *in vitro* and *in vivo* tumour models lack human stromal cells and so are inadequate for the assessment of novel tumour microenvironment targeted therapies.
Methods: We have developed a novel 3D matrix reconstitution assay, where the paracrine interaction between tumour derived fibroblasts and epithelial cells are restored, allowing the real-time assessment of tumour-microenvironment driven tumour growth in the presence of targeted therapies.
Results: Primary tumour associated fibroblasts and mesenchymal stem cells drive growth and proliferation of non-small cell lung cancer cell lines of adenocarcinoma (A549, NCI-H358, NCI-H460) and squamous subtypes (SK-MES1) as well as early stage disease (NCI-H322M). The paracrine interaction promotes 3D structure formation and invasion into laminin rich basement membrane extract. Using fluorescent cell labelling and real-time viability assays the 3D matrix reconstitution assay allows the rapid and scalable assessment of targeted therapies in comparison to platinum doublet standards of care in the context or tumour micro-environment driven growth.
Conclusion: The 3D matrix reconstitution assay provides a more accurate model for pre-clinical testing of therapies which target the tumour microenvironment or the downstream pathways of their paracrine interaction with tumour cells.

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TGF-β1 as a therapeutic target in high risk endometrial carcinomas

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Background: Endometrial cancer is among the three most common cancers in females in industrialized countries. In the majority of cases the tumor is confined to the uterus at time of diagnosis and presents a good prognosis. However, after primary surgery, from 15% to 20% of these tumors recur and have limited response to systemic therapy. We thus aimed to perform gene expression profiling associated with high risk of recurrence in endometrial carcinomas, and evaluate new therapeutic approaches targeting the molecular pathways involved in the acquisition of an aggressive tumor phenotype.
Material and Methods: We performed cDNA microarray analysis in 60 human endometrial carcinomas and compared the gene expression profiles associated with low (IA and IB endometrioid tumors) and high (II, III, IV and IC endometrioid, serous papillary and clear cell carcinomas) risk of