Drug resistance and modifiers

617 POSTER Pharmacodynamic analysis of surrogate tissue responses to the demethylating agent 2′-deoxy-5-azacytidine (Decitabine)
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The DNA methyltransferase inhibitor 2′-deoxy-5-azacytidine (decitabine) can sensitize drug resistant tumour xenografts grown in nude mice to a range of cytotoxic chemotherapeutic drugs including carboplatin (Plumb et al, 2000, Cancer Res., 60, 6039). This treatment also induces CpG-island methylation and increased expression of the HMLH1 gene in drug resistant human tumour xenografts. These data have led to an ongoing Phase I clinical trial of decitabine and carboplatin in patients with advanced solid tumours. Decitabine was given iv over 6 hours on Day 1, Carboptatin at a fixed dose of AUC5 was given on Day 6 (iv over an hour) and treatment was repeated every 4 weeks. Patients have been treated at three dose levels of Decitabine: 45, 90 and 135mg/m². Maximum tolerated dose was identified as Decitabine 135mg/m² with Grade 4 febrile neutropenia (1 patient), and Grade 4 neutropenia necessitating delay of cycle 2 for >7 days (1 patient). Pharmacodynamic objectives of this trial were; 1) to study the time course and relationship between DNA methylation in surrogate tissues and the dose and pharmacokinetic behavior of decitabine, 2) to investigate changes in methylation of specific CpG-islands at gene promoters induced by decitabine in surrogate tissues. DNA extraction from peripheral blood mononuclear (PBM) cells showed a dose dependent increase in levels of 5-methyl-2′-deoxycytidine up to day 10 which then reversed and had returned to near starting levels by day 22. The demethylation observed remained similar in subsequent cycles of decitabine treatment. The MAGE1A CpG-island is biallelically methylated in adult somatic normal tissues. Decitabine treatment induced dose dependent demethylation of the MAGE1A CpG-island in DNA isolated from PBM cells of treated patients. The reduction in 5-methyl-2′-deoxycytidine levels in human PBM cells is equivalent or greater to that observed in murine PBM cells, at decitabine doses in mice when concomitant demethylation of the HMLH1 gene and chemosensitisation occurs in drug resistant xenografts. Analysis of DNA methylation of tumours from patients before and after treatment. The MAGE1A CpG-island is biallelically methylated in adult which then reversed and had returned to near starting levels by day 22. The

619 POSTER Analogies in imatinib-resistant threonine-to-isoleucine mutation in BCR-ABL, KIT and PDGFRA: a combined experimental/computational approach
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Background: Current strategies to target resistant subtypes of chronic myeloid leukaemia (CML), including Imatinib, suffer from limited clinical efficacy, with the evolvement of drug resistant clones. The development of drug resistance occurs in a multistep process, involving genetic and epigenetic changes. In this work, using a computational approach, we aim to explore the analogies between BCR-ABL and two other important tyrosine kinases, KIT and PDGFRA, in terms of point mutations that confer drug resistance. Our recent studies on BCR-ABL, using a combination of atomistic and coarse-grained molecular dynamics simulations, have shown that the ATP-binding pocket of the wild-type kinase is structurally conserved in the resistant forms. In this work, we have built homology models of KIT and PDGFRA and used computational methods to predict the analogies between these kinases with BCR-ABL.

Materials and Methods: Computational free energy perturbation techniques were applied both to calculate the stability of wild-type and T-to-I mutants of BCR-ABL, KIT and PDGFRA, and to predict the relative binding affinities between the mutant forms and Imatinib.

Results: We were able to qualitatively and quantitatively predict drug resistance in KIT and PDGFRA, and to predict the relative binding affinities between the mutant forms and Imatinib. The analogies between BCR-ABL and KIT or PDGFRA are best observed at the level of structural changes in the catalytic domain.

Conclusions: These results confirm the potential role of computational methods in the design of new drugs against drug resistant kinases and highlight the importance of drug resistance mechanisms of decreasing drug binding affinity.