Poster Presentation: Experimental/Molecular Therapeutics, Pharmacogenomics

Drug Discovery – Drug Design

PO-402 ESTABLISHMENT OF HUMAN MONOCLONAL ANTI TUMOUR ANTIBODIES WITH HIGH AFFINITY TO CD9 USING AN IN VITROB CELL AFFINITY MATURATION PLATFORM

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Introduction Several attempts to develop an anti-CD9 antibody for cancer treatment failed pre-clinically due to the life-threatening side effect of platelet aggregation. We currently have a patient derived anti-CD9 antibody, named AT1412, under development (see abstract Schotte et al.). This patient, diagnosed with metastatic melanoma, was treated with autologous ex vivo expanded T cells and is still in remission 10 years after treatment. The antibody derived from the patient’s B cells targets a novel epitope on CD9 with low affinity, mediates ADCC against melanoma cells in vitro and, affects growth of tumour cells in mouse models. In contrast to previously described anti-CD9 antibodies AT1412 does not aggregate platelets. To improve the anti-tumour efficacy of the antibody we generated variants using a novel affinity maturation platform.

Material and methods We developed an in vitro selection technique that mimics the natural process of affinity maturation of antigen specific B cells. Bcl-6/Bcl-XL immortalised primary human B cells are B-cell receptor positive, antibody-producing germinal centre like B cells. Low frequencies of AID induced somatic hypermutations in B cell clones are detected. This allows for the selection of sub clones with altered antibody binding characteristics. Sub clones of the AT1412 B cell clone were seeded and expanded. Antibody containing supernatants were collected and tested for binding to CD9 by Surface Plasmon Resonance (SPR).

Results and discussions Out of 800 B cell sub clones, 9 produced an antibody with a higher affinity to CD9 as compared to the parental clone. Purified recombinant antibodies were generated to confirm enhanced binding to CD9 expressing cells by flow cytometry and SPR. One antibody variant combining two mutations reached similar affinity levels as commercial anti-CD9 mAbs. Extensive epitope mapping suggests that all high affinity variants recognise the same epitope as the parental AT1412 antibody. These high affinity antibodies mediate much stronger ADCC against melanoma cells than the wild type antibody. Importantly, the high affinity variants still do not aggregate platelets.

Conclusion We developed a tool which extends the applicability of mutational signatures analysis to low counts of mutations. Application of our SigMA algorithm to detect HR defect and other mutational signatures in panel sequencing data will increase the number of cases that may benefit therapeutic agents that target specific classes of genomic alterations.

PO-403 DISCOVERY OF A NEW POTENT AND MUTANT-SELECTIVE EGFR INHIBITOR THAT OVERCOMES T790M-MEDIATED RESISTANCE IN LUNG CANCER

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Introduction Despite remarkable activity in epidermal growth factor receptor (EGFR)-mutant lung cancer patients, the clinical efficacy of EGFR tyrosine kinase inhibitors (TKIs) is limited by the emergence of acquired resistance which is mostly caused by secondary T790M mutation. Fortunately, newly developed, mutant-selective EGFR-TKIs against T790M have been proven as an effective therapeutic approach although only osimertinib received the FDA approval until now.

Material and methods H1975, PC-9/GR and PC-9/ER cells which show the resistance to 1st generation EGFR-TKIs through T790M were used. Ba/F3 cells with exon 20 insertion mutation or C797S point mutation were generated by site-directed mutagenesis and stable transfection.

Results and discussions We discovered the OBX-012, another novel, mutant-selective EGFR-TKI. It showed substantial selectivity for mutant EGFR, especially T790M mutation (IC50 value; 24 nM for L858R and 2 nM for L858R/T790M vs 566 nM for wild-type EGFR), in cell-free kinase assay. Compared with other mutant-selective EGFR-TKIs such as olumitribin and osimertinib, OBX-012 exhibited the similar potency and selectivity for mutant EGFR. The treatment of OBX1-012 was highly effective against human EGFR-mutant lung cancer models with or without EGFR T790M, not only in vitro but in vivo.
CISPLATIN AND RUTHENIUM(III) COMPLEXES – COMPARISON OF CELLULAR RESPONSE OF TREATED MDA-MB231 BREAST CELLS

**Introduction** Since the discovery and clinical success of the platinum(II) anticancer drug, cisplatin, researchers are putting much effort to develop more efficient metal-based therapeutic compounds, with fewer side-effects and greater cytoselectivity. Ruthenium complexes arose as promising anticancer agents, due to the success of some ruthenium drug candidates in clinical trials. Here we report comparison of *in vitro* cytotoxic activity and mechanisms of action of cisplatin and four newly synthesised ruthenium(III) complexes with bidentate anionic schiff base derived from 5-methylsalicilaldehyde and methylamine: (complexes 1–4).

**Material and methods** Cytotoxicity was tested on four human cancer cell lines (K562, A549, EA.hy926, MDA-MB231) and one human non-tumour cell line (MRC-5), by MTT assay. Being the most cytotoxic of all four tested complexes, complex 1 ([Na[RuLCl2], L=N-propyl-5-chlorosalicylidenimino]) is selected for further analyses of molecular mechanisms underlying its activity toward MDA-MB231 cells.

**Results and discussions** The average IC₅₀ values were in the low micromolar range 2–23 µM, depending on cell line. Investigated complexes displayed an apparent cytoselective profile, as they reduced the viability of tested tumour cell lines more efficiently than of the non-tumour MRC-5 cells. Cisplatin resistant MDA-MB231 cells showed to be ten times more sensitive to C₁ (IC₅₀=2 µM) than to cisplatin. 24 hour treatment of MDA-MB231 cells with IC₅₀ values of C₁ and cisplatin induced minor cell cycle alterations, while 48 hour treatment induced substantial accumulation of cells in Sub-G1 region, up to 22.4% (C₁) and 86.4% (cisplatin), versus control 4.8%. Acridine orange/ethidium bromide dual staining treatment induced substantial accumulation of cells in Sub-G1 region, up to 22.4% (C₁) and 86.4% (cisplatin), versus control 4.8%. Acridine orange/ethidium bromide dual staining confirmed the Annexin V-FITC/PI assay results of notable reduction in cell number after the treatment with C₁ and cisplatin. While cisplatin-treated cells prominently die of necrosis, C₁-treated cells after 24 hour treatment show apoptotic morphology, but after prolonged treatment, necrosis becomes predominant. Decrease in the intracellular levels of reactive oxygen species was comparable in the cisplatin-treated and C₁-treated cells, with cisplatin displaying more conspicuous effects at higher dose. C₁ entered the cells more efficiently compared to cisplatin. Intracellular C₁ concentration after 4 hour treatment exceeded that of cisplatin by 7.8 times approximately.

**Conclusion** Present study pointed out interesting activity of this type of ruthenium(III) complex and need for further biological studies and its chemical structure optimisation.

**PO-404**

REPOSITIONING EXISTING DRUGS AS NOVEL THERAPEUTICS FOR HIGH-RISK PAEDIATRIC LEUKAEMIA

**Introduction** Despite remarkable improvements made in the treatment of childhood acute lymphoblastic leukaemia (ALL), prognosis remains dismal for a certain subgroups of high-risk (HR) patients including infants with leukaemia harbouring rearrangement of the Mixed Lineage Leukaemia (MLL/KMT2A) gene. Development of more effective, less toxic therapeutics is therefore urgently needed. The aim of this study was to identify compounds that target HR leukaemia cells based on drug-repurposing, whereby an approved drug is applied to treat a disease other than the one for which it was originally intended.

**Material and methods** New Abstract

**Results and discussions** The screen identified that two FDA-approved drugs, auranofin and disulfiram, originally developed for treatment of rheumatoid arthritis and chronic alcoholism respectively, had preferential cytotoxicity against leukaemia cell lines compared to solid tumours and normal cells (p<0.0001). Both compounds subsequently showed potent activity in paediatric high-risk leukaemia patient-derived xenograft (PDX) cells cultured *in vitro*, including xenografts derived from MLL-rearranged ALL and Philadelphia-positive ALL subtypes. The compounds induced apoptosis within 12 hours of treatment through an increase in intracellular reactive oxygen species (ROS) (p<0.01), which was accompanied by induction of Nrf2, a master regulator of the antioxidant response. Incubation with ROS scavenger N-acetyl cysteine prior to treatment with either drug prevented the increase in cellular ROS levels (p<0.05) and rescued cells from apoptosis (p<0.0001). The drugs showed synergy with each other, and auranofin also potentiated the established chemotherapeutic cytarabine in resistant HR leukaemia cells (p=0.016).

**Conclusion** In summary, we have identified two FDA-approved drugs that demonstrated potent, synergistic anti-leukaemia activity through ROS induction as well as chemosensitise cells that are resistant to current chemotherapeutics, potentially opening up new avenues for clinical treatment of HR paediatric leukaemia. We will be testing these therapies *in vivo* using relevant PDX models of HR paediatric ALL.

**PO-406**

THE OLIVE-BASED OLEOCANTHAL AS A DUAL HER2-MET INHIBITOR FOR THE CONTROL OF BREAST CANCER

**Introduction** Also in *vivo*. However, OBX1-012 like other EGFR-TKIs failed to show the efficacy for exon 20 insertion mutation or C797S point mutation.

**Conclusion** These results identify OBX1-012 as one of highly effective, mutant-selective EGFR-TKIs for treatment of T790M-mediated resistance.