

PO-205 **FUNCTIONAL CHARACTERISATION OF BRIP1/FANCI IN TUMOUR SUPPRESSION AND THERAPY RESPONSE**

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Introduction The helicase BRIP1 is critically required for genomic maintenance, and *BRIP1* helicase deficient germline variants are associated with Fanconi Anaemia (bi-allelic) and various cancers (mono-allelic), in particular ovarian and breast cancer. Cellular based assays and biochemical studies have elucidated the function of BRIP1 in replication stress responses, including interaction partners and enzymatic substrate specificity. However, functional characterisation of BRIP1 for these processes *in vivo* are largely unexplored, including the importance of BRIP1 as a tumour suppressor for breast cancer, and therapy responses. This project aims to elucidate the functional roles of BRIP1 as a tumour suppressor for breast cancer and in therapy responses.

Material and methods We have developed breast tissue-specific knockout mice for BRIP1, and will monitor the development of breast tumours. In addition, we have developed mice deficient for BRIP1 helicase activity (K52R) to further investigate the functional role of BRIP1 as a tumour suppressor. In addition, we will use the mouse models for intervention studies, testing known and novel therapies for BRIP1 deficient breast cancer. Moreover, we will investigate BRIP1 germline variants identified in high-risk breast cancer families by large scale sequencing efforts. This comprehensive list of patient derived mutations will be introduced endogenously in a primary epithelial cell line using a CRISPR-CAS9 approach. To monitor the effect on BRIP1 activity, these cell lines will be tested in cancer relevant functional assays for which BRIP1 deficient cells are known to have a phenotype.

Results and discussions This project is at an early stage, and we will present our preliminary data from our BRIP1 mouse models, and from our *in vitro* CRISPR assay for testing BRIP1 gene variants of uncertain significance (VUS).

Conclusion This project is expected to yield novel insight into the regulation of critical genomic stability pathways and provide important knowledge for developing improved germ line variant risk prediction and treatment opportunities for patients.

PO-206 **MODELLING T CELL ACUTE LYMPHOBLASTIC LEUKAEMIA USING CRISPR/CAS9 MEDIATED GENOME EDITING IN XENOPUS TROPICALIS**

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Introduction T cell acute lymphoblastic leukaemia (T-ALL) is an aggressive haematological malignancy that originates from malignant transformation of T cell progenitors. It accounts for 15% of paediatric and 25% of adult ALLs. Most frequent genetic alterations associated with this neoplasia are activating

mutations in *NOTCH1* and loss of function mutation for *FBXW7* and *PTEN*. Current chemotherapeutic approaches demonstrate good cure and survival rates in patients. However prognosis of relapsed patients is still dismal. Therefore there is an urgent need to identify new targets for the development of more effective therapeutic compounds.

Material and methods CRISPR/Cas9 editing technology was used to generate mosaic mutant animals in *Xenopus tropicalis*, a true diploid amphibian. sgRNAs were designed to induce activating (*i.e.* truncating) mutations in *notch1** and loss-of-function mutations in *fbxw7* and *pten*. Cas9 recombinant protein and different sgRNA combinations were injected in 4 cell stage embryos. Animals were raised for 6 weeks. Liver, spleen, kidneys and thymus were dissected and further processed.

Results and discussions Combined injections of sgRNAs that targeted *notch1* and *pten* resulted in mosaic mutant animals that exhibited pale appearance, enlarged spleen and haemorrhagic hindlimbs. Furthermore, sudden unexplained full penetrant mortality was observed around the time of metamorphosis. From diseased animals, genomic DNA was extracted from dissected thymi and the targeted gene loci were PCR-amplified and deep sequenced. Interestingly, several thymi showed clonal enrichment of a single cell population with a unique INDEL mutation pattern in one *notch1* allele and two *pten* alleles, indicative of a leukemic cell population in the mosaic mutant animals. Exploiting a concept of clonal enrichment and negative selection for mutations in genes essential for T-ALL formation, we are using multiplexed gene inactivation to identify T-ALL dependency factors and hence explore novel routes for targeted therapy. We are currently verifying the feasibility of this experimental concept by multiplexed injections of *pten* and *notch1* together with *myc*. Furthermore, we are validating our model by conducting flow cytometric and immunohistochemical analysis of the blood.

Conclusion We generated a genetic T-ALL model in *X. tropicalis* by co-targeting of *notch1** and *pten*. We expect this model to provide new opportunities for identification and validation of novel driver genes and dependency factors for T-ALL.

PO-207 **FERRITIN-ENGINEERED NANOPARTICLES AS TARGETED DRUG DELIVERY SYSTEM FOR CANCER TREATMENT**

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Introduction Cancer remains still one of the major causes of death worldwide, therefore continuous improvements in tumor-fighting strategies are necessary. Targeting drugs directly to the tumour site, to overcome the systemic side effects, represents a great challenge. Nanoparticles have increasingly been used as drug delivery system showing intriguing therapeutic efficacies.

Material and methods A genetically engineered nanocarrier based on human ferritin heavy chain (HFt) able to incorporate and deliver drugs was developed. These nanoparticles contain a short motif sequence (MP) cleavable by matrix metalloproteases between the HFt subunit and a masking sequence rich in proline (P), alanine (A) and serine (S): HFt-MP-PASE40. A topoisomerase I inhibitor was loaded into these nanocarriers (HFt-MP-PASE-Topo). Cell viability of different pancreatic cancer cell lines was evaluated *in vitro*. *In vivo* the therapeutic efficacy of HFt-MP-PASE-Topo was investigated on both a pancreatic cancer cell-line-derived xenograft and a patient-derived pancreatic cancer xenograft (PDX).

Results and discussions *In vitro* studies showed a potent cytotoxic activity of HFt-MP-PASE-Topo with an IC50 that ranges between 0,005 μ M to 0,05 μ M. *In vivo* studies further demonstrated the therapeutic efficacy of HFt-MP-PASE-Topo in pancreatic cancer-bearing mice and in PDX model. *In vivo* treatments exhibited a robust decrease in tumour growth furthermore the animal overall survival significantly increased in HFt-MP-PASE-Topo treated mice.

Conclusion Altogether, our results indicate that HFt-MP-PASE-Topo may constitute a promising tool in anticancer therapeutics.

PO-208 HUMAN ZEBRAFISH XENOGRAPTS AS THERAPY SENSORS FOR BREAST CANCER

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Introduction Despite great advances in biomarker-driven therapies, we still lack methods to predict how a specific cancer in a unique patient will respond to a given therapy. This exposes some patients to unnecessary toxicities and delays access to other potentially effective therapies.

Material and methods Recently, we developed and optimised zebrafish-larvae-xenografts for personalised medicine. As a proof-of-principle, we screened the current standard of care for colorectal cancer (CRC), from 1st to 3rd lines of treatment, following the international cancer therapy guidelines. After showing a similar response to therapy between mouse and fish xenografts, we demonstrate the feasibility of generating zebrafish Patient Derived Xenografts (zPDX) and provide proof-of-concept experiments that compare response to therapy between patients and their matching zPDX (Fior et al, 2017).

Results and discussions We are now replicating the same technology for triple negative breast cancer (TNBC). Surprisingly, we found that TNBC cells can respond differently to compounds of the same family, such as taxanes (docetaxel vs paclitaxel) and anthracyclines (epirubicin vs doxorubicin). These findings may have clinical implications since nowadays they are considered equivalent. Moreover, we show that bevacizumab, an anti-VEGF therapy without any biomarker available and contradicting results in the clinic, can be screened in zebrafish xenografts.

Conclusion We are generating breast zPDX and comparing response to therapy in patients to their matching zPDX both in early and advanced disease. Altogether, our preliminary results suggest that zebrafish-xenografts constitute a promising *in vivo* assay for screening chemotherapy in breast cancer.

PO-209 CRISPR/CAS9 BASED DEVELOPMENT OF RNAI RAT MODELS FOR DRUG DISCOVERY

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Introduction Genetically engineered mouse models have become the premier organism for dissecting cancer mechanisms and evaluating novel drug targets *in vivo* due to the availability of mouse embryonic stem cells that could be genetically manipulated *in vitro*. Despite the utility of mouse models, the rat has historically been the major model species in many biomedical fields, notably toxicology and carcinogenicity testing; and for many scientists, the rat still remains the preferred rodent due to their larger size for surgical manipulation, repeat blood sampling, and their cognitive and physiological characteristics that more closely resemble humans than their mouse counterparts. Now, with the advent of CRISPR/Cas9 technology, generation of genetically engineered rats is now a possibility.

Material and methods Here, we take advantage of our two-step engineering approach and exploit the efficiency of CRISPR-based targeting to develop RNA interference rat models that enable inducible and reversible gene silencing to simulate therapeutic regimes. When combined CRISPR-Cas9 gene modification, we can not only generate cancer *de novo* in a few weeks time, but also mimic drug therapy via RNAi in the same animal, giving us advanced capabilities to perform pre-clinical studies *in vivo*.

Results and discussions We demonstrate that our approach allows us to rapidly generate RNAi rat models and mimic the function of the targeted small molecule inhibitors, such as BET inhibitors targeting *Brd4*. We compare our results to our *Brd4* RNAi mice and demonstrate organism variances that provide valuable insight to cross-species differences. These results demonstrate that our high-throughput system currently used to generate RNAi mice is also applicable to the rat system and, by extension, other mammalian models.

Conclusion Inducible RNAi rat models will undoubtedly be powerful tools that can be used to model human cancers, to mimic the action of putative drugs, and to assess the potential of therapeutic targeting strategies *in vivo* prior to the costly drug development, ultimately guiding the development of safer and more effective drugs.

PO-210 SYNERGY BETWEEN THE KEAP1/NRF2 AND PI3K PATHWAYS DRIVES NON-SMALL CELL LUNG CANCER WITH AN ALTERED METABOLISM

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