generate multiple expression profiles for the related ROI. We intend to use this flexible, high-dimensional spatial profiling to identify the spatial transcriptomic signatures and explore phosphorylation sites in cancer-targeted therapies.

Results: The spatial transcriptomics analysis of this study is in view.

Conclusions: Our findings will contribute to understanding how the spatial landscape of the tumour microenvironment enhances the efficacy of anti-tumour drugs and what subset of patients are more likely to benefit from such therapy.

Legal entity responsible for the study: Susan Heavey's Laboratory, Department of Targeted Interventions, University College London.

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100P Study on the effect of ursolic acid on MMPs and antimetastatic activity in TNBC cells

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Background: The matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that degrade multiple components of the extracellular matrix. The significant role of MMPs in tumor invasion, neoangiogenesis and metastasis presented it as ideal pharmacological targets for cancer therapy. The effects of ursolic acid (UA) on matrix metalloproteinase (MMP) gene expression, cell invasion and cell migration were investigated MDA MB- 231 cell line.

Methods: Cytotoxicity of UA towards cancer cells was evaluated by MTT assay. MDA MB- 231 cells were cultured for 48 hrs with different concentrations (0, 5, 15 and 25μM) of UA and mRNA expressions of MMP-1, MMP-3, MMP-7, MMP-9 and MMP-11 were analyzed by real time PCR. Cell migration wound healing assay was done at 0, 6, 24, and 48 h periods for cell migration evaluation. To asses the cell invasion MDA MB-231 cancer cells were seeded on collagen based matrigel chamber and treated with 1, 5, 10 and 15μM UA, whereas untreated cells considered as control. After48 hrs cells invaded through matrigel quantified by colorimetric method.

Results: UA inhibited the proliferation of MDA MB -231 cells in a dose dependent manner and time dependent manner. Our results showed that UA significantly affect all MMPs except MMP-3 and 7. The expression of MMP 1, 3 and was reduced significantly 0.34, 0.11 and 0.68 fold in response to 25μM UA after 48hrs incubation in MDA MB-231 cells in comparison to control cells. Wound healing assay results exhibited that cells treated with 15μM UA significantly hindered the motility of these cells and 7.27% wound area remained unfilled after 48hrs. The cancer cells invasion was 89.75% at 25μM UA through matrigel after 48 hrs.

Conclusions: In conclusion, UA reduced the mRNA expressions of MMP-1, MMP-3 and MMP-9 in MDA-MB-231 cells. Ursolic acid also inhibited the invasion and migration of TNBC cells.

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101P Potential immune-oncological effect of liposomal-doxorubicin in breast cancer via tumor microenvironment alteration

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Background: After invasive ductal carcinoma, invasive lobular carcinoma (ILC) is the most frequent breast cancer and accounts for nearly 15% of all breast cancers (BC). Doxorubicin (DXR) is a very effective chemotherapeutic drug utilized to treat BC but induces drug resistance and severe side effects. Recently, liposomes have gained popularity as effective tumor microenvironment (TME) modulatory anticancer agents for various malignancies, including BC. This study propose a liposomal-DXR combination to produce an immune-responsive regulatory effect on competing endogenous RNAs (ceRNAs) through alteration in TME and investigate the immunological and anticancer impact of liposomal-DXR combination via regulation of ceRNAs and immune regulatory cytokines at BC cells.

Methods: MDA-MB cells were treated with varying concentration of liposomal-DXR combination and dose response curve was calculated. Total RNA was extracted and quantified by qRT-PCR and TNF-α ELISA kit. Computational target interaction was analyzed.

Results: Treatment resulted in a dose and time-dependent cell-viability and migration inhibition. Treatment altered the level of AKT1, ESR1, and ARID1A genes. Alteration in miR-17-5p compared to control cells indicated the alteration in ceRNAs network. TNF-α was found repressed in treated MDA-MB cells.

Conclusions: Present study propose the mechanism of liposomal-DXR as potential TME modulatory, immunoregulatory anti-ILC agent through altering ceRNAs circuit, AKT1, ESR1, and ARID1A genes, and immune regulatory TNF-α in BC cells.

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102P Regulation of the occurrence of mammary tumors in mice C3H/Sn by action steroid in early postpartum

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Background: Previously, we established that mice of the C3H/Sn line, which multiply intensively, develop mammary gland tumors in 100% of cases before the age of 12 months. In virgin mice of this line, tumors occur in no more than 30%. During pregnancy, the level of steroids is higher and obviously this indicates a more intensive activation of mutated genes by steroid hormones than in virgin mice. The role of hormones at more distant stages of development of the body and organs has been revealed. The possibility of preventing the occurrence of tumors is considered in this work.

Methods: Mice of the C3H/Sn and BALB/c lines became pregnant at the same time. Synchronously born C3H/Sn and BALB/c mice were given to BALB/c females for nursing. Some of the mice were given per os approximately 1 μl of a solution of estradiol and corticosterone at the dose of 10^{-9} M/L for 2 weeks.

Results: BALB/c mothers successfully nursed 15 C3H/Sn mice to puberty. After giving birth, the mice were divided into two groups: 1) intact - 7 pcs. 2) 8 pcs received a solution of estradiol and corticosterone (10-9 M/L) for 2 weeks once a day starting from the first day after delivery. No further action was taken on them. Adult mice were observed up to 3 generations. In 1 group of intact mice, mammary gland tumors were detected in 1 mouse out of 7. In the second group, in 6 out of 8. That is, treatment with estrogens in the early postnatal age leads to the manifestation of the effect in the distant future. It seems that the action of steroids can be biphasic: I — activation. II - is more strongly expressed during sexual maturation of animals, when the level of hormones increases. Such a mechanism of action in the case of unmutated genes ensures the development of mammary glands in children when they become adults. When mutated, it contributes to the development of cancer. Translation from laboratory experiments to the clinic can be based on the reverse scheme: strict limiting of the use of steroids with breast milk during the early postpartum period. Theoretically, no negative consequences are expected, except for breast hypoplasia.

Conclusions: The long-term effect of steroids in the early postnatal period on the development of mammary glands in the future state requires further study.

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103P The effect of carboplatin on sodium-dependent phosphate transporter NaPi2b expression in the OVCAR-4 ovarian cancer cell line

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Background: The platinum and taxane drugs as a neoadjuvant and adjuvant chemotherapy are the most used for treatment of ovarian cancer (OC). In later stages of ovarian cancer, targeted therapy with monoclonal antibodies may be included in the treatment regimen. Sodium-dependent phosphate transporter NaPi2b is a perspective target for the ovarian cancer treatment with monoclonal antibodies since the overexpression of NaPi2b was found in about 90% of the cases of OC. Currently NaPi2b-specific therapeutic monoclonal antibodies XMT-1536 (NCT03319628) and XMT-1592 (NCT04396340) are successfully undergoing clinical trials for the treatment of ovarian and lung cancers. Previously it was shown that NaPi2b protein abundance decreased in OC tumors of patients after neoadjuvant therapy mainly including

carboplatin, thus, the subsequent use the therapy of monoclonal antibodies might be not effective. Currently it is unknown which drug of the ovarian cancer neoadjuvant chemotherapeutic regimen is responsible for the decrease of the Napi2b protein abundance. Thereby, the aim of the research was to evaluate the effect of carboplatin on the NaPi2b mRNA and protein level in the OVCAR-4 ovarian cancer cell line.

Methods: The NaPi2b mRNA and protein level before and after carboplatin treatment of OVCAR-4 cells was determined by real-time PCR and Western-blotting respectively. The Statistical analysis was performed in GraphPad Prism software.

Results: Low abundance of NaPi2b protein was revealed in OVCAR-4 cells after the carboplatin treatment in IC25 and IC50 concentrations within 48 and 72 hours. At the same time NaPi2b mRNA level decreased after carboplatin treatment in IC25 and IC50 concentrations only within 72 hours. A weak correlation was observed between NaPi2b mRNA level and NaPi2b protein abundance in OVCAR-4 cells before and after the carboplatin treatment.

Conclusions: Thus, we revealed that carboplatin decreased the NaPi2b mRNA and protein abundance. The created model can be used to study the mechanisms underlying the regulation of NaPi2b gene expression. The NaPi2b level in ovarian cancer cells after neoadjuvant therapy may be a predictive marker for prescribing monoclonal antibodies therapy in patients with ovarian carcinoma.

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104P Development of a new generation of imatinib using structural biology techniques at ambient temperature

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Background: Chronic myeloid leukemia (CML) is a kind of blood cancer and most CML patients have associated with a chromosomal anomaly with the BCR-ABL fusion oncogene, which occurs as a result of translocation between the Abelson murine leukemia (ABL1) gene on chromosome 9 and breakpoint cluster region (BCR) gene on chromosome 22. Imatinib mesylate is the first small molecule developed to target the BCR-ABL fusion protein. Imatinib reduces BCR-ABL activity by binding to the inactive conformation of tyrosine kinases. Despite a high response rate in CML patients with imatinib therapy, almost one-third of patients still have an inadequate response to Imatinib. In other words, mutations in the imatinib-binding pocket or other regions of the BCR-ABL kinase result in various resistances to imatinib in CML patients. Therefore, there have revealed a need to develop a more potent new molecule with an imatinib function that is more resistant to mutations.

Methods: In this study, ABL kinase domain gene was purchased from Genscript Biotech. The gene was inserted to pET11a vector plasmid construct. The plasmid was transformed into E.coli, strain BL21 (Rosetta-2). Transformed E. coli were grown overnight on agar plates. The colonies were collected from agar plates and started large volume of culture in rich LB media. To be able to procure further purified protein, we were used Ni-NTA affinity chromatography. The purified protein solution was added to crystal screen conditions in Terasaki plates. Then, X-ray diffraction images were collected from the formed crystals in order to acquire the best 3D structure. These diffraction datas were collected from XtalCheck module (Rigaku Oxford Diffraction) at ambient temperature.

Results: We will have revealed the structures determined at ambient temperature and high resolution with the help of X-ray crystallography technique. Additionally, we will have re-evaluated structures and designed new target small molecule with approaching from the perspective of integrative structural biology.

Conclusions: The results propose that may developed a new generation of Imatinib that is more specific, high affinity and resistant to possible mutations to treat CML disease and improve patients' lives.

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105P RADseq for tumour mutation burden estimation and mutation signature analysis

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Background: Tumour mutation burden (TMB) is a biomarker for cancer immune checkpoint blockade (ICB) response, and mutation signatures provide a "life history" of tumour evolution. Researchers have repurposed cancer gene panels (CGPs) to cheaply estimate these parameters. The low coverage of CGPs, however, may be problematic in the exploration of TMB as a biomarker for ICB. Restriction enzyme associated DNA sequencing (RADseq) uses restriction enzymes to achieve coverage at random loci throughout the genome. It was hypothesised that RADseq could recapitulate mutation signatures and estimate TMB in breast cancer samples.

Methods: In silico RADseq libraries were generated by scanning the human genome for restriction enzyme cut sites and filtering for regions with adjacent cut sites. The resulting fragments were overlapped with mutations from whole genome sequencing (WGS) of breast cancer genomes. RADseq libraries were evaluated in terms of the accuracy of TMB estimation and mutation profile recapitulation, and compared to a CGP used for TMB estimation in terms of these parameters. A mouse breast cancer cell line was transfected with APOBEC enzymes, which are known to cause mutations in breast cancer, and profiled using RADseq.

Results: Using cosine similarity (CS) to the WGS profile as a measure of mutation profile recapitulation quality, RADseq libraries were able to recapitulate WGS mutation signatures, while the CGP performed relatively poorly in this regard (e.g. enzyme_1 CS=0.90 ± 0.06 vs CGP CS=0.28 ± 0.19). The CGP had higher TMB estimation error than an enzyme library of comparable coverage (CGP range 0.0034-8.83 mutations/mb vs enzyme_2 range 0.0029-7.50, Wilcox $p<0.001$). Using RADseq, an increased TMB and the APOBEC mutation signature was detected in a mouse cell line transfected with APOBEC3A, both of which were absent in control cell lines.

Conclusions: CGP methods do not accurately capture "genome-wide" mutation parameters such as TMB and mutation signatures. This warrants investigation into whether these methods can be used in TMB estimation in ICB trials. By comparison, RADseq may offer a cheap, effective solution for TMB estimation. RADseq may also be of use to researchers seeking to study the evolution of mutation signatures in model systems, as we have demonstrated.

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106P Ultra low-coverage whole genome sequencing for precision oncology in solid tumors

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Background: Copy number alterations (CNAs) are common genetic features in cancer with prognostic and therapeutic implications. At our institutional Molecular Tumor Board we introduced an ultra low-coverage Whole Genome Sequencing (ulcWGS, <0.5x coverage) to estimate numerical karyotype and CNAs. We performed this technique on 128 solid tumor samples from 2018 to 2022. Here we report 3 exemplary cases in which we detected potentially targetable CNAs via ulcWGS.

Methods: DNA was extracted from microdissected Formalin-Fixed, Paraffin-Embedded tissue slides. ulcWGS libraries were prepared using the NEBNext Ultra II Kit (New England Biolabs, Ipswich, MA, USA). Sequencing was performed on an Illumina MiSeq Instrument and raw reads were analyzed with a proprietary read-depth based software developed recently for calculated karyotyping of Acute Myeloid Leukemia. We used immunohistochemistry (IHC) and targeted sequencing (VariantPlex, ArcherDX/Invitae, Boulder, CO, USA) (VP) to validate CNAs.

Results: In patients reported in the table ulcWGS uncovered amplifications (amp) in FGFR1, ERBB2 and MDM2, respectively. In patient 1, IHC staining confirmed moderate to high (3+) membrane positivity for FGFR1, despite negativity of VP analysis. In patients 2 and 3, high-level amplifications were also confirmed via VP. Due to unavailability of clinical trials at the time of referral, just patient 2 received a matched therapy, particularly off-label trastuzumab deruxtecan (T-Dxd). At a 6-month follow-up, the patient showed a sustained stable disease on imaging and a tumor marker decrease.