Material and methods We have designed an interventional project included in the HoTBreast trial: the extended use of HT as an NA for breast cancer patients who refuse surgery or are considered inadequate to receive it, in the context of close surveillance. In this group of extended NA treatment patients, different hormone treatment strategies (aromatase inhibitor and tamoxifen) are allowed to be used, depending on tumour response and patient quality of life. During treatment, clinical and imaging (ultrasound) follow-up will be implemented every two to three months in the first 3 years and every four to six months after that. The treatment option can be changed at any time if considered appropriate, based on disease response or progression and the patient’s quality of life. This group will be randomly divided into two subgroups, one who will receive hormone treatment alone and a second one who will receive HT and aspirin. Aspirin plays the role of an anti-inflammatory controller and it is not clear what the relation between chronic inflammation processes and breast cancer is – this is a new approach for cancer treatment with a strategy targeting the tumour microenvironment. Our secondary objective is to understand if the local disease presents the same response as the systemic one (in cases where this also exists) or if it can be used as an independent predictor of disease behaviour.

Results and discussions We believe that HoTBreast Trial can give some answers about the best practice for treating early BC. We also expect that it can shed some light on the question of the role of surgery in early BC and the use of aspirin in BC patients. We acknowledge the hypothesis that aspirin can improve the treatment of BC patients in a safe, well tolerated and inexpensive way.

Conclusion Efforts should be made to better communicate to patients that NA HT is a valid option and sometimes the most appropriate for the specific patient. The decision should depend on the patient, based on an informed understanding of the benefits and risks of avoiding surgery.

**PO-259**

**IDENTIFICATION OF A CLINICALLY MEANINGFUL SITE-SPECIFIC STEROID ROADMAP IN PROSTATE CANCER.**

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Introduction In prostate cancer the genotype of the tumour does not always predict clinical behaviour and disease aggressiveness after castration therapy. Additional factors might be important. Here we hypothesise that there is a site-specific steroid profile roadmap after castration that is of clinical significance.

Material and methods Steroid profiles in blood plasma (BP) and bone marrow aspirate plasma (BMA) from 120 castrated patients with prostate cancer were evaluated with mass spectrometry. We studied the steroid ligand-receptor interactions in prostate cancer adrenal metatases (PCAM) because they represent a steroid-rich environment. Adrenalectomy specimens from 3 castrated patients with PCAM were analysed.

Results and discussions It is known that normally BP/Adrenal vein plasma testosterone (T) concentration ratio is approximately 1:1 and cortisol (C) ratio is roughly 1:3. We measured similar T levels in BP and BMA. The levels of C were significantly lower in BMA compared to BP. Our data suggest that T levels are uniform but there is a C concentration gradient between the different compartments. Cortisol acts through GR and in the presence of T is a partial AR inhibitor.

All PCAM samples had functional androgen receptor (AR) (wild-type (wt), no copy number changes) and glucocorticoid (GR), but no progesterone receptor (PR). No DHT was detected. Patient 1 tumour was able to synthesise T and had above castrate intra-tumoral T levels. The tumour lacked enzymes to synthesise, but was able to catabolize C. The levels of C were within the normal BP range. Gene expression of AR was normal. Patient 2 and 3 samples had castrate T and high C levels (3-fold BP), but AR gene expression was remarkably high. All patients had PTEN and RB deletions, while patients 2 and 3 had mutant (m) p53.

Addition of 40 ng/dl T increased growth in vitro in a xenograft PCAM model (propagated in matrigel) derived from patient 1. When C was concomitantly added at high levels such as those in patients 2 and 3, PCAM growth returned to baseline (no steroids), suggesting that almost all T or C activity were lost. Moreover, proliferation in VCAP cells (High AR/GR+) decreased when high C was added in low T, but remained well above baseline, generating the hypothesis that this steroid combination mostly inhibits AR/GR tumours with non-amplified AR (e.g. early in castration).

Conclusion There is a clinically meaningful site-specific steroid roadmap. The PCAM steroid profile may serve as a useful model to elucidate tumour interactions with the steroid environment.

**PO-260**

**DEEP PHENOTYPING OF COLORECTAL CANCERS BY HIGH-DIMENSIONAL MASS CYTOMETRY REVEALS TUMOR-SPECIFIC IMMUNE LANDSCAPES.**

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Introduction Immune checkpoint blockade has revolutionised cancer treatment. However, clinical outcomes are highly variable as only a proportion of cancer patients benefit from this treatment. To understand the mechanisms that determine responses to current immunotherapies and for the design of alternative approaches, it is crucial to characterise the immune landscape of the tumour microenvironment. An in-depth understanding of anti-tumour immunity requires a comprehensive analysis of the immune cell populations that participate in the process of tumorigenesis. The aim of this study is to unravel local and systemic profiles of lymphoid and myeloid immune subsets in colorectal cancer (CRC) using high-dimensional immunophenotyping by mass cytometry.

Material and methods The expression of 36 immune cell markers was simultaneously assessed at the single-cell level by mass cytometry in tumour tissues (n=19), tumor-associated lymph nodes (n=17), adjacent normal mucosa (n=4), and peripheral blood samples (n=9) derived from 18 CRC patients. In total, 9 million cells were included in the analysis. Cytoscore and HSNE (Hierarchical Stochastic Neighbour
Embedding) analysis tools were used to identify and visualise the composition of the lymphoid and myeloid cell populations of the innate and adaptive immune compartments in the tissues.

**Results and discussions** We identified 220 immune cell subsets, including tumor-resident CD8+CD45RO+CD103+PD-1+, CD4+CD45RO+CD103+PD-1+ and CD4+CD45RO+CD103+PD-1+ T cell populations that were not found in tumour-associated lymph nodes, adjacent normal mucosa, and peripheral blood samples of CRC patients. In addition, we identified an overrepresentation of CD3+CD7+CD56+CD45RO+CD127+ intermediate innate lymphoid cells in mismatch-repair deficient cancers and an enrichment of CD14+CD45RO+CD103+PD-1+ myeloid populations in mismatch-repair proficient cancers. Unsupervised clustering of the samples based on the composite immune profile separated the aforementioned groups.

**Conclusion** High-dimensional immunophenotyping of colorectal cancers reveals tumor-specific immune signatures and charts the complexity of innate and adaptive immune cell populations in the tumour microenvironment. Furthermore, previously unappreciated immune cell subsets further differentiate the two main pathways of tumorigenesis in this cancer type. This work may support the delineation of alternative and tailored immunotherapeutic approaches to improve treatment in colorectal cancer.

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**PO-261**

**MULTIMODAL MASS SPECTROMETRY IMAGING TO UNDERSTAND DRUG DELIVERY, METABOLISM, RESPONSE AND AMP, RESISTANCE IN PANCREATIC DUCTAL ADENOCARCINOMA.**

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**Introduction** The desmoplastic Tumour MicroEnvironment (TME) in Pancreatic Ductal Adenocarcinoma (PDA) plays a key role in drug delivery, metabolism and resistance; drug interventions in turn regulate tumour metabolism. Mass Spectrometry Imaging (MSI) techniques may provide information about regional tumour metabolic profile and drug delivery to enhance our understanding of TME heterogeneity and its impact on drug efficacy and resistance.

**Material and methods** Multimodal MSI acquisition of the tissue distribution of gemcitabine, the ATR inhibitor AZD6738, their metabolites and the endogenous metabolome was performed in a KPC GEMM mouse model of PDA.

Targeted analysis of the compounds and their metabolites were used to evaluate drug delivery. Small molecule quantification including haem and metabolic markers such as ATP/ADP/AMP, were used together with H and E and staining for known molecular biomarkers including Pan-CK and αSMA to characterise tissue architecture.

Untargeted analyses including statistical identification of discriminative or colocalised metabolites were used to identify de novo endogenous metabolite biomarkers and cellular phenotypes driving tumour heterogeneity.

**Results and discussions** MSI revealed significant intratumoural heterogeneity of drug delivery and drug metabolism. Highest delivery of the parent compounds (AZD6738 and dFdC) were found to colocalise with haem in areas confirmed histologically to be necrotic and haemorrhagic. dFdC metabolism appeared related to TME metabolic heterogeneity. The active and inactive metabolites of gemcitabine (including dFdCTP and dFdU) demonstrated differential distribution, both from the parent compounds and each other in the tissue.

Unsupervised clustering based segmentation and colocalisation analysis of the MSI metabolomic data enabled identification and characterisation of these distinct tissue regions based on similarities in their metabolic profile.

**Conclusion** We have shown that MSI allows spatial resolution of drug delivery, metabolism and that MSI-based metabolomics analysis enables detection of greater tumour heterogeneity than visible by traditional pathology methods such as H and E. Combining this with biomarker information from Imaging Mass Cytometry (IMC) may enable identification of the cell types and phenotypes responsible for the differential metabolic effects observed with combination therapies. Innovative, information-rich technologies such as IMC and MSI may drive greater understanding of the impact of tumour heterogeneity on drug efficacy *in vivo* and ultimately in patients.

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**PO-262**

**REMOELLING OF THE TUMOUR MICROENVIRONMENT BY PEGVORHYALURONIDASE ALFA (PEGPH20): A NOVEL, FIRST-IN-CLASS BIOLOGIC THAT ENZYMICALLY DEGRADATES TUMOUR HYALURONAN (HA) TO IMPROVE ANTI-TUMOUR EFFICACY**

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**Introduction** Hyaluronan (HA) is a naturally occurring glycosaminoglycan that can accumulate in the tumour microenvironment (TME). In pancreatic and other cancers, high levels of HA are associated with poor clinical outcome. In mouse models, high levels of HA in the TME can increase interstitial fluid pressure and compress tumour vasculature, thereby impairing delivery of anti-cancer therapeutics. Here, a novel, first-in-class biologic that enzymatically degrades HA, PEGPH20, increased tumour vascular volume (VV) and improved anti-tumour efficacy when combined with chemotherapy or checkpoint inhibitor antibodies in multiple HA-accumulating tumour models.

**Material and methods** Various human and mouse cancer cell lines were transduced with hyaluronan synthase 3 (HAS3) to increase HA production, and implanted in the mammary fat pat (4 T1/HAS3) or adjacent to the tibial periosteum (all other lines). Tumour VV was measured by high resolution ultrasound coupled with micro-bubbles (A549/HAS3 and WT-CLS1/HAS3), or IHC (CT26/HAS3), following PEGPH20 treatment (0.0375 mg/kg or 1 mg/kg, IV, 2qw). For BxPC3/HAS3 studies, mice were dosed with vehicle or PEGPH20 (4.5 mg/kg, IV, 2qw)±NAB, paclitaxel (NAB, 10 mg/kg, IV, 2qw)±gemcitabine (GEM, 180 mg/kg, IP; 24 hour after PEGPH20 ±NAB, qw). For AsPC1/HAS3 studies, mice were dosed with vehicle or PEGPH20 (0.0375 mg/kg, IV, 2qw)±NAB, paclitaxel (NAB, 10 mg/kg, IV, 2qw)±GEM (180 mg/kg, IP; 24 hour after PEGPH20 ±NAB, qw). For checkpoint inhibitor studies (CT26/HAS3, 4 T1/HAS3, Pan02/HAS3), mice were dosed...