antibody response. The search for tumor-specific targets among membrane proteins is important for the development for new effective anticancer drugs because membrane proteins represent 60% of drug targets. Transmembrane proteins in cancer cells exhibit an altered pattern of post-translational modifications and generally contain a large extracellular domain with multiple glycosylation and sulfhydryl sites with unknown function. We analyze the features of membrane protein which are required for recognition by monoclonal antibodies using the sodium-dependent phosphate transporter NaPiIIb as a model. The EMD4 is a 250-360 amino acid extracellular domain with multiple sulfhydryl sites. The 4 cysteines and 6 asparagines have been substituted EMD4 by 36P Using site-directed mutagenesis. The epitope recognition and ability to take up corresponding to this domain.

Methods: We used an ex vivo differentiation system for MDSCs and TAM by modelling the tumour microenvironment from C57BL/6j mouse bone marrow cells in conditioning medium (1). We also differentiated resting macrophages (M0) as controls using standard techniques. Flow cytometry and microscopy confirmed their phenotype by assessing their morphology and presence of characteristic lineage markers. Three global experiment of mass spectrometry-based quantitative (shotgun) proteomics were performed. Construction of functional interactions from up- or down-regulated proteins was conducted with the Ingenuity Pathway Analysis (IPA) Tool from Qiagen. Targeted proteins were evaluated using western blot. Bibliography: J) Liechtenstein T, Perez-Janices N, Gato M, et al. Oncotarget. 2014;5(17):7843-7857.

Results: We observed morphological differences in ex vivo differentiated myeloid populations. High-throughput proteomics uncovered protein expression patterns characteristic of populations modelling tumour-infiltrating subsets, result of the tumour microenvironment pressure. MAPK1 was identified as upstream regulator using IPA tool. We identified upregulation of STAT3 phosphorylation in all tumor-associated subsets. Both TAM and MDSC have strongly upregulated phosphorylation on S727. Moreover, strong increase in STAT3 Y705 phosphorylation was observed in TAM versus both monocyct and granulocytic MDSC.

Conclusions: In the present study, we identified differences in proteomic signatures between myeloid cells modelling M-MDSC, G-MDSC and TAMs related to lineage, and cancer-driven polarization.

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constrained and glycosylated tumor-specific conformationally exposed epitope within NaPi2b MD4D representing an excellent targets for specific therapeutic antibodies.

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27P Oxidized low-density lipoprotein induces cell death and inflammation in cardiomyocytes exposed to nivolumab by TLR4/NF-κB and NLRP3/myd88 pathways

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Background: Oxidized low-density lipoprotein (Ox-LDL) is oxidatively modified form of LDL and it contributes to atherosclerotic plaque formation and progression. Recent findings reported that cardiovascular events (myocarditis and atherosclerosis) were higher after initiation of immune check-point inhibitors (ICIs), potentially mediated by accelerated progression of atherosclerosis. Optimization of cardiovascular risk factors and increased awareness of cardiovascular risk, prior to, during and after treatment, should be considered among patients on an ICi. We evaluated whether ox-LDL-induced apoptosis depends on in part on the activation of NF-κB-signaling pathway and NLRP3 during exposure of cardiomyocytes to nivolumab.

Methods: Human fetal cardiomyocytes (HFC cell line) in co-culture with hpBMC, were exposed to a clinically relevant concentration of nivolumab (100 nM) alone or combined with Ox-LDL at 1, 10 and 50 μg/mL for 24h. After the incubation period, we performed the following tests: determination of cell viability, through analysis of mitochondrial dehydrogenase activity, study of lipid peroxidation (quantifying cellular malondialdehyde and 4-hydroxynonenal), intracellular Ca²⁺ homeostasis and apoptosis. Moreover, pro-inflammatory studied were also performed (activation of NLRP3 inflammasome, expression of TLR4 and NF-κB). In order to evaluate the pathways involved in Ox-LDL damage, TLR4 and NLRP3 inhibitors (TAK-242 and dapsanurile, respectively) were added during cell viability and apoptosis studies.

Results: Nivolumab exerts cytotoxic and pro-apoptotic effects in co-culture of cardiomyocytes and hpBMC. Ox-LDL increases nivolumab-induced cardiotoxicity in a manner that is sensitive to TLR4 and NLRP3. Incubation of cardiomyocytes with Ox-LDL (10 and 50 μg/mL) for 24 hours increased TNF and NF-κB expression significantly. Ox-LDL had pro-apoptotic effects in a concentration-dependent manner with the involvement of lipid peroxidation but not of intracellular calcium.

Conclusions: Ox-LDL exacerbates apoptosis and inflammation in cardiomyocytes during exposure to nivolumab turning the light on their role in ICi-induced cardiotoxicity.

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28P Diagnosis of early-stage carcinoma through exosomal FOXP3 detection

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Background: Early diagnosis of cancer is imperative to stop the spreading of the disease and for the long-term survival of the patients. Focus on detecting symptomatic patients as early as possible gives the best opportunity for successful treatment. But the failure to detect the rare cancer stem cells (CSCs) population that uniquely initiates and sustains the disease poses a major challenge to early tumor detection. Recent reports indicate the contribution of exosomes, secreted by CSCs to orchestrate the formation and maintenance of tumor-promoting microenvironment.

Methods: Breast CSCs (BCSCs) were purified from breast cancer cell lines and human breast tumor tissues following which CSC-derived exosomes (CDEs) were isolated. CDEs were isolated from the tumor blood samples and characterized through DLS, AFM, and western blot analysis. CDE-FOXP3 was detected through flow cytometry, western blot, and mass spectrometry analysis.

Results: We observed a higher level of FOXP3 expression in MDA-MB-468 CDE-derived exosomes (CDEs) as compared to exosomes isolated from non-tumorigenic MCF10A derived stem cells. Interestingly, CDEs isolated from tumor patients also showed a comparable FOXP3 expression. A strong positive correlation between CSC percentage and CDE-FOXP3 expression in tumor patients was also observed. Furthermore, we report significant elevation of CDE-FOXP3 in peripheral blood of 18 clinically diagnosed breast cancer patients compared to 19 healthy individuals in a randomized, open-label, case-control study. The clearly detectable non-overlapping ranges of CDE-FOXP3 in breast cancer patients and healthy cohorts highlight the potential of CDE-FOXP3 to be a low-cost blood-based detection marker eligible for a phase-I clinical study.

Conclusions: Detection of CDE-FOXP3 in the peripheral blood of breast cancer patients will allow us to detect CSCs in a non-invasive method. Its expression is distinguishable from healthy donors due to clearly definable ranges and remains unchanged even during chemotherapy, suggesting future prospects of the same in the application as an early diagnostic marker. Collectively our data demonstrate that detecting CDE-FOXP3 can become a quick non-invasive way to diagnose and screen early-stage breast cancer in a cost-effective manner.

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29P All Wales Medical Genomics Service: Implementation of a comprehensive DNA and RNA next generation sequencing panel to transform Welsh cancer diagnostics for solid and haematological cancers

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Background: The All Wales Medical Genomics Service (AWMGS) provides cancer genomic testing (solid tumours and haematological cancers) to all NHS Wales Health Boards, serving a population of 3.5 million people, using multiple NGS panels and a range of other genomic assays e.g. FISH, ddPCR. As part of a continuous service improvement programme, the existing testing strategy was evaluated and a redesign was proposed to enable standardisation of the technical and analysis pathways, ensuring scalability and future-proofing of this rapidly expanding service. To support the proposed redesign, suppliers were invited to tender for the procurement of a comprehensive somatic NGS panel for sequencing on the Illumina NovaSeq 6000TM.

The chosen solution (Illumina TruSight Oncology 500) met the required tender scope and additionally indicated significant improvements to the proposed service may be achieved by facilitating the detection of structural variants (SVs), single nucleotide variants (SNVs) and copy number variants (CNVs) using a single workflow.

Methods: Developing the new service required the implementation of additional IT infrastructure, a new bioinformatics solution, in-house validation of the Illumina TSO500 panel and the development of novel reporting pathways.

Results: The Illumina TSO500 panel test performance was measured against a comprehensive set of key performance indicators, and importantly, results were compared to ongoing AWMGS standard of care diagnostic NGS panel results to ensure concordance. Results presented will include the assessment of the panel to detect SVs, SNVs and CNVs at a range of DNA and RNA input concentrations for multiple sample types, including formalin fixed paraffin embedded tissue (FFPE), bone marrow samples and peripheral blood samples.

Conclusions: The redesign of the existing solid tumour and haem services has involved joint working between NHS, academic and commercial partners. Validation results have shown that the new service can introduce significant diagnostic service improvements that will facilitate the delivery of an efficient rapid genomic testing service for cancer patients in Wales.

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