Summary/Conclusions: Our global protein expression database for CLL cells is thus the most complete database of its type. By combining advanced computational biology to relate protein expression to whole genome sequencing data, the database will provide a framework for elucidating the effect of genomic alterations on protein expression. The construction of this comprehensive CLL database also provides the platform for applying SWATH/MS-based analysis of individual patient samples to identify novel drug targets and predictive biomarkers of disease progression and treatment response.

E1023

THE IMPACT OF THE TUMOUR MICROENVIRONMENT ON B-CELL RECEPTOR (BCR) EXPRESSION AND SIGNALLING IN CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

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Background: Chronic lymphocytic leukaemia (CLL) is thought to be driven following antigen engagement of the B cell receptor (BCR). This has been highlighted further by the BCR Kinase inhibitors Ibrutinib and Idelalisib, which have proved instrumental in the treatment of CLL. However, these inhibitors appear to only suppress the disease without being curative. A number of patients have developed resistance to ibrutinib following mutation of the *BTK* or *PLCγ2* gene, whilst other patients are unable to tolerate these drugs due to adverse events or progress whilst on therapy for unknown reasons. B-cell activating factor (BAFF) and interleukin-4 (IL-4) are known to increase CLL cell viability *in vitro*, and are thought to be present within CLL lymph nodes. Therefore we have investigated the role of BAFF and IL-4 in regulating BCR signalling in CLL cells and promoting resistance to ibrutinib and idelalisib.
Aims: To understand how IL-4 and BAFF regulate BCR pathway inhibition by ibrutinib and idelalisib.
Methods: Experiments were carried out using primary CLL cells. All isolates used contained >85% $CD19^+CD5^+$ cells. Protein expression was evaluated by flow cytometry or immunoblotting and signalling capacity was detected by calcium flux analysis in response to soluble αIgM by flow cytometry. Cell viability was assessed using annexin v/propidium iodide staining.
Results: As previously shown CLL cells treated with BAFF (500 ng/ml) or IL-4 (10 ng/ml) only for 48h significantly protected against basal apoptosis. This anti-apoptotic effect was further augmented when cells were treated with BAFF and IL-4 in combination. CLL cells treated *in vitro* with ibrutinib and idelalisib induced approximately 24.3% and 28.8% apoptosis respectively following treatment for 48h. However, apoptosis induced by the BCR-kinase inhibitors was reversed following pre-treatment with IL-4 or BAFF. CLL cells incubated *in vitro* with IL-4 for 24h significantly augmented sIgM expression and αIgM induced phosphorylated ERK and calcium flux. These IL-4 induced effects on sIgM expression were more prominent in Unmutated(U)-CLL samples, and could be reversed using the JAK3 inhibitor tofacitinib (CP-690550). Whilst, IL-4 had little effect on sIgD. Interestingly pre-treating CLL cells with IL-4 significantly reversed the ability of ibrutinib (1 μM) and idelalisib (1 μM) to inhibit αIgM-mediated signalling at 24h. In contrast BAFF treatment showed no clear effect on sIgM expression and downstream signalling. However, in a proportion of CLL samples treated simultaneously with BAFF and IL-4, BAFF reduced IL-4 specific increases in sIgM augmentation. Next we pre-treated CLL cells with tofacitinib (10 μM) for 1 hr prior to IL-4 treatment. Tofacitinib significantly reversed the effects observed with IL-4 and restored the ability of ibrutinib and idelalisib to inhibit αIgM induced signalling. This effect was specific to the inhibition of IL-4 since tofacitinib (≤10 μM) did not induce CLL cell death. Finally we showed that BAFF, and IL-4 to a greater extent, resulted in an increase in miRNA155 expression which has previously been shown to be associated with more progressive disease and enhanced BCR signalling.
Summary/Conclusions: Our data suggests that IL-4 augments sIgM recovery and down-stream signalling in CLL, which may compromise the effectiveness of BCR kinase inhibitors. However these effects were not observed for BAFF. Furthermore, co-treatment of BCR-kinase inhibitors in combination with tofacitinib may indicate a promising treatment strategy for the treatment of CLL.

E1024

INHIBITION OF USP7 INDUCES SELECTIVE CANCER CELL DEATH IN CHRONIC LYMPHOCYTIC LEUKEMIA THROUGH PTEN AND INDEPENDENTLY FROM P53 STATUS

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Background: Standard immune-chemotherapy allows to achieve 60-70% response rate in CLL patients, but complete remission remains uncommon. Unsatisfactory therapeutic options still exist for higher risk groups, and in particular those harboring *TP53* mutations or deletion.
Aims: We propose USP7, a known de-ubiquitinase with multiple roles in cellular homeostasis, as a potential therapeutic target in CLL.
Methods: Primary CLL cells were enriched in $CD19^+$ fraction using the Milteny anti-CD19 kit and were used to investigate USP7 levels. P5091 (USP7 inhibitor) efficacy was tested in CLL cell lines and patients. Proliferation and apoptosis were evaluated respectively with CTG technology and Annexin V-FITC/Propidium PE detection by flow cytometry. The specificity of the USP7 inhibitor was verified with a pool of 5 different siRNAs able to efficiently silence USP7.
Results: Our data show that the de-ubiquitinase USP7 is aberrantly expressed in CLL. In particular, USP7 is over-expressed in about 70% of CLL $CD19^+$ lymphocytes, both at the mRNA and protein levels. We also analyzed USP7 expression levels in an expansion cohort of a publicly available CLL patients (n=217) and 12 normal samples, where USP7 was over-expressed in CLL when compared to normal samples (****p<0.0001). We proved that USP7 is regulated at post-transcriptional level by miR-338-3p and functionally activated by Casein Kinase 2 (CK2) through phosphorylation at serine 18 residue. USP7 inhibition by P5091 as well as by specific siRNA, induces cell growth arrest and apoptosis mediated by the restoration of the nuclear pool of PTEN. Notably in primary CLL cells, P5091 treatment strongly promoted apoptosis. Moreover, USP7 inhibitor treatment of primary CLL was associated with increases ubiquitination of endogenous PTEN and consequently PTEN relocalization into the nucleus. Strikingly, TP53 deleted CLL samples and cells lines were equally subject to P5091 apoptosis induction, confirming that the USP7 inhibitor acts in a p53 independent manner. These data showed potent activity of P5091 against primary CLL.
Summary/Conclusions: We demonstrate the efficacy of a small molecule inhibitor of USP7, P5091, *in vitro* in cell lines and *ex vivo* in primary CLL samples in a P53-independent manner. Our preclinical study therefore, supports the evaluation of USP7 inhibitor as a potential CLL therapy.

E1025

DIRECT AND INDIRECT MODULATION OF MACROPHAGES IN THE TUMOR MICROENVIRONMENT UPON GENOTOXIC STRESS ALTERS PHAGOCYTIC FUNCTION

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Background: Resistance against chemotherapy is a central problem in the current treatment of B cell malignancies. Up until now, little is known about the impact of the tumor microenvironment on therapeutic outcome. This microenvironment consists of many different kinds of cells varying between tumors, significantly manipulating malignancy and enabling relapses after treatment. Macrophages are present in the microenvironment of most tumors-designated as tumor associated macrophages (TAM)-and are often correlated to a poor prognosis. We postulate that macrophages and their reprogramming are an essential element in the malignant progression of tumors and their response to DNA damage as occurring in chemotherapeutic treatment and are a promising target to improve therapy response.
Aims: We aim to define the functional characteristics of macrophages in their cross talk with malignant B cells and characterize their role for disease progression and antitumor therapy. Our particular interest lies on the investigation of the functional mechanistic and kinetics underlying macrophage repolarisation. Here, we intend to identify key regulators and their upstream signaling pathways by focusing on the DNA damage pathway.
Methods: To assess macrophage leukemia cell interaction we established *in vitro* and *ex vivo* co-cultures of macrophages and leukemic cells, specifically investigating macrophage antibody dependent cellular phagocytic (ADCP) capacities. Leukemic cell clearance and macrophage phenotype characterization are determined by flow cytometry. We addressed both primary CLL patient cells as well as humanized mouse model of BCL2/MYC double hit lymphoma.
Results: Functionally we prove that low dose application of the *in vitro* equivalent of Cyclophosphamide, Mafosfamide significantly increases the phagocytic function of macrophages. Besides this direct effect on macrophages, a combination of cyclophosphamide and alemtuzumab was demonstrated to induce a macrophage based increase in tumor clearance by cytokine secretion of stressed malignant B cells. Here, we could determine IL10 as an inducer of increased phagocytosis in tumor associated macrophages. Moreover we show that IL10 modulates differentiation of macrophages which strongly increases their ADCP compared to classical M1/M2 differentiated macrophages. Analyzing the functional contribution of the DNA-damage pathway we could identify pronounced secretion of IL10 in p53-deficient leukemia cells. We show *in vitro* that the DNA damage pathway plays an important role in the ASAP mechanism as a down regulation of various DNA damage key players in the leukemic cells diminishes the stimulating effect of Cyclophosphamide and antibody combinations.
Summary/Conclusions: Repolarisation of macrophages towards increased phagocytosis is essential in immunochemotherapies. We demonstrate that the DNA damage pathway is important for this stimulating effect and that the mech-