dendritic, T, NK and B cells and hereby, suppresses anti-cancer immunity in a multifold manner. Blocking the HLA-G checkpoint reverses this immune suppression and e.g. shift macrophages from a tumor-supportive (M2) towards a tumor-suppressive (M1) phenotype. To avoid off target activity, DSP216 is designed to binds cells in an ‘AND gate’ fashion, which means that DSP216 should not or minimally bind to cells that express only CD47 or HLA-G, but should bind strongly to cells that express CD47 AND HLA-G due to enhanced avidity. Here, we tested if DSP216 follows this unique binding behavior and if DSP216 can prevent HLA-G mediated polarization of M0 macrophages to M2 macrophages.

Materials and Methods Binding of DSP216 to HLA-G+ CD47+ cancer cells was tested by flow cytometry. The binding was blocked with CD47 and HLA-G blocking antibodies to test if the binding was specific. Undesired binding of DSP216 to red blood cells (RBCs) and PBMCs was tested by flow cytometry. The effect of DSP216 on the polarization of M0 macrophages co-cultured with HLA-G+ CD47+ cancer cells was tested by monitoring the expression of CD163, CD206 and HLA-DR by flow cytometry.

Results DSP216 bound dose-dependently to HLA-G+ CD47+ cancer cells and blocking the binding with HLA-G or CD47 blocking antibodies showed that binding was specific to CD47 and HLA-G. When DSP216 was mixed with RBCs and PBMCs, it didn’t bind to single positive CD47+ RBCs and bound very weakly to CD47+ PBMCs. These experiments show that there is indeed strong binding through enhanced avidity when both targets, HLA-G and CD47, are present and weak binding if CD47 but no HLA-G is expressed. In co-culture of M0 macrophages with HLA-G+ CD47+ cancer cells, DSP216 prevented HLA-G induced upregulation of the M2 specific markers CD163 and CD206 and induced expression of the M1 specific marker HLA-DR. As tumor associated macrophages (TAMs) with an M2 like phenotype play an important role in the creation of an immunosuppressive tumor microenvironment, this is a first indication that DSP216 can inverse an immune suppressive tumor microenvironment.

Conclusions DSP216 binds exclusively to HLA-G+ CD47+ cells and not to single positive cells, like RBCs and PBMCs. DSP216 prevents HLA-G mediated polarization of M0 macrophages to M2 macrophages and instead promotes polarization to M1 macrophages. DSP216 is a novel bifunctional Fc-fusion therapeutic that has the potential to reverse tumor suppressive signaling by tumor associated macrophages and unleash the anti-tumor activity of various cells from innate and adaptive immune response.

Disclosure Information L.J. Jacob: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; KAHHR medical. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; KAHHR medical. F. Consultant/Advisory Board; Significant; KAHHR medical.

P01.07 GALECTIN 9 DEPENDENT IMMUNE EVASION MACHINERY AS A POTENTIAL TARGET FOR PERSONALISED IMMUNOTHERAPY OF CANCER

W Sumbayev*, 1Y Yasinska, 1S Schlichtner, 1GS Lall, 1E Fader-Kan, 1BF Gibbs, 1University of Kent, Chatham Maritime, UK; 2University Hospital Bern (Inselspital), Department of Paediatric Surgery, Bern, Switzerland; 3University of Oldenburg, Oldenburg, Germany

Background Cancers affect a large number of people worldwide and sometimes have mortality rates of over 90% (pancreatic ductal adenocarcinoma (PDAC), high grade glioblastomas). Currently, immunotherapeutic strategies fail in number of cancer cases due to a lack of understanding of the molecular mechanisms operated by these cancers to evade immune surveillance. Recent evidence demonstrated that the protein galectin-9 is highly expressed in majority of cancer cells. Importantly, this protein is a crucial part of the immune evasion machinery operated by human malignant tumours. Understanding the biochemistry of this immune evasion machinery is required for designing highly efficient and affordable personalised targeted immunotherapy of human cancers.

Materials and Methods We used, glioblastoma, PDAC, breast and renal cancer cell lines as cancer cell models. Jurkat T lymphocytes, TALL-104 cytotoxic T cells and primary human and mouse CD3-positive T lymphocytes were employed for immune evasion studies. C57 BL16 mice were used for in vivo studies. Western blot analysis, on-cell and in-cell Western, ELISA, qRT-PCR, chromatin immunoprecipitation (ChIP) assays as well as a variety of biochemical tests were used to conduct the studies.

Results We found that T cells trigger galectin-9 expression and secretion (in most cases requires Tim-3 protein (T cell immuno-noglobulin and mucine domain containing protein 3) in a variety of human cancer cells including but not limited to glioblastoma, PDAC, breast and renal cancers. Galectin-9 triggers exhaustion and often death of cytotoxic T cells. Other immune checkpoint proteins including VISTA (V-domain Ig-containing suppressor of T cell activation) and PD-L1 (ligand of programmed cell death protein 1) promote apoptotic death of cytotoxic T cells induced by galectin-9. Activity of T helpers, required to induce cytotoxic T lymphocytes is also suppressed by galectin-9 in cooperation with VISTA and PD-L1. We discovered that expression of all these immune checkpoint proteins is differentially controlled by the autocrine transforming growth factor beta type 1 (TGF-beta)-Smad3 pathway as well as several types of interferons.

Conclusions The immune evasion machinery triggered by TGF-beta-Smad3 and interferon pathways and operated by galectin-9, Tim-3, VISTA and PD-L1 should be considered as a potential target for personalised immunotherapy of cancer. Importantly, blood and tumour sample tests could help mapping the exact immune evasion networks employed by each tumour and the pathways responsible to produce respective immune checkpoint proteins. As such, personalised targeted
immunotherapy could be adapted. Further investigations required to map the immune evasion machinery for each type of cancer and design the best targets for personalised immunotherapy of human malignancies.

Disclosure Information V.V. Sumbayev: None. I. Yasinska: None. S. Schlichtner: None. G.S. Lall: None. E. Fasler-Kan: None. B.F. Gibbs: None.

P02 Tumor microenvironment and microbiome in immunotherapy

A BIOINFORMATIC ANALYSIS: THE OVEREXPRESSION AND CLINICAL SIGNIFICANCE OF HVEM IN LIVER HEPATOCELLULAR CARCINOMA

Background The occurrence and development of liver cancer is related to the immune evasion caused by abnormal expression of immune costimulatory molecules in liver cancer cells. High expression of herpesvirus entry mediator (HVEM) was associated with tumor progression and reduced patient overall survival in multiple cancers. Overexpression of HVEM may lead to binding with inhibitory co-receptor B- and T-Lymphocyte Attenuator, downregulating T cell activation and proliferation, and mediating immune evasion in tumor immune microenvironment. However, clinical significance of HVEM overexpression in liver hepatocellular carcinoma (LIHC) and its relation to immunity remains unknown.

Materials and Methods We obtained expression profiles of HVEM in pan-cancer and LIHC through The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression. Expression of HVEM in LIHC was then validated by immunohistochemical staining in Human Protein Atlas (HPA). Additionally, we evaluated the prognostic value and association of clinicopathological factors to HVEM in LIHC with clinical survival data from TCGA. A risk prognostic nomogram model using Cox regression analysis was constructed to identify important LIHC prognostic indicators. Finally, we evaluated the correlation of HVEM with 24 immune cell infiltrates based on single sample gene set enrichment analysis.

Results Paired data from TCGA LIHC dataset indicated HVEM overexpression in LIHC compared to normal liver tissues (p < 0.001, normal = 50, tumor = 50). Unpaired data showed similar results (p = 0.001, normal = 160, tumor = 371). Additionally, immunohistochemical staining of HVEM in normal liver tissues was weak in HPA database but strong in LIHC tissues. High HVEM expression was strongly associated with worse overall survival [Hazard Ratio (HR) = 1.60 (1.13–2.26), p = 0.008], disease-specific survival [HR = 1.63 (1.03–2.26), p = 0.035], and progress free interval [HR = 1.35 (1.00–1.82), p = 0.048] in LIHC patients. Alpha-fetoprotein levels, Child-Pugh score, fibrosis ishak score, histological grade, T stage, M stage, and N stage are benchmarks associated with cancer progression. Logistic regression analysis revealed significantly higher HVEM expression in LIHC patients with greater cancer progression according to those benchmarks. Additionally, Cox risk regression analysis indicated that HVEM might be an independent prognostic factor for LIHC patients (p = 0.016). Nomogram calibration was performed on the constructed nomogram, revealing a C-index of 0.67, indicating good predictive ability. Functional enrichment and Gene Ontology analyses and Kyoto Encyclopedia of Genes and Genomes pathway analysis showed HVEM association with immune-related genes and pathways respectively. HVEM was positively correlated with the abundance of tumor-infiltrating immune cells like DC, B cells, CD8 T cells, macrophages, NK cells, Th1 cells, and Th2 cells, and negatively correlated with the abundance of TCM and TEM, suggesting association between HVEM and infiltration by immune cells.

Conclusions In conclusion, our findings demonstrated high expression of HVEM in LIHC patients and is a potentially dangerous prognostic predictor of LIHC. We also found a correlation between HVEM immune-related signaling pathways and immune cell abundance, which may affect progression and prognosis of LIHC patients.

Disclosure Information K. Xiao: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; China Scholarship Council (NO.202106550003). C. Soh: None. K. Li: None. P. Xue: None. S. Zhu: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; China National Natural Science Foundation (Grant No. 81973640).

EASY AND SCALABLE TRANSFER OF AN IMMUNO-ONCOLOGY PANEL ACROSS A WIDE RANGE OF CANCER TISSUE TYPES

Background Therapies based on checkpoint inhibitors have emerged as promising strategies in the treatment of cancer patients. The selection of the most efficient combination of immunotherapies along with the accurate prediction of cancer prognosis are unmet endeavors. Thus, scientists and clinicians need deeper knowledge of the immune profile of each patient as well as a detailed characterization of the tumor microenvironment (TME) to develop targeted therapies. Multiplex immunofluorescence (mIF) has become an important tool in the immune profiling of the TME. Ideally, multiplex IF assays should allow for the study of multiple markers in the same tissue sample while preserving the spatial information of tissue morphology. Despite a fast-growing need of multiplex methods, the broad implementation of mIF still remains challenging due to several technical barriers. Assays require robust validation but also a large degree of flexibility to be used across multiple tissue types.

Materials and Methods Here we present COMET™, an innovative solution to easily develop mIF assays that work as a fully automated staining and imaging platform allowing for hyperplex staining with up to 40-markers in just a few hours, without human intervention. COMET™ performs sequential immunofluorescence assays, which consist of sequential cycles of staining, imaging, and elution of two markers per cycle. With the aim of phenotyping different immune and cancer cells and their relation to immunity remains unknown.

Results Paired data from TCGA LIHC dataset indicated HVEM overexpression in LIHC compared to normal liver tissues (p < 0.001, normal = 50, tumor = 50). Unpaired data showed similar results (p = 0.001, normal = 160, tumor = 371). Additionally, immunohistochemical staining of HVEM in normal liver tissues was weak in HPA database but strong in LIHC tissues. High HVEM expression was strongly associated with worse overall survival [Hazard Ratio (HR) = 1.60 (1.13–2.26), p = 0.008], disease-specific survival [HR = 1.63 (1.03–2.26), p = 0.035], and progress free interval [HR = 1.35 (1.00–1.82), p = 0.048] in LIHC patients. Alpha-fetoprotein levels, Child-Pugh score, fibrosis ishak score, histological grade, T stage, M stage, and N stage are benchmarks associated with cancer progression. Logistic regression analysis revealed significantly higher HVEM expression in LIHC patients with greater cancer progression according to those benchmarks. Additionally, Cox risk regression analysis indicated that HVEM might be an independent prognostic factor for LIHC patients (p = 0.016). Nomogram calibration was performed on the constructed nomogram, revealing a C-index of 0.67, indicating good predictive ability. Functional enrichment and Gene Ontology analyses and Kyoto Encyclopedia of Genes and Genomes pathway analysis showed HVEM association with immune-related genes and pathways respectively. HVEM was positively correlated with the abundance of tumor-infiltrating immune cells like DC, B cells, CD8 T cells, macrophages, NK cells, Th1 cells, and Th2 cells, and negatively correlated with the abundance of TCM and TEM, suggesting association between HVEM and infiltration by immune cells.

Conclusions In conclusion, our findings demonstrated high expression of HVEM in LIHC patients and is a potentially dangerous prognostic predictor of LIHC. We also found a correlation between HVEM immune-related signaling pathways and immune cell abundance, which may affect progression and prognosis of LIHC patients.

Disclosure Information K. Xiao: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; China Scholarship Council (NO.202106550003). C. Soh: None. K. Li: None. P. Xue: None. S. Zhu: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; China National Natural Science Foundation (Grant No. 81973640).