HI-TRON Mainz Kick Start Seed Funding 2021

Aim
HI-TRON Mainz opened a call for Kick-Start Seed Funding projects. The funding program aims at facilitating the rapid transfer of scientific findings into clinical applications and to make use of the synergies between scientists based in Mainz and Heidelberg. The HI-TRON Mainz Kick-Start Seed Funding Program provides seed funding to cross-institutional projects with a clear clinical translational focus and path towards implementation in patient care. The selected HI-TRON Mainz Kick Start Seed Funding projects are listed below.

Project 1: To enhance anti-tumor immune responses by inhibiting a pair of immune checkpoints (Corresponding PI: Guoliang Cui)

Compared with the traditional cancer therapies, such as chemotherapy and radiation therapy, immune checkpoint blockade-based immunotherapy has higher effectiveness and fewer side effects in several types of cancers. Those immune checkpoints act as ‘brakes’ to suppress the protective immune responses against cancers. Identification and inhibition of those ‘brake’ molecules will unleash anti-cancer immunity. We have identified one of those immune system “brakes” in human and mouse tumors (referred to as “a new IC” in the figure below), which suppresses anti-tumor immune responses. Based on these data, we propose that blocking this pair of molecules will provide an opportunity to suppress melanoma growth. This project will attempt to elucidate the mechanisms through which this molecule pair regulates anti-tumor immune responses. In pursuing these studies, we hope to discover new ways to revive the tumor-killing function of our immune system and deliver new drug targets for the treatment of colon cancer and other types of cancers.
Project 2: FIDELIO
Detection and validation of gene Fusion-Induced neoepitopes and cognate T-cell receptors in DEdifferentiated LipoSarcoma (Corresponding PI: Stefan Fröhling)

Liposarcomas are malignant soft-tissue tumors that are prone to local recurrence and distant metastasis. Medical therapy of patients with advanced liposarcoma, whose median survival is less than two years, represents a challenge since only a minority respond to conventional cytotoxic drugs and no approved targeted therapies exist. The most common histologic subtypes, well-differentiated and dedifferentiated liposarcoma (DDLS), are characterized by focal amplification of chromosome 12q, involving CDK4 and MDM2 genes. Using whole-exome/genome and RNA sequencing in DDLS patients within the DKFZ/NCT/DKTK MASTER program, we discovered that the structural changes on chromosome 12q generate open reading frames, leading to several hundred presumably transcribed and translated chimeric genes and neoantigens. Thus, DDLS may be accessible for immunotherapy. However, these events' randomness and diversity make the presence of a common neoantigen unlikely and instead favor individualized T-cell receptor (TCR) or vaccination-based treatment strategies. The proposed project is based on preliminary data showing that the administration of individualized, fusion peptide-derived vaccines can induce measurable, peptide-specific T-cell responses in the blood of patients with DDLS. We now aim to systematically analyze gene fusion-derived neoepitopes and characterize patient-specific T-cell receptors in DDLS patients. This will lay the foundation for a scalable high-throughput pipeline to be used in future clinical trials with individualized TCR-transgenic T cells or personalized vaccines.
**Project 3: Regulation of immune evasion in the tumor microenvironment by coagulation signaling**
(Corresponding PI: Claudine Graf)

**Current state of research:** During malignancies systemic alteration of immune responses modulates blood coagulation, platelet-leukocyte interactions and pathological thrombus formation. This process is exacerbated by tumor cell specific expression of the coagulation proteins tissue factor, prothrombin and thrombopoietin leading to a hyperthrombotic state and thrombocytosis. Beyond regulation of blood clotting, coagulation proteases are causatively linked to tumor immune evasion as demonstrated for thrombin and activated factor X (FXa) in preclinical models. Thrombin acts on platelets to release the immunosuppressive and fibrosis inducing TGF-β, whereas FXa induces an immunosuppressive macrophage phenotype via autocrine FXa-protease-activated receptor 2 (PAR2) signaling, which converges with other innate immune signaling pathways to control dendritic cell phenotypes and to impair cytotoxic T-cell (CTL) responses. Both pathways are druggable with direct oral anticoagulants (DOACs), and their effects show synergism with checkpoint inhibitors. Complementary studies from tumor patients likewise showed increased monocyte-FX expression when compared to healthy controls indicating the translational relevance of our experimental studies. However, the detection of FXa in monocytes / TAM as a biomarker or the usage of DOACs as immune-modulators in tumor patients has not been clinically established.

**Objectives:** We aim to investigate the immunomodulatory function of coagulation signaling on the efficacy of clinically relevant immunotherapy in patients with or without FXa inhibitor co-medication. To this end, we aim to characterize the FX expression in peripheral blood monocytes and tumor-associated macrophages (TAM) of tumor patients at diagnosis and under treatment as a biomarker for tumor progression. Complementary studies in mice investigate the impact of platelet-monocyte aggregate abundance on monocyte phenotype and their abilities to differentiate into antigen presenting cells and T-cell priming. We posit that variable hyperthrombotic states and thrombopoietin expression of tumors modulate platelet counts and that tumor-educated platelets drive monocyte-differentiation into a tolerogenic state. As FXa activates prothrombin to thrombin, inhibition of FXa likewise inhibits thrombin generation. A better understanding of how coagulation signaling and different anticoagulants affects these processes will eventually allow for modifications of immunotherapies by combined usage with FXa inhibitors.

Project 4: B-cells as a target in immunotherapy of NASH/BASH and liver cancer (Corresponding PI: Mathias Heikenwälder)

Obesity can lead to metabolic syndrome (e.g. abdominal obesity, insulin resistance) as well as hepatocellular carcinoma (HCC), which is the fastest growing cancer in the Western world, with strongly rising incidence in developing countries. Using long-term choline-deficient high-fat diet (CD-HFD) or Western Diet (WD), we could recapitulate the most important features of metabolic syndrome in humans - including the pathophysiology of non-alcoholic fatty liver disease (NAFLD), its severe pathology termed “non-alcoholic steatohepatitis (NASH)” and NASH-to-HCC transition. Besides, we have also established a model of alcoholic steatohepatitis (ASH) and NASH-ASH driven liver cancer, termed BASH (both alchoholic steatohepatitis forms). Today, the majority of people with fatty liver disease display BASH, due to a combination of high caloric diet, alcohol consumption and sedentary life style. Recently, B-cells have been considered as important mediators and players in innate and adaptive immune responses associated with metabolic diseases. Our preliminary data illuminate new mechanistic aspects on the role of B-cells in NASH development, liver fibrosis and liver cancer. Our data show that secretion of IgA drives FcγR/FcεR-signaling on inflammatory monocytes in the development of fibrosis, NASH and liver cancer. Recently, we could show that liver cancer patients with an underlying NASH/BASH do not respond to immunotherapy, but in contrast actually display adverse effects (e.g. reduced overall survival) (Dudek et al., Nature 2021; Pfister et al., Nature 2021). Thus, adjustment of existing therapies and better stratification is needed to optimize therapy outcome. B-cells might become an important target cell in this context - as besides immunoglobulin expression also B-cell derived antigen presentation contribute to hepatic dyslipidemia, NASH/BASH and liver cancer. To develop new, or adjusted immunotherapies for HCC patients (caused by NASH/BASH), we plan to use a B-cell directed immunotherapy and intend to target different B cell types or inhibit specific immunoglobulins in order to study how this therapy either prevents or cures HCC - e.g. in combination with an immunotherapy approach. We aim (ia) to perform Single cell RNA-Seq analysis of sorted hepatic and gastrointestinal CD45+ cells from different liver cancer mouse models (ib) to perform Single cell RNA-Seq analysis of CD45+ cells from human gastrointestinal and liver samples of NASH/BASH patients, liver cancer patients. We aim to (ii) to target newly identified, selected genes expressed in B-cells, and reinject genetically modified B-cells. We aim to (iii) to perform therapeutic B-cell- or immunoglobulin-A (IgA) depletion in mice with NASH/BASH and investigate liver cancer development in the presence or absence with mono- or combinatorial immunotherapy (anti-PDL1 and anti-VEGF). This study is lead by two PIs: Prof. Mathias Heikenwälder; Division Chronic Inflammation and Cancer, German Cancer Research Center (DKFZ), Heidelberg; Prof. Ari Waisman; Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg University of Mainz; Focus Program Translational Neuroscience (FTN) Mainz; Research Center for Immunotherapy (FZI) Mainz, Mainz, Germany. The project greatly profits from the complementary expertise in immunology and cancer of the two individual PIs.

Figure: B-cells play a role in NASH development, fibrosis and liver cancer. A) Representative H&E staining of 6month-old mice illustrating NASH, WT and μMT CD-HFD, and JH/- CD-HFD showing absence of NASH. (B) Quantification of serum ALT in male 6 month-old mice. (C) NAFLD score (NAS). (D) Graph summarizing C57BL/6 mice without liver tumors (NT) and with liver tumors (T) in WT μMT/- and JH/- CD-HFD-fed mice. (E, F) Representative Sirius red staining illustrating the status of peri-sinusoidal fibrosis and incidence of fibrosis.
Project 5: Investigating the interaction of B- and T-cell mediated immune responses to pancreatic cancer toward an immunotherapy approach (Corresponding PI: Jörg D. Hoheisel)

Pancreatic cancer is the currently most lethal tumor entity; mortality is close to incidence. In Western countries, it will become the second most frequent cause of cancer-related death behind lung cancer by 2030 due to the lack of means for early detection and therapeutic options. The DKFZ Division of Functional Genome Analysis has been working on pancreatic cancer for many years in close collaboration with the European Pancreas Center at Heidelberg University Hospital.

New data suggests that the humoral immune response and the T-cell reaction to pancreatic cancer may affect each other in a specific way. The project aims at understanding this interaction in sufficient detail to exert an influence on this process in a targeted manner. Thereby it may be possible to trigger a strong immune reaction to pancreatic cancer. Toward this end, the DKFZ group teams up with colleagues at the Immunotherapy Development Center of TRON, bringing together expertise from the areas of molecular biology, medical research and immuno-oncology. The project might lay the basis for a new approach of immunotherapy, which could improve the dismal prognosis of pancreatic cancer patients.
Project 6: TCR-sequencing of human CD8+ T cells specific for H. pylori antigens presented by tumor cells (Corresponding PI: Martin Löwer)

*Helicobacter pylori* (*H. pylori*), which infects about 50% of the world population, is associated with several gastropathies including chronic gastritis, peptic ulcer disease and mucosa-associated lymphoid tissue lymphoma. It causes gastric cancer in 1-3% infected humans. We hypothesize that the *H. pylori*-derived peptides are subject to CD8+ T-cell recognition and that *H. pylori*-specific CD8+ T cells can be isolated from the peripheral blood of patients with gastric cancer.

We will identify specific *H. pylori* antigens from literature, databases and our sequence-prediction algorithms using the genomic sequence and epitope prediction tools. Blood samples from gastric cancer patients will be screened for reactive T-cells using Elispot assays. In parallel, we will use NGS data from TCGA and GTEx as well as public mass-spec datasets to gain insight into the tumor specificity of the predicted antigens. We will use *H. pylori*-specific T-cells from selected *H. pylori*-reactive blood samples and stimulate them with selected *H. pylori* peptides. Next, the pool of antigen-specific reactive T-cells will be enriched followed by single-cell TCR sequencing. The subsequent bioinformatics analysis will allow the definition of *H. pylori*-specific TCR sequences. Alpha and beta chains will be cloned in a specific vector available in house to produce IVT RNA for each TCR chain, which will be tested for reactivity and specificity in primary T-cells and Jurkat cells, using a pool of autologous antigen-presenting cells preloaded with *H. pylori*-specific peptides.

The project will result in a proof-of-concept that antigens of bacterial origin can be presented on the cell surface of host cells in patients with gastric cancer. The detection of *H. pylori*-reactive T cells may give rise to a new class of immune-oncology biomarkers and interventional targets. These TCRs may be a starting point for an immunotherapy against *H. pylori* and gastric cancer.
Project 7: Membrane proteins and lipids as targets for immunotherapy in pediatric cancer patients (Corresponding PI: Jörg Faber)

The treatment of pediatric patients can be defined as a success of medicine, with 82% of the patients under 15 years of age surviving their cancer for more than 15 years in Germany. However, cancer remains the leading cause of death by disease in children in developed countries and new therapy approaches are urgently needed.

Immunotherapy is changing the landscape of cancer treatment for adult patients, but is generally not used for the treatment of pediatric patients with solid tumors, with the exception of monoclonal antibodies against the ganglioside GD2 in neuroblastoma. Immunotherapy clinical trials in pediatric patients are still rare. This is due not only to the need to protect young patients from not well defined risk and toxicities, but also to our limited knowledge on the expression of molecular targets in pediatric tumors and in normal tissues of children. Pediatric cancers have indeed a unique etiology. While adult cancers usually occur as the result of a gradual accumulation of somatic mutations, pediatric cancers are rather considered as a developmental disease.

To accelerate the inclusion of pediatric patients in immunotherapy trials, in this project we will analyze the distribution of molecular targets in pediatric solid tumors associated with a poor prognosis, such as neuroblastoma, osteosarcoma, Ewing’s sarcoma and kidney tumors and in intracranial high-grade tumors, such as diffuse midline glioma and medulloblastoma. We will focus on plasma membrane targets that can be addressed by monoclonal antibodies and CAR-T cells in therapeutic protocols. Importantly, we will not only consider protein, but also lipid targets, which are not detectable by the pipelines generally used for targets identification. An important part of this project will be the establishment of a high quality set of normal pediatric tissues for testing the tumor specificity of the molecular targets.

The project is led by the Center for Pediatric and Adolescent Medicine of the University Medical Center (UM) Mainz (PI Prof. Dr. Jörg Faber and scientific coordination PD Dr. Claudia Paret) in collaboration with the Institute of Pathology (PI Prof. Wilfried Roth and Dr. Larissa Seidmann), the Institute of Neuropathology (PI PD Dr. Katrin Frauenknecht) of the UM Mainz, TRON gGmbH (PI Dr. Akilli-Oeztuerk) and the group of Lipid Pathobiochemistry of the DKFZ (PI PD Dr. Roger Sandhoff).

We anticipate that this project will facilitate the access of the pediatric population to immunotherapy clinical studies that are currently enrolling only adult patients and to Investigator Initiated Trials (IIT).

Figure: Expression of gangliosides in primary tumor cells of a Diffuse Midline Glioma (A) with Histone H3-K27M mutation (B) by flow cytometry (C) and mass spectrometry (D)
Project 8: Generating Neoepitopes by ADAR1-mediated RNA base-editing: A New Tool for Immunotherapy (Corresponding PI: Riccardo Pecori)

Successful immunotherapy depends on the potentiation of previously anergic, tumour-specific T cells, towards tumour cell killing. Current strategies include the reactivation of T cells via inhibition of their quiescent state through immune checkpoint inhibitors (ICIs). Despite their remarkable clinical successes, many patients do not respond to immunotherapy or develop therapeutic resistance. This aspect defines an urgent need for novel strategies that sensitize tumors to immunotherapy. Tumour mutational burden is the best predictor of responsiveness to immunotherapy, as its increase correlates with increased neoepitope/antigen formation, and therefore increased sensitivity to T cell attack. Therefore, specifically increasing neoepitope formation in cancer cells constitutes a promising strategy to sensitize tumors to immunotherapy. Neoantigens arise either from DNA mutations or are generated by post-transcriptional modifications at the RNA level (e.g., RNA splicing or retained introns caused by splicing errors). RNA editing, the most common post-transcriptional modification in mRNA, can induce non-synonymous substitutions generating neoantigens. The RNA editing enzyme adenosine deaminase acting on RNA 1 (ADAR1) deaminates adenosine to inosine (A-to-I) within double-stranded RNA (dsRNA) structures. In many tumours, ADAR1 is overexpressed and targets coding regions at the mRNA level, thus facilitating the generation of neoepitopes. Antisense oligonucleotides (ASOs) enable re-targeting of endogenous ADAR1 to coding regions leading to amino acid recoding. To pave the way towards novel, ADAR1-mediated tumor immunotherapy approaches, we will elucidate the efficiency of ADAR1-dependent neoantigen generation on the MHC ligandome level (A) and investigate approaches to specifically enhance neoepitope formation by using ASOs with the aim to increase the level of tumour “neoantigenicity” (B) and thus render the tumour sensitive to ICIs.

The project is enabled by a tight collaboration between PD Dr. Riccardo Pecori (DKFZ) and Prof Dr. Stefan Tenzer (University Medical Center Mainz / HI-TRON) merging their expertise in RNA base-editing and immunepeptidomics technologies.
Project 9: Effects of a new signaling pathway in cancer cells on T cell mediated therapeutic outcome upon checkpoint inhibitor treatment (Corresponding PI: Fulvia Vascotto)

Immune checkpoints are key regulators in the control of anti-cancer immunity. In recent years, checkpoint inhibitors (CPI) have proven the possibility to mount immune responses against cancer inducing profound and durable clinical responses. Nevertheless, a large proportion of cancer patients unfortunately fall into relapse. Thus, there is an urgent need to deeper understand the underlying resistance mechanisms of cancers during checkpoint inhibitor therapies, in order to improve the clinical outcome of patients. Reinvigoration and release of suppressive mechanisms on intratumor CD8+ T cells, considered as the main anti-tumor effector cells, are the major aims of combination therapies. Based on our previous findings, we will investigate the tumor immune microenvironment (TiME) modeling *in vivo* and *in vitro* using murine tumor models and human primary tumors to study comprehensively the role of a novel axis involved in the control of T cell activity. Our aim is to generate evidence that this new axis in combination with checkpoint inhibitors can generate a new therapeutic approach for cancer patients.

Figure: Modelling of the proposed MoA of the new signaling pathway involving intratumoral cytotoxic T cells in cancers.
Project 10: Utilization of the HLA-independent anti-tumor T-cell repertoire for cancer immunotherapy (Corresponding PI: Thomas Wölfel)

Down-regulation or loss of HLA expression represents an important immune-escape mechanism and is a serious limitation for immunotherapy strategies relying on HLA-restricted effectors. HLA-independent anti-tumor αβT-cell responses have been observed in patients with HLA-negative disease. So far two of their target antigens have been identified, tyrosinase-related protein 2 (TRP2) and the GM-CSF receptor alpha chain (CSF2RA). The project aims at the preclinical testing of patient-derived, HLA-independent, anti-tumor T-cell receptors (TCR) against TRP2 and CSF2RA in mice to demonstrate their anti-tumor effects after adoptive transfer and to exclude relevant off-tumor and off-target effects applying in vivo tracking of TCR-transduced T cells and immunohistochemistry on tumor and normal tissues. In addition, it is planned to identify further HLA-independent, tumor-associated T-cell targets (A) via cDNA library-expression screening and (B) via reverse targeting of promising tumor-associated surface molecules. The project’s ultimate goal is to pave the way for clinical adoptive therapy studies with HLA-independent anti-tumor TCR and, finally, prove both relevance and therapeutic use of the naturally occurring HLA-independent anti-tumor T-cell repertoire. This would also support future efforts to augment such T-cell responses in patients and to circumvent adoptive transfer requirements.

Figure: HLA-independent αβT-cell receptors recognize intact molecules on the surface of their target cells and thus do not depend on the presence of HLA molecules.