DKFZ-MOST Cooperation in Cancer Research

8th German-Israeli Cancer Research School

CANCER IMMUNOTHERAPY

November 20–24, 2016, Neve Ilan, Israel

PROGRAM AND BOOK OF ABSTRACTS
Foreword

The German-Israeli Cooperation in Cancer Research was founded in 1976 and belongs to the longest lasting scientific cooperations of both countries. To date, more than 160 joint projects have been funded. Importantly, the cooperation has also strongly fostered friendship between scientists from Germany and Israel and other involved partners (www.dkfz.de/israel).

In 2006, during the 30th Anniversary of the Cooperation, the idea of a “German Israeli Cancer Research School” was conceived. Our aim was to bring together young scientists with the senior scientists in the field of cancer research from both countries, Israel and Germany, for the exchange of knowledge and ideas in a friendly and casual atmosphere.

The 8th German-Israeli Cancer Research School in November 2016 is dedicated to “Cancer Immunotherapy”. On this occasion, we will review the spectacular recent progress in this area with respect to the clinical application of – for instance – checkpoint inhibitors and adoptive T cell therapies, discuss the latest insights in the regulation of immunity in health and disease, and – most importantly – aim at identifying new opportunities in the pre-clinical and clinical tumor immunology research.

The meeting offers an exciting program of seminars by 16 cancer immunotherapy/tumor immunology experts, 8 from Israel and 8 from Germany. This year’s keynote lecture will be delivered by Prof. Zelig Eshhar, a famous Israeli evolutionary biologist who brought forward the ‘Handicap Principle’ and who has recently received the Israel Prize for his contributions to the field.

An important aspect of this year’s program is that all participating PhD students will present their own project at prime time during the meeting. On each morning 8 PhD students will give 10-minute flash-talks, after which they can display their work in further details during the subsequent poster session. The best posters will be awarded.

We are indebted to Profs. Apte, Rotter, Offringa and Dr. Poschke for organizing the scientific program and to Hadar Huberman and Susanne Schunk for handling all administrative matters.

We look forward to an exciting and stimulating 8th German Israeli Cancer Research School in Israel.

Peter Angel Varda Rotter Hagit Schwimmer
DKFZ-Coordinator Israeli Coordinator MOST Coordinator
Israel-Cooperation of the Schools of the Schools
Program

**SUNDAY, NOVEMBER 20, 2016**

**ARRIVAL** in the afternoon

19:00 Welcome Dinner

**MONDAY, NOVEMBER 21, 2016**

08:30 Gilad Bachrach  
Recognition of bacteria by NK cells

09:00 Angel Porgador  
Natural killer immunity and cancer immunome

10:00 Break

10:30 Flash talks by PhD students and junior postdocs

12:00 PhD students and junior postdocs poster session and lunch buffet

13:30 Thomas Woelfel  
Analyses of the anti-tumor T cell repertoire in melanoma patients

14:15 Gerald Willinsky  
Identification of tumor-specific rejection antigens for TCR gene therapy

15:00 Michael Platten  
Immunotherapy of brain tumors

15:45 Break

16:15 Barbara Seliger  
Many mechanisms of tumors leading to target-less T cells

17:00 Isabel Poschke  
Immunotherapy of pancreatic cancer in the neo-adjuvant and adjuvant setting

17:45 Happy hour

19:00 Dinner, followed by a social activity
TUESDAY, NOVEMBER 22TH, 2016

08:30  Ron Apte
       Microenvironment IL-1 is a major determinator of the balance between immunity and inflammation at tumor sites

09:15  Vigo Heissmeyer
       Roquin-deficient T cells create a tumor-inducing micro-milieu in the pancreas

10:00  Break

10:30  Flash talks by PhD students and junior postdocs

12:00  PhD students and junior postdocs poster session and lunch buffet

13:30  Afternoon excursion to Jerusalem

19:00  Dinner

20:00  Special Lecture:
       Amotz Zahavi
       Natural selection functions by two mechanisms, signal selection by the handicap principle and the selection for efficiency for all other traits.
**Wednesday, November 23rd, 2016**

08:30  Dirk Busch  
Adoptive T cell therapy: from single cells to immunity

09:15  Michal Lotem  
Co-stimulation of CD8 T cells, beyond APC: lymphocyte interactions

10:00  Break

10:30  Flash talks by PhD students and junior postdocs

12:00  PhD students and junior postdocs poster session and lunch buffet

13:30  Jacob Schachter  
Immunotherapy – the new platform for the treatment of cancer

14:15  Yoram Reiter  
Engineering immune molecules and cells for novel cancer immunotherapy

15:00  Michael Bachmann  
Retargeting of immune cells to tumor cells

15:45  Break

16:15  Keynote Lecture:  
Zelig Eshhar  
The chimeric antigen receptor T cells: from the mouse Cage to the patient health

17:00  Gal Markel  
Immune resistance mechanisms in melanoma

17:45  Happy hour

19:00  Closing dinner party
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Microenvironment IL-1β regulates the balance between inflammation and immunity during tumor growth.

Irena Kaplanov, Rachel Kornetsky, Yaron Carmi, Elena Voronov and Ron N. Apte

The Shraga Segal Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences Ben-Gurion University of the Negev, 84105 Beer-Sheva, ISRAEL

Interleukin-1 (IL-1) is a potent cytokine in the tumor microenvironment, being produced by the malignant and/or infiltrating myeloid cells. IL-1β, the most pronounced secreted agonist of the IL-1 family, is abundant at tumor sites. We have used the model of 4T1 breast cancer cells, which upon orthotopic injection induces local tumors and spontaneous lung metastases. In wild-type (WT) mice, tumor progression and death of tumor-bearing mice occurred, while in IL-1 β deficient (IL-1 β KO) mice, regressing tumors were observed, with no lung metastasis and complete survival of mice. We assessed the myeloid cell infiltrate in initial phases of tumor growth, when the direction of the malignant process is determined. A dramatic reduction in CCL2 levels and a significant differentiation arrest of LY6C<sup>high</sup>CCR2<sup>+</sup> inflammatory monocytes into mature macrophages were observed in tumors in IL-1 β KO mice, as compared to WT mice. In addition, the relative increase in the proportion of dendritic cells among tumor infiltrating leukocytes was observed in IL-1β KO mice. Tumor infiltrating macrophages were shown to produce IL-10, while dendritic cells produce IL-12. The favorable myeloid cell infiltrate and cytokines in tumor sites in IL-1 β KO mice resulted in the recruitment of activated CD8<sup>+</sup> T cell into tumor sites and eradication of the malignant cells. In contrast, in WT mice, IL-10 producing suppressive macrophages supported tumor progression. Furthermore, anti-IL-1β therapy in tumor-bearing WT mice, synergized with anti-PD-1 antibodies in modification of the myeloid cell infiltrate and reduction in tumor invasiveness. Thus, anti-IL-1 β therapy should be effective for cancer treatment.
Retargeting of immune cells to tumor cells

Michael Bachmann

Helmholtz-Zentrum Dresden-Rossendorf e.V. (HZDR), Institute of Radiopharmaceutical Cancer Research, and University Cancer Center (UCC) Carl Gustav Carus TU- Dresden, Dresden, Germany

In recent years, bispecific antibodies (bsAbs) and chimeric antigen receptors (CARs) emerged as promising candidates for an antigen-specific cancer immunotherapy. Both bsAbs and CARs are able to redirect T cells for efficient tumor cell lysis. The development of a novel bsAb or a CAR is pretty time consuming and cost efficient. Therefore, we recently introduced a novel modular platform (UniTARG) that can be rapidly and easily adapted for redirection of T cells to any tumor associated antigen (TAA) in both a bsAb or CAR related manner. The UniTARG system separates the respective effector arm and the anti-TAA binding domain to two separate molecules: (I) a universal effector unit recognizing a unique short peptide epitope (E5B9), and (II) an exchangeable target module (TM) comprising an anti-TAA binding moiety fused to the peptide epitope. The respective universal effector system represents either a bsAb (with specificity for CD3 and the peptide epitope (E5B9)) termed UniMAB or a second generation CAR (directed to the E5B9 epitope) termed UniCAR. TMs will form an immune complex with the respective effector system. The complex can cross-link T cells and tumor cells similar to conventional bsAbs or CARs resulting in an immune synapse like structure which activates the immune effector cells and finally leads to the killing of the target cells. For redirection of T cells to any kind of TAA only the binding moiety of the TM has to be adapted what saves costs and time. To increase tumor specificity and to reduce the risk of tumor escape variants, the modular character of the UniTARG system further offers the possibility to apply simultaneously different monospecific or even bispecific TMs recognizing two TAAs. TMs can be prepared from a variety of molecules including for example from conventional Abs, nanobodies, soluble TCRs and even small molecules such as the PSMA-HBED-CC Ligand which is otherwise clinically used as PET tracer. As most TAAs are not exclusively expressed on tumor cells, especially conventional CARs have a high risk to attack healthy tissues expressing the target even at low levels as has already seen in clinical studies. In contrast, UniCAR T cells armed via TMs will automatically be turned off after the TM has been eliminated from the blood stream of the patient. If necessary, they can be re-armed even against a different target if escape variants develop under therapy. Proof of concept of the UniTARG system, for both leukemic and solid tumor cells will be provided.
Involvement Fusobacterium nucleatum in colon cancer

Jawad Abed¹, Chamutal Gur², Ofer Mandelbim³, Gilad Bachrach⁴

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Natural Killer (NK) cells play a key role in controlling tumor growth and in protection against viral infections. Much less is known about the interactions of NK cells with bacteria. *F. nucleatum* is an oral anaerobe commonly involved in periodontal disease. However, during colon cancer, blood-borne fusobacteria bind Gal-GalNAc overexpressed in colon cancer and colonize the colon tumor. On the tumor cells a fusobacterial surface Fap2 activates the TIGIT immune cell suppressor receptor and inhibits the tumor’s elimination by NK cells and Tills. Our results demonstrate a mechanism by which bacteria can accelerate tumor development.
Choice of better T cells for adoptive immunotherapy

Dirk Busch, MD

Institute for Medical Microbiology, Immunology and Hygiene
Technische Universität München, Germany

Adoptive transfer of antigen-specific T cells has demonstrated astonishing clinical results in the treatment of infections and some malignancies. The definition of optimal targets and antigen receptors as well as the differentiation status of transferred T cells are emerging as crucial parameters for generating cell products with predictable efficacy and safety profiles. Our laboratory has demonstrated that defined subsets within the memory CD8+ T cell compartment fulfill all key characteristics of adult tissue stem cells and are essential for robust and long-term maintained responses upon adoptive transfer. Enrichment of these memory stem T cells for adoptive T cell therapy, using primary (unmodified) or genetically engineered T cells, can be highly effective, even when low numbers of T cells are transferred. Since such potent T cell products also bear some risk of toxicity, safeguards that allow selective depletion of transferred cells in the case of un-tolerable side effects may be needed. In this context, we explored the capacity of a truncated version of Epidermal Growth Factor Receptor (EGFRt) co-expressed with adoptively transferred T cells. EGFRt is functionally inert, as it cannot bind EGFR-ligands and lacks signaling components, and is non-immunogenic. However, it still binds to Cetuximab, an EGFR-specific antibody already used for clinical applications. We can show in a pre-clinical animal models that EGFRt-expressing engineered T cells can be effectively depleted via Cetuximab treatment in vivo. For example, B cell aplasia, which is a common long-lasting side effect of CD19 CAR-T cell treatment, can be reverted by antibody-mediated in vivo depletion.
The chimeric antigen receptor T cells: from the mouse Cage to the patient health

Zelig Eshhar

The Marshall and Renette Ezralow Professor of Chemical and Cellular Immunology
Weizmann Institute

Our laboratory pioneered and developed the “T-Body” approach that was later renamed as CAR T-cell and has recently been used in clinical trials to fight cancer. The genetically-engineered T cells have already shown to effectively kill, and eliminate patients with lymphomas and leukemias where about 45% of the treated patients recovered. We applied CAR T cells to solid tumors constituting a major challenge because of damage to healthy tissues due to cross reactivity of the antibodies used to redirect the CART cells. Using human xenografts in immunodeficient mice we focused on and optimized different treatment modes. In these models we suggested the treatment of choice and maximized its safety.
Roquin-deficient T cells create an inflammatory, pre-cancerous micro-milieu in the pancreas

Desheng Hu¹, Jessica Zöller², Mathias Heikenwalder²³, Vigo Heissmeyer¹⁴

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Investigating a family of RNA-binding proteins, Roquin-1 and Roquin-2 we found that these post-transcriptional regulators prevent spontaneous activation and inappropriate T cell differentiation. On the molecular level these factors control the expression of costimulatory receptors like ICOS and Ox40, cytokines like IL-6, IFN-γ and TNF and of transcription factors like IRF4 and c-Rel as well as modulators of transcription like IκBζ and IκBN. Conditional deletion of Roquin-1/2 encoding genes in peripheral T cells causes elevation of IL-17, IL-6, and TNF levels in the serum and accumulation of Tfh cells in secondary lymphoid organs and of Th17 cells in the lung.

Surprisingly, in the pancreas we find evidence for adaptive and innate inflammation as well as for the formation of acinar to duct metaplasia and development of PanIN lesions. These phenotypes correlated with an occurrence of high affinity autoantibodies against antigens of the pancreas and increased STAT3 signaling in the pancreatic tissue.

Generating compound genotypes of mice our current efforts are directed to find out which molecular targets of Roquin and which Roquin-controlled T cell differentiation pathways are essential for the generation of the inflamed, tumor-inducing milieu in the pancreas. Moreover, we will dedicate our efforts to comparative analyses of human pancreatitis and pancreatic cancer.
Co-stimulation of CD8 T cells, beyond APC: lymphocyte interactions

Michal Lotem, MD

Head, Center of Melanoma and Cancer Immunotherapy
Hadassah Hebrew University Hospital, Jerusalem, Israel

Activation of antigen-specific T cells is primarily dependent on encounter with the cognate antigen presented in conjunction with MHC molecules. The magnitude and duration of the response is controlled by a wide repertoire of cell surface receptors on lymphocytes, which bind to their ligands. The complex signaling provided by these interactions are designated "signal 2".

In this talk, the diverse repertoire of immune modulatory receptors on T lymphocytes will be addressed. The partnering cells involved in T cell modulation will be described including tumor cells and cells of lymphoid origin. We will touch on aspects of immune modulation including exhaustion, secondary antigen presentation, control of plasticity and redundancy of receptors and ligands.

Lastly, we will discuss the clinical application of immune modulatory receptors in cancer medicine.
CEACAM1 is a member of the carcinoembryonic antigen family of proteins. The presence of CEACAM1 on melanoma tumors predicts poor outcome. We show that CEACAM1 promotes the aggressiveness of melanoma cells by two distinct ways: a) it protects melanoma cells by inhibiting cytotoxic T cells in an antigen-restricted manner, and b) it directly facilitates the proliferation of melanoma cells. Thorough investigations show that different biological mechanisms enable each of the roles of CEACAM1 in melanoma. CEACAM1 blockade with a monoclonal antibody alleviates the immuno-protective effects, providing the scientific rationale for a first in man dose escalation phase I clinical trial in multiple cancer indications. Finally, soluble CEACAM1 is elevated in the serum of melanoma patients, it reflects disease burden and shows some promising signs as prognostic and monitoring tool.
Towards implementation of T-cell Therapy in Pancreatic Cancer

Isabel Poschke¹, Michael Volkmar¹, Oliver Strobel², Frank Bergmann³, Niels Halama⁴, Dirk Jäger⁴, Ugur Sahin⁵, Markus Büchler², Rienk Offringa¹,²

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In contrast to the general belief that pancreatic ductal adenocarcinoma is a poorly immunogenic tumor, we found cumulative evidence for an adaptive immune response in this aggressive cancer type. Immunohistochemistry reveals prominent T-cell infiltrates in the majority (~70%) of tumor biopsies, and these tumor-infiltrating lymphocytes (TILs) can be isolated and expanded ex vivo with similar efficiency as those isolated from melanoma. Furthermore, comparison of the T-cell receptor repertoire between TIL and PBMC isolates from patients points at the selective expansion of T-cell subsets in the tumors. Finally, in ~50% of tumor specimen, T-cell infiltration is accompanied by the presence of tertiary lymphoid structures that comprise areas rich in CD3+ T-cells and CD208+ dendritic cells as well as areas rich in B-cells and follicular dendritic cells.

Based on these findings, our current efforts aim at:

• Evaluating neoadjuvant treatment with agonist immunostimulatory antibodies as a means to mobilize this pre-existing immune response in patients with primary resectable disease*
• Analysis of the anti-tumor reactivity and antigen-specificity of TCR-species that are prominently enriched in the tumor as compared to PBMC.
• Exploration of TIL therapy for treatment of recurrent disease.

* Clinical trial in context of FP7 EU IACT program; Immunostimulatory Antibodies for Cancer Treatment
Immunotherapy of brain tumors

Michael Platten $^{1,2,3}$

$^1$ DKTK CCU Brain Tumor Immunology, German Cancer Research Center, Heidelberg, Germany, $^2$ Neurology Clinic and National Center for Tumor Diseases, Heidelberg, Germany, $^3$ Neurology Clinic, University Hospital Mannheim, Germany

With the success of checkpoint inhibitors in clinical trials with - often remarkable - durable responses even in metastatic situations cancer immunotherapy has experienced an unprecedented renaissance. Numerous studies have shown that these checkpoint inhibitors work by unleashing an endogenous T cell response targeting mutated antigens in tumors. As checkpoint blockade comes at a cost of increased autoimmunity and as tumors with a low burden of non-synonymous mutations - including gliomas - are unlikely to respond to checkpoint inhibition alone it is important to understand and identify the signature of relevant immunogenic mutated antigens (neoepitopes) and the T cell receptors recognizing these epitopes to employ a more specific immunotherapy that also works in tumors not responding to checkpoint blockade alone. This signature is very likely to be highly patient-specific. This presentation discusses the current understanding and targeting of key pathways involved in T cell trafficking to the CNS and modulating antigen-specific T cell responses in gliomas and models. It will also cover our efforts in identifying and characterizing neoepitope–specific vaccines for brain tumor patients and translating them to early clinical trials exemplified by a peptide vaccine trial targeting mutant IDH1 in patients with astrocytomas (EudraCT: 2014-000503-27, Clinicaltrials.gov: NCT02454634).
Natural Killer Immunity and Cancer Immunome

Angel Porgador

1The Shraga Segal Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel
2National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer Sheva, Israel

Cancer-associated immunome is a key player in cancer prognosis and response to therapy. The cancer-associated immunome, particularly within the cancer microenvironment, is associated with cancer prognosis. The presence and phenotype of intratumoral natural killer (NK) cells in the cancer microenvironment is tightly associated with cancer prognosis. NK cell activity is a balance between signals delivered by inhibitory and activating receptors. Major activating receptors include NKG2D and the natural cytotoxicity receptors (NCRs): NKp46 (NCR1), NKp44 (NCR2) and NKp30 (NCR3). The issue of altered expression of activating/inhibitory isoforms of immune-associated genes needs will be discussed; in particular, the splicing-enabled paradoxical role of NKp44/NCR2 suggested immune checkpoint will be detailed with references to cancer and pregnancy.
Engineering Immune Effector Molecules for Novel Immunotherapy of Cancer and Inflammation

Yoram Reiter

Faculty of Biology and Technion Integrated Cancer Center, Technion-Israel Institute of Technology, Haifa 32000, Israel

The ability to generate highly specific T-Cell receptor Like (TCRL) antibodies which bind HLA-peptide complexes on the surface of cells opens new possibilities in expanding the wealth of therapeutic modalities which targets structures which are derived from intracellular-derived targets. These antibodies can bind specifically to, and kill, the diseased cells. Thus, transform disease-specific targets that are expressed inside malignant cells into targets that can be recognized on the cell surface by soluble TCRL antibodies. Similar approaches can be applied when such TCRL antibodies target autoantigens associated with autoimmunity and inflammation. These approaches expand the pool of novel therapeutic antibodies beyond the limits of currently available antibody-based approaches. The scientific rational as well as specific examples will be presented and discussed.
Immunotherapy-The New Platform For The Treatment of Cancer

Jacob Schachter

Head of the Research and Treatment of melanoma and skin cancer. Deputy Oncology Unit at the Chaim Sheba Hospital. Specialty: oncology and radiotherapy

There are two new basic approaches to the treatment of advanced melanoma:
Targeted therapy based on BRAF status
Immunotherapy.

There are advantages and disadvantages to each technology and this will be discussed in my presentation.
The main topic of the presentation will focus on immunotherapy as the future basic platform for the treatment of advanced cancer.
Molecular mechanisms regulating HLA class I antigens in tumor cells

Barbara Seliger¹, Katharina Biehl¹, Jürgen Bukur¹, Evamaria Gonschorek¹, Diana Handke¹, Arndt Hartmann², Simon Jasinski-Bergner¹, Marifili Lazaridou¹, Claudia Lennicke¹, Stefanie Meyer³, Anja Mueller¹, Christine Stoehr², Bernd Wullich⁴, Ofer Mandelboim⁵

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Abnormalities of classical and non-classical HLA class I antigens are a frequent event in tumors and often associated with a poor prognosis of patients. The molecular mechanisms underlying these defects are mainly caused by deregulation of components of the HLA class I antigen processing machinery (APM) and HLA-G rather than by structural alterations. Epigenetic and transcriptional changes resulting in deficient HLA class I APM and altered HLA-G expression have been analysed, but little information exist about posttranscriptional and posttranslational control mechanisms. Using a combination of in silico analyses, high throughput technologies, RNA affinity purification combined with mass spectrometry microRNAs (miRNAs) and RNA-binding proteins have been identified to regulate HLA class I APM components and/or HLA-G and were of clinical relevance. In addition, oncogenic features, altered (interferon) signal transduction as well as changes in the metabolism control HLA class I APM component expression. Based on these results posttranslational mechanisms have not only an important impact on the regulation of HLA class I antigens, but also provide novel strategies to revert HLA class I-mediated immune escape.
Identification of tumor-specific rejection antigens for T cell receptor gene therapy

Gerald Willimsky

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T cell receptor (TCR) gene therapy, the grafting of antigen specificity onto patients' T cells followed by their use for cancer therapy, can be very effective. Choice of suitable target antigens and obtaining optimal-affinity TCRs are important parameters. Advanced methods of systems medicine have simplified the discovery of recurrent and individual-specific point mutations and small insertions/deletions in tumors that provide truly tumor-specific antigens (TSAs). We identify immunogenic TSAs repeatedly occurring in cancers of different origin as optimal target for TCR gene therapy providing the best possible risk-benefit ratio. We obtain TCRs from transgenic mice with a diverse human TCR repertoire restricted to HLA-A2. The generation of human TCRs is critical because mouse TCRs are immunogenic in humans, and the use of tumor-free mice avoids immunosuppressive effects on the priming of TSA-specific T cells that could alter the amplification of high-affinity T cell clones.

So far we demonstrated CD8+ T cell responses to a number of TSAs recurrently mutated in various human cancers or derived from tumor viruses. For example, TCRs either recognizing the 3rd most frequent mutation Rac1P29S in melanoma or specific for oncoproteins derived from human papilloma virus (HPV) have been isolated and are currently further characterized. TCRs that recognize immunogenic, processed and presented epitopes, and reject large experimental tumors, will be pursuit into the clinic.
Analyses of the anti-tumor T-cell repertoire in melanoma patients

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An effective cancer immunotherapy may depend on individualized monitoring and therapeutic approaches to identify and utilize the primary targets of individual patients’ T-cell responses. Using T cell-driven cDNA expression screening and cloning procedures and more recently whole exome and transcriptome sequencing we have performed in-depth analyses of the anti-tumor CD8+ T-cell repertoire in individual melanoma patients. Our analyses demonstrated a high degree of individuality and complexity for the cellular antitumor response in patients, which was due to HLA polymorphism and also to the existence of highly tumor-specific mutated antigens. Moreover, in the face of HLA class I loss in metastatic lesions leading to resistance towards conventional T-cell responses, we observed CD8+ αβ T cells recognizing autologous melanoma cells in an HLA-independent fashion via their T-cell receptors (TCR). These T cells lysed naturally occurring autologous melanoma cell variants exhibiting total loss of HLA class I expression. We began to explore the prevalence, diversity and potential relevance of HLA-independent, αβ TCR+ T cells recognizing tumor cells and to establish a basis for their use in immunotherapy.
Abstracts of Posters

(in alphabetical order)
Dynamics of tumor-reactive lymphocytes in the melanoma mouse model

*Lena Maetani Appel*

German Cancer Research Center / Theoretical systems biology / Heidelberg

Dynamics of tumor-reactive lymphocytes in the melanoma mouse model

In recent years immunotherapeutic approaches with the goal to harness the patient’s T cell response have become the standard therapy for many cancers. Still, the underlying mechanisms of the antitumor immune response are poorly understood which is reflected in the unpredictability of the individual patient’s response to a particular immunotherapeutic approach.

To further our mechanistic insights, we are devising a mathematical model of the anti-tumor T cell response in the B16-OVA melanoma mouse model. Following induction of the B16-OVA tumor and the transfer of naïve OT-I T cells which are able to recognize an OVA-derived peptide, their proliferation was monitored using the cell dye Carboxyfluorescein Diacetate Succinimidy Ester (CFSE) and cell numbers in all relevant compartments were determined (tumor, blood, draining and non-draining lymph nodes, spleen). In conjunction with mathematical modeling these data allow us to quantify the time-dependent proliferative activity of the tumor-infiltrating lymphocytes in these compartments and to study their migration behavior.

Preliminary results indicate that the proliferative activity of the transferred OT-I cells in the draining lymph nodes declines significantly well before the tumor growth is controlled by them while proliferation in the tumor remains rather constant and necessary to explain the high number of infiltrating OTI cells. Further experiments are devised and evaluated at the moment, which suggest that other mechanisms, like differences in the migration behavior of the involved T cell subsets, should as well be taken into account to explain the experimental results.
Systematic screening of cytotoxic agents targeting oncogenic signalling pathways for combinatorial immunotherapy in tumor models

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Combinations of immunostimulatory compounds and cytostatic agents are currently being extensively tested against different tumor types. Clearly synergistic anti-tumor responses are rare, however. Furthermore the impact of the compounds on the immune cells itself is insufficiently understood, as is the risk of unanticipated side effects of combination regimens. Therefore, our aim is to study combinatorial immunotherapeutic approaches in a systematic fashion by making use of robust in vitro and in vivo immune cell assays.

In order to select the most effective and immune response potentiating compounds, we are currently screening cytotoxic/cytostatic compounds, such as chemotherapeutic agents and small molecule inhibitors against MAPK or PI3K/AKT signaling for their efficacy against murine and human tumors cell lines and their effects on T cell proliferation and effector functions.

In tumor experiments combination of selected MEK inhibitors with an agonist CD40 antibody show T-cell dependent synergy in different syngeneic transplantable tumor models. In more advanced models, such as the autochthonous BRaf-driven model for metastatic melanoma, the effect is much less prominent, presumably due to a strongly immune suppressive tumor microenvironment and the lack of mutated neoantigens. In order to optimize our models with respect to prediction of clinical outcome, we are developing electroporation-based autochthonous tumor models, which are equipped with immunogenic foreign antigens and can be used to study the metastatic disease.
Stromal CD38 regulates progression of primary melanoma and generation of spontaneous metastasis

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The progression of primary melanoma, the deadliest skin cancer, and generation of metastasis is supported by tumor microenvironment (TME) which includes non-cancerous cells e.g., fibroblasts. Since the TME plays an important role in melanoma pathogenesis, its targeting is a promising therapeutic approach. Thus, it is important to identify proteins in the melanoma TME that may serve as therapeutic targets. Here we show that the nicotinamide adenine dinucleotide glycohydrolase CD38 is a suitable target for this purpose. Loss of CD38 in the TME as well as inhibition of its enzyme activity restrained progression of primary melanoma generated by two transplantable models of melanoma, B16F10 and Ret-mCherry-sorted (RMS) melanoma cells. Pathological analysis indicated that loss of CD38 increased cell death and reduced the amount of cancer-associated fibroblasts (CAFs) and blood vessels. Co-implantation of CD38 deficient fibroblasts together with B16F10 cells reduced, whereas wild-type fibroblasts increased, tumor progression, suggesting that CD38 in CAFs regulated melanoma growth. Loss of CD38 also inhibited spontaneous occurrence of RMS pulmonary and brain metastasis as well as B16F10 and RMS brain tumors progression after intracranial injection. Collectively, our results suggest that targeting CD38 in the melanoma TME provides a new therapeutic approach for melanoma treatment.
Experimental performance evaluation of T cell epitope prediction tools

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With the advent of tumor sequencing programs and checkpoint blockade agents, mutation-derived neo-epitopes have come into the spotlight as targets for anti-tumor immune responses. To assess if a mutation is likely to result in a neo-epitope, T cell epitope prediction servers are used for prediction of peptide binding to the human leukocyte antigen (HLA) molecules of the respective tumor patient. However, prediction results vary between available prediction servers, and there are vast differences in the accuracy of predictions for different HLA alleles.

The aim of this study was therefore to evaluate the performance of eight MHC class I prediction algorithms for important HLA alleles by experimental validation of potential epitopes. The servers were queried to predict potential 8-11mer epitopes of the model antigens HPV16 E6 and E7. This process was facilitated by development of a web-based tool that combines the prediction results in one sortable file.

Predicted peptides were synthesized and tested in competition-based cellular binding assays to determine the actual binding affinity. Based on acquired data, analysis of receiver operating characteristic (ROC) curves was performed and the area under the curve (A_{ROC}) was calculated as a measure of prediction quality. While predictions for HLA-A2 and -A3 were very accurate, predictions for HLA-A11 and -A24 were less precise. No single prediction server outperformed the others, but different prediction servers were found to be best for different HLA types and peptide lengths.

Based on these results, we recommend to always employing a minimum of two prediction servers to generate reliable results.
Blocking the Recruitment of Tumor-Associated Macrophages to Tumor Sites with Fully Human Antibodies

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Tumor-associated macrophages (TAMs) are one of the most abundant tumor infiltrating immune cells and have been found to be pro-tumorigenic, pro-angiogenic and immunosuppressive. A novel therapeutic approach to target TAMs is the blockage of macrophage recruitment to the site of the tumor. In this study, fully human antibodies are being developed to target key molecules which show strong chemotactic activity for macrophages and can lead to the entrapment of TAMs in the hypoxic tumor region. Thereby, macrophage migration should be ablated and the function of the innate immune system in cancer restored. Binding antibody fragments are being selected by means of phage display from lymph node derived antibody libraries. These libraries have the major advantage that the antibodies have undergone natural affinity maturation which can result in improved functional properties and specificities.
DNMT and HDAC inhibitors globally induce cryptic TSSs encoded in long terminal repeats

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Several mechanisms of action have been proposed for DNA methyltransferase and histone deacetylase inhibitors (DNMTi and HDACi); mainly based on candidate gene approaches. However, less is known about their genome-wide transcriptional and epigenomic consequences. By mapping global transcription start site (TSS) and chromatin dynamics, we observed the activation of thousands of cryptic, currently non-annotated TSSs (TINATS) following DNMTi and/or HDACi treatment. The resulting transcripts encode truncated or chimeric open reading frames translated into products with predicted abnormal functions or immunogenic potential. TINAT activation after DNMTi coincided with DNA hypomethylation and gain in H3K4me3, H3K9ac, and H3K27ac histone marks. In contrast, HDACi induced only canonical TSSs in association with histone acetylation, but TINATs via a yet unknown mechanism. Nevertheless, both inhibitors convergently induced unidirectional transcription from identical sites since TINATs are encoded in solitary long-terminal repeats of the endogenous retrovirus-9 family, epigenetically repressed in virtually all normal cells.
Novel p53 target genes secreted by the liver are involved in non-cell-autonomous regulation

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The tumor suppressor p53 is a transcription factor that prevents cancer development and is involved in regulation of various physiological processes. This is mediated both by induction of cell cycle arrest and apoptosis and by controlling the expression of a plethora of target genes, including secreted proteins. It has been demonstrated that p53 may exert its effect in non-cell-autonomous fashion by modulating the expression of genes that encode for secreted factors. In this study, we utilized our microarray data to identify and characterize novel p53 target genes expressed in human liver cells and associated with steroid hormones processing and transfer. We identified the steroid hormones binding factors, sex hormone binding globulin, corticosteroid-binding globulin and cytochrome P450 family 21 subfamily A polypeptide 2, as novel p53 target genes. Their expression and secretion was increased following p53 activation in various hepatic cells. We observed that p53 wild type mice exhibited higher levels of corticosteroid-binding globulin compared with their p53 null counterparts. We demonstrated that the induction of the steroid hormones binding factors can be mediated by binding to specific p53 responsive elements within their promoters. In addition, utilizing conditioned medium experiments we have shown that p53 dependent induction of sex hormone binding globulin secretion from liver cells enhances apoptosis of breast cancer cells. Moreover, depletion of sex hormone binding globulin abolished the induction of breast cancer cells death. The newly identified p53 target genes suggest a novel non-cell-autonomous tumor suppressive regulation mediated by p53 that is central for maintaining organism homeostasis.
Response to immune checkpoint blockade in experimental glioma

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Immunotherapy for brain tumors is entering the clinical arena with novel immunotherapeutic strategies which potentiate T cell responses by releasing T cell suppression using immune checkpoint inhibitors. However, clinical trials with these inhibitors indicate that only a fraction of patients respond to the therapy. Consequently, there is increasing interest in identifying biomarkers that predict the efficacy of immune checkpoint inhibitors in order to select for patients that benefit from checkpoint-targeted therapy. In this study, we investigated the efficacy of monoclonal antibodies targeting CTLA-4 and PD-1 in a syngeneic glioblastoma model. Subsequently, we assessed the infiltration of immune cell subsets (CD4 and CD8 effector T cells, regulatory T cells, macrophages and myeloid derived suppressor cells) as well as their phenotype in responder mice compared to non-responder mice. Here, we observed no significant difference in the CD8/CD4 T cell ratio of tumor infiltrating lymphocytes in responder and non-responder mice. However, depletion of CD4 T cells resulted in a complete abrogation of the therapeutic effect of immune checkpoint blockade, indicating a key role of CD4 T cells in response to therapy. Interestingly, the percentage of tumor infiltrating CD11b⁺ myeloid cells as well as CD11b⁺F4/80⁺ macrophages was significantly increased in non-responder mice compared to responder mice. Moreover, CD11b⁺ tumor infiltrating cells in non-responder mice showed a significant higher percentage of PDL1⁺ cells compared to responder mice suggesting the presence of a suppressive myeloid cell compartment in non-responder mice. In conclusion, this data points out the importance of understanding the mechanisms of checkpoint blockade therapy and proposes a complex interaction of myeloid cells and T cells as a key factor of therapy response.
Smelling Cancer Cells: A Potential In-vivo Diagnostic and Monitoring Device

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The development of non- or minimal invasive methods to detect cancer and monitor its treatment continues to be a major challenge in oncology. Circulating DNA and Tumor Cells (ctDNA and CTCs, respectively) are critical for understanding the biology of cancer and are promising to act as noninvasive biomarkers to effectively monitor disease progression and therapy response as well as to serve as a liquid biopsy in cases where tissue biopsy is unavailable. In the current study, we aim to develop an "in-vivo mobile lab" enable of sensing internal molecular alterations that are linked with the presence of CTCs and ctDNA. Mainly, analysis of volatile organic compounds (VOCs) released from- or in response to their incidence. The envisioned mobile lab shall have an ultra-miniaturized array of artificially intelligent nanoarrays with modified biocompatible membrane. The mobile lab that is based on ultra-small circuitry shall be connected wirelessly with external receiver. Preliminary studies have shown the artificially intelligent nanoarray can identify specific volatolomic signatures that are associated with pre-defined lung cancer mutations, including EGFR, ALK, KRAS and P53. These results and our ongoing experiments on blood samples are of great promise and may give us a non-invasive insight into the blood vessels, which eventually lead to better diagnostic and treatment tools.
Activity of engineered Antigen-specific T cells as a function of the relationship between affinity, avidity and antigen density

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The use of engineered Ag-specific T cells in Adoptive cell transfer therapies has recently gained significant focus due to initial clinical success. Such engineered cells are generated using a chimeric Ag receptor (CAR) based on common formats composed from Ag-recognition elements, such as αβ-TCR genes or Ab variable domain fragments, fused with T cell-signaling moieties. We recently combined these recognition elements using Abs that recognize peptide-MHC (TCR-like Abs), and compared such a high-affinity TCR-like Ab CAR to a native low-affinity TCR. Unexpectedly, the high-affinity CAR was less effective than the low-affinity TCR, suggesting an upper affinity threshold for TCR-based effective functional outcomes of engineered T cells. That is, exceeding this threshold leads to reduced functionality. To further characterize this affinity threshold and to achieve optimal T cell function, we characterized a series of anti-Tyr single chain fragment binding domains, ranging from 4nM to 1000nM Kd, and constructed Tyr-specific second generation CARs with ranging affinities. The interplay between CAR affinity, Ag density and CAR expression (i.e. avidity) and their effect on T cell functionality will then be studied. Hence, the precise combination of affinities and avidities (termed functional avidity) of T cells that lead to optimized functional outcome can be of crucial importance in adoptive cell transfer immunotherapies that utilize CARs.
Development of dendritic cell vaccination for combined melanoma immunotherapy

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Malignant melanoma is known for its fast progression and poor response to current treatments. Despite melanoma immunogenicity, the overall results of immunotherapeutic trials are largely disappointing, indicating that new approaches for melanoma treatment are urgently needed. Tumor escape could be due to a profound immunosuppression induced by chronic inflammation in the melanoma microenvironment, which is characterized by the long-term secretion of inflammatory mediators. Moreover, the development of melanoma-specific effector T cells may be hampered by insufficient tumor antigen delivery, processing and presentation. In the ret transgenic mouse model of spontaneous melanoma that mimics human melanoma development, we observed an accumulation of inflammatory factors and activated myeloid-derived suppressor cells (MDSCs) in melanoma lesions, which was associated with tumor progression. Upon administration of paclitaxel at ultra-low, non-cytotoxic doses in tumor-bearing mice, we demonstrated a reduction of inflammatory mediators in melanoma lesions together with decreased MDSC frequencies and immunosuppressive functions, leading to the restoration of T cell reactivity and prolonged mouse survival.

We have recently established the production of constructs encoding major histocompatibility complex (MHC) class I molecules that couples the peptide presentation and activation of dendritic cells (DC). We have generated such constructs encoding melanoma-associated antigens tyrosinase and tyrosinase related protein (TRP) - 1. Upon the construct electroporation into DC, cells were injected into naïve C57BL/6 mice. We detected a significant activation of CD8 T cell responses, reflected by increased specific killing of target cells in in vitro and in vivo assays. To complement our DC vaccine repertoire, we have developed and characterized MHC class II constructs encoding for tyrosinase and TRP-1 peptides. A strong stimulation of CD4 T cell response in C57BL/6 mice was detected in the proliferation assay. In addition, DC electroplated with MHC class I restricted constructs were applied in melanoma-bearing ret transgenic mice to evaluate tumor specific T cell activation and anti-tumor efficiency. Furthermore, developed DC vaccination will be combined with the neutralization of immunosuppression by ultra-low dose paclitaxel. We suggest that combined melanoma immunotherapy based on the simultaneous targeting of melanoma escape mechanisms such as insufficient anti-tumor T cell reactivity and immunosuppressive tumor environment could lead to a significant improvement of existing melanoma therapies.
SLAMF6 - roles and modes of action in normal immunity and cancer immunotherapy

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The SLAM family of receptors (SFRs) is a set of six receptors expressed only on hematopoietic cells. It has been recently shown by our lab that treatment of melanoma-reactive human CD8+ T cells with seSLAMF6 (soluble ectodomain of SLAMF6) results in a marked improvement of their capacity to proliferate, produce IFN-γ and mediate cytotoxicity in response to MHC I-matched (”cognate”) melanoma cells. seSLAMF6 also improves resistance of CD8+ T cells to reactivation-induced cell death. It also has a stimulatory effect on mouse CD8+ T cells in vitro, and stimulates marked melanoma tumor clearance in vivo.

We propose that, in normal CD8+ T cells, SLAMF6 is a bona fide inhibitory receptor suppressing T cell activation. It is triggered as a result of constitutive SLAMF6-SLAMF6 interactions between CD8+ T cells or at the surface of individual CD8+ T cells, thereby enabling SLAMF6 tyrosine phosphorylation and coupling of SLAMF6 to undetermined intracellular effectors. We hypothesize that this inhibitory effect is prevented by seSLAMF6, which disrupts SLAMF6-SLAMF6 interactions. Such an effect does not occur with anti-SLAMF6 MAb, because dimeric antibodies mimic or, perhaps, even enhance SLAMF6-SLAMF6 interactions.

To elucidate further the mechanism by which seSLAMF6 modulates CD8+ T cell functions, two approaches will be taken:

1a) intracellular effectors and targets of seSLAMF6 in CD8+ T cells: In the absence of SAP adaptors, SFRs are super-inhibitory as a result of augmented coupling to inhibitory effectors such as SHP-1, SHP-2, SHIP-1 or Csk. To clarify further the mechanism by which seSLAMF6 modulates CD8+ T cell functions, the impact of expression of these various inhibitory effectors on CD8+ T cell functions, in the absence or the presence of seSLAMF6, will be examined, using siRNAs targeting these effectors.

To identify the downstream intracellular pathways regulated by SLAMF6, we will study the impact of seSLAMF6 on various biochemical signals – including protein tyrosine phosphorylation, and activation of the kinases Erk, Akt, p38 and JNK.

1b) impact of Fc fusion variant of seSLAMF6 and anti-SLAMF6 MAb: To test further the notion that seSLAMF6 promotes CD8+ T cell responses because it is a monomeric reagent that blocks SLAMF6-SLAMF6 interactions, we will compare its impact to those of a dimeric variant of seSLAMF6 and a dimeric anti-SLAMF6 MAb.
Deep proteomic analysis of the response to immunotherapy in melanoma patients

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Up until recent years patients diagnosed with metastatic melanoma had very poor survival rates. However, recent developments in the immunotherapy field had improved dramatically their prognosis. T-cell based adoptive cell transfer (ACT) is one type of immunotherapy strategy, which involves in the transfer of ex-vivo expanded tumor infiltrating lymphocytes (TILs) back to the patient. Though the success rates are good, as any other treatment - not all patients respond well. In order to predict the response to the treatment, as well as to improve the success rates by combination therapies, it is important to understand the molecular mechanisms underlying the response to these treatments.

Here we used high-resolution mass spectrometry analysis to perform a deep proteomic profiling of tumor samples derived from stage IV melanoma patients that either respond or did not respond well to TILs treatment. We examined 42 tumor samples and quantified 8900 proteins with high confidence using SILAC-based (stable isotope labeling with amino acid in cell culture) quantification method. We then applied statistical analysis and advanced machine learning algorithms to portray the molecular pathways that differ between the two groups, which mostly focused on central metabolism. Finally, we set to validate our results using in-vitro models of patient derived tumor cell lines and their autologous TILs. Altogether we characterized the metabolic state that is associated with good response, aiming to further use it as a predictive tool in future treatments.
Therapeutic vaccination against HPV-induced tumors in a MHC-humanized mouse model

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Therapeutic vaccinations against the viral oncoprotein E7 of human papillomavirus (HPV)16 have shown efficacy against a model of cervical cancer in C57BL/6 mice. However, only murine epitopes can be studied in this model. To overcome this challenge in translatability, we use mice that are humanized for MHC molecules, called A2.DR1. In this model, we aim at the development of a vaccine formulation that effectively induces high numbers of HPV16-specific CD8+ T cells to mediate anti-tumor effects.

We make use of minimal epitopes to induce only immune responses against epitopes that have been proven by mass-spectrometry to be naturally presented on HPV16+ human cancer cells. Our vaccination approaches include emulsion-based vaccines, RNA vaccines, constructs exhibiting amphiphilic properties, silica-nanoparticles and the use of various TLR-agonists together with the minimal epitope HPV16 E711-19.

Our results show that robust immune responses against this HLA-A2-restricted epitope can be induced in A2.DR1 mice. The numbers of E711-19-specific CD8+ generated by our different vaccination formulations vary greatly, with amphiphilic constructs showing the most promising responses to date.

Additionally, we develop an A2.DR1-compatible HPV16 E7-expressing tumor model. We propose our preclinical vaccination experiments in this A2.DR1 model as a means to achieve improved translatability, due to the possibility to use the same epitopes for vaccination as would be used in humans.
Activated CD8+ T cells can be a vaccine in triple negative breast cancer

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Triple negative breast cancers which is 15\% of all breast cancers, are characterised by a lack of ER\textalpha, PR expression and HER2 overexpression. The triple negative breast cancers are associated with high mortality rates due to both the aggressive nature of the disease and limited treatment options available.

The tumor cells often activates an antitumor immune response and is detected by both T and B cell antitumor immune responses. Such as, immune cells infiltrating human tumors are detected in many different tumors, although the prognostic significance of infiltrates varies among tumor type, tumor-infiltrating lymphocytes (TIL) contain tumor-specific T cells. In our work will emphasis on CD8+ T cells. Through activation in-vitro of CD8+ T cells with tumor sepecific antigens, we will follow the response of the tumor cells to the activated CD8+ T cells.
Cancer-Associated Fibroblasts inhibit T cell mediated killing of breast cancer cells

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Breast cancer is a leading cause of death in women in the western world. Breast tumors are characterized by a rich stromal compartment, prominently populated by fibroblasts. Cancer-associated fibroblasts (CAFs) are key players in the tumor microenvironment of many types of cancer, involved in a variety of processes that promote tumor initiation and progression. Evading immune destruction is a widely accepted hallmark of cancer, and tumors are generally characterized by an immunosuppressive milieu. T cells are key players in the process of tumor cell recognition and killing, and their functionality can tip the balance between tumor elimination and tumor escape. CAFs were suggested to affect T cell immunosuppression, but their direct effect on T cells has not been elucidated. Here we demonstrate that CAF-derived secreted factors inhibit the ability of T cells to kill tumor cells in vitro, while secreted factors derived from normal fibroblasts did not affect T cell functionality. Moreover, CAF-derived factors induced in T cells a downregulation in the expression of cytotoxicity-associated cytokines, IFN-γ and TNFα. These results suggest that CAFs have a direct immunosuppressive effect on T cells, and emphasize the need for elucidating the interactions between these two cell types for the improvement of cancer immunotherapies.
Genetic random DNA barcode generator for in vivo cell tracing

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Fate mapping is a powerful method commonly used for the analysis of lineage relationships in multicellular systems such as tumors. Current techniques however are limited in numbers of markers (e.g. fluorescent proteins) or require manipulations of the cells (e.g. retroviral transfection, transplantation) and therefore have a shortage in resolution or may not reflect physiological conditions. To overcome these limitations, we have devised a system that combines the advantages of non-invasive Cre-mediated in vivo labeling with high resolution DNA barcoding. We generated mice that carry an artificial DNA substrate termed *Polylox* that is randomly recombined upon Cre-recombinase induction. This new tool provides a diversity of thousands of barcodes enabling the tracing of cell populations at single-cell level. We applied it for endogenous barcoding of hematopoietic stem cells (HSCs). At this research school, I would like to share our most recent findings on HSC differentiation and lineage potential realized under unperturbed conditions in vivo. In addition, it will be considered how the barcoding system could be applied to trace tumor progression and to address questions on clonal heterogeneity of primary tumors, metastatic and relapsing tumor cells. It would be a great opportunity to discuss these experiments and our data with the expert lecturers and participants of this cancer school.
The T cell receptor (TCR) controls the cellular adaptive immune response to antigens, but our understanding of TCR repertoire diversity and response to challenge is still incomplete. For example, TCR clones shared by different individuals with minimal alteration to germline gene sequences (public clones) are detectable in all vertebrates, but their significance is unknown. Although small in size, the zebrafish TCR repertoire is controlled by processes similar to those operating in mammals. Thus, we studied the zebrafish TCR repertoire and its response to stimulation with self and foreign antigens. We found that cross-reactive public TCRs dominate the T cell response, endowing a limited TCR repertoire with the ability to cope with diverse antigenic challenges. These features of vertebrate public TCRs might provide a mechanism for the rapid generation of protective T cell immunity, allowing a short temporal window for the development of more specific private T cell responses.
Hepatitis C Virus-induced OX40L on monocytes activates Natural Killer cell proliferation and IFN-γ production

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Natural Killer (NK) cells are important effector cells in Hepatitis C Virus (HCV) infection, a virus that chronically infects around 3% of the world population and is a major cause of liver disease and hepatocellular carcinoma. The exact mechanisms, however, through which NK cells are activated in response to HCV remain elusive.

Using the well-established HCV replicon cell-culture model we show that after co-culture of HCV replicon-carrying Huh6 hepatoblastoma cells with peripheral blood mononuclear cells (PBMCs), NK cells increase expression of the high-affinity IL-2 receptor chain CD25, proliferate rapidly and produce IFN-γ. Activation of NK cells was dependent on IL-2, most likely produced by T cells and on cell-cell contact mediated signals from monocytes. Monocytes from replicon-carrying co-cultures showed increased expression of OX40L and concurrently its receptor OX40 was increased on NK cells. Blocking of OX40L in those co-cultures abrogated the virus-induced activation and effector functions of NK cells.

Together, our data reveals a novel mechanism of monocyte mediated NK cell activation against virus infected cells involving the OX40/OX40L axis with potential relevance for therapeutic intervention.
Adenosine A1 receptor dysfunction is associated with leukopenia: a possible mechanism for sepsis-induced leukopenia

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Introduction: Most patients survive the initial hyper-inflammatory phase of severe sepsis and reach an intensive care unit with immunosuppression. Adenosine, a potent modulator of inflammation and immunity is strongly elevated in blood of septic patients. We have previously shown that adenosine A1 receptor (A1R, Gi receptor) dominates the pro-inflammatory phase of bacterial peritonitis and that activation of this receptor by a specific agonist induces A2AR (Gs) expression and dominance during the resolution phase of inflammation. In this study we aimed to elucidate the role of adenosine and its receptors in sepsis-associated leukopenia. We hypothesized that elevated adenosine levels in sepsis affects the normal development of lymphocytes through down-regulation of A1R.

Methods: Polymicrobial severe sepsis was induced in C57BL/6 (C57) mice by cecal ligation and puncture (CLP) with an 18G needle. Blood and bone marrow (BM) cell counts, as well as flow cytometry were performed at 24 h post sepsis induction.

Results: CLP-treated mice exhibited significantly lower number of WBC compared to sham controls (9.15±2.65 vs. 3.21±1.58 cells x103/µl). Sham A1R-/- mice showed lower WBC counts compared to sham WT littermate (4.5±3.24 cells x103/µl, p<.05). No significant difference was observed in WBC count between the sham A1R-/- and CLP WT groups. Similarly, desensitization of A1R by agonist (CCPA) or elimination of A1R with A1R antagonist (DCPCX) were associated with leukopenia. The T-cell was the main cell population affected by CLP and A1R elimination. Apoptotic rate of nucleated BM cells in sham A1R-/- mice was similar to the rate observed in WT sham group, and almost 2-fold greater than the early apoptosis rate (Annexin V+, 7-ADD-) shown in WT sham group (3.64±1.23% vs. 1.86±0.59%, respectively, p<.05). Interestingly, CLP-A1R-/- mice were shown to produce less interleukin (IL)-15 in lavage fluid compared to CLP-WT mice (8.8±6.0 vs. 35.3±9.8 pg/ml, respectively p<.001). Conclusions: The similarities in the phenotype induced by sepsis and suppression of A1R support our hypothesis that dysfunction/down-regulation of the Gi-A1R at the onset of sepsis changes the effect of adenosine towards Gs-A2AR/A2BR-mediated leukopenia and immune paralysis. Suppression of IL-15 might be a part of the mechanism of leukopenia observed in CLP-treated mice and A1R-/- mice.
PTEN loss, epigenetics and immune escape in melanoma.

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Based on our previous findings and the current literature we hypothesize, that a loss of phosphatase and tensin homolog (PTEN) leads to PI3K/AKT pathway dependent and independent immune escape mechanisms, involving rearrangements in the epigenetic landscape of melanoma cells that will be further examined in this project.

We screened 11 human melanoma cell lines for the expression of PTEN protein, the activation status of the PI3K/AKT pathway and the mRNA expression of a variety of immune-modulatory genes like checkpoint ligands, NKG2D ligands and components of the antigen presenting machinery. We observed a correlation between the loss of PTEN protein expression and the mRNA levels of the checkpoint ligand PD-L1, as well as a correlation between the phosphorylation of AKT and the mRNA expression of the NKG2D ligand ULBP-4 in those melanoma cell lines in vitro.

To examine the exclusive roles of PTEN loss and PI3K/AKT pathway activation in more detail, we are currently establishing a CRISPR/Cas mediated knock down of PTEN and the introduction of an AKT activating mutation (E17K), in those cell lines.

To study the complex interaction of PTEN loss and immunogenicity in melanoma, we will employ a spontaneous melanoma mouse model (LLA-TG3). LLA-TG3 mice have a fully functional immune system and spontaneous immune responses against LLA-TG3 tumors have been described. Moreover we developed an inducible melanocyte specific PTEN knock out in these mice (LLA-TG3/PTEN°°/°°/Tyr::Cre). This model enables us to study a potential feedback regulation and inter-dependence between PI3K/AKT signaling, epigenetic rearrangements and immune escape mechanisms in melanoma in vivo.
Survival in acute myeloid leukemia is associated with NKp44 splice variants

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NKp44 is a receptor encoded by the NCR2 gene, which is expressed by cytokine-activated natural killer (NK) cells that are involved in anti-AML immunity. NKp44 has three splice variants corresponding to NKp44ITIM+ (NKp44-1) and NKp44ITIM- (NKp44-2, and NKp44-3) isoforms. RNAseq data of AML patients revealed similar survival of NKp46+NKp44+ and NKp46+NKp44- patients. However, if grouped according to the NKp44 splice variant profile, NKp44-1 expression was significantly associated with poor survival of AML patients. Moreover, activation of PBMC from healthy controls showed co-dominant expression of NKp44-1 and NKp44-3, while primary NK clones show more diverse NKp44 splice variant profiles.

Cultured primary NK cells resulted in NKp44-1 dominance and impaired function associated with PCNA over-expression by target cells. This impaired functional phenotype could be rescued by blocking of NKp44 receptor. Human NK cell lines revealed co-dominant expression of NKp44-1 and NKp44-3 and showed a functional phenotype that was not inhibited by PCNA overexpression. Furthermore, transfection-based overexpression of NKp44-1, but not NKp44-2/NKp44-3, reversed the endogenous resistance of NK-92 cells to PCNA-mediated inhibition, and resulted in poor formation of stable lytic immune synapses.

This research contributes to the understanding of AML prognosis by shedding new light on the functional implications of differential splicing of NKp44.
A double-humanized patient-derived xenograft mouse model for the assessment of immunotherapy drug effects and durability

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While millions of bases in the genome are found to be mutated in cancers, only a few hundreds have associated phenotype and clinical significance. Thus, deciphering the cancer genome is a bottleneck in the utility of cancer genomics. Patient-derived xenografts (PDXs) are an ideal tool to associate clinical features with observed mutations and treatments, as they produce quicker and cheaper results without patient related toxicity. Unfortunately, these models are oversimplified, and lack elements of the host, specifically immune surveillance and metastatic spread through blood vessels. In addition, due to known heterogeneity of cancer cells, the fidelity of PDXs derived from physical tissue resection could be misleading.

An improved humanized PDX model utilizing the same-patients' immune system will be incorporated to develop a double-xenograft model combining same-patient tumor (represented by tumor biopsy material and circulating tumor cells from peripheral blood) and a immune system following hematopoietic stem cell transplantation. This model will provide a novel basis for comparison of drug effects and will enable multifaceted comparison of combined anti-cancer treatment efficacy, including immunotherapy drugs. Currently, experiments are underway to determine the effects of Keytruda as well as experimental immunomodulation compounds on tumor biology in the double-humanized PDX model.

This research aims to develop an advanced personalized mouse model, which incorporates metastatic and immune surveillance features of the cancer patient, for assessing targeted therapy in the current era of personalized medicine.
The stress kinase GCN2 does not mediate suppression of antitumor T cell responses by tryptophan catabolism in experimental melanomas

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Tryptophan metabolism is a central pathway in regulation of immune responses and is known to shape the immunosuppressive tumor microenvironment. During the past decade inhibitors targeting the rate-limiting enzymes that mediate tryptophan depletion, namely indoleamine-2,3-dioxygenase (IDO) and tryptophan-2,3-dioxygenase (TDO), have entered clinical trials. Tryptophan depletion in the local microenvironment by IDO and TDO is generally regarded as the key immunosuppressive effector mechanism. In T cells, the stress kinase general control non-derepressible 2 (GCN2) has been identified as a molecular sensor of tryptophan deprivation that induces apoptosis and mitigates T cell proliferation upon activation.

We investigated whether GCN2 attenuates tumor rejection in experimental B16 melanoma using T cell-specific Gcn2 knockout mice. Our data demonstrate that GCN2 in T cells did not affect immunity to B16 tumors. Even augmentation of tumoral tryptophan metabolism in B16 tumors by Tdo overexpression did not differentially affect GCN2-proficient vs. GCN2-deficient T cells in vivo. We found that GCN2 target genes were not upregulated in tumor-infiltrating T cells and the novel technique MALDI MS imaging revealed that, despite high turnover rates, intratumoral tryptophan concentrations do not drop to levels sufficient to activate GCN2.

In conclusion, our results demonstrate that the molecular tryptophan sensor GCN2 is dispensable for suppression of T cell-mediated tumor rejection.
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