Challenging Notch Signaling in B-cell Chronic Lymphocytic Leukaemia

Josipa Skelin¹, Lidija Milkovic², Biljana Jelic Puskaric³, Ika Kardum-Skelin³, Maja Matulic⁶, Delfa Radic-Kristo⁴,⁵, Mariastefania Antica¹

¹Division of Molecular Biology, Ruđer Bošković Institute, Croatia
²Division of Molecular Medicine, Ruđer Bošković Institute, Croatia
³Department of Clinical Cytology and Cytogenetics, Merkur University Hospital, Croatia
⁴Hematology, Merkur University Hospital, Croatia
⁵School of Medicine, University of Osijek, Croatia
⁶Faculty of Science, University of Zagreb, Croatia

Background

B-cell chronic lymphocytic leukaemia (B-CLL) is a lymphoproliferative disorder renowned by the accumulation of malignant cells blocked in the progression of differentiation. The number of patients is expected to grow with population aging, and, considering the heterogeneity of B-CLL, the priority in research should be defining the molecules and signaling pathways underlying its development and progression. NOTCH1 has been implicated in T-ALL, but its influence on B-CLL has still to be determined. Further, B-CLL has been characterized by an increased expression of AIOLOS, an important transcription factor in lymphopoiesis, which has been involved in survival of mature neoplastic B-cells.

Objective

The aim of our study is to find a relationship between RNA and protein expression of NOTCH1, its downstream target genes HES1 and DELTEX and AIOLOS in B-CLL patients and to determine the leukemic survival when challenged by receptor-ligand interaction in a coculture with cells expressing Delta like1.

Methods

Lymphocytes were separated on a density gradient. mRNA was quantified by qPCR. Protein expression was determined by flow cytometry. Cocultures of B-CLL samples with OP9DLL1 cells.

Results

The NOTCH1 receptor was expressed in all but one sample, with its levels being lower than in PBL or sorted B-cells of healthy individuals. Its intracellular domain was detected on a protein level, showing activation in all samples but surprisingly HES1 was mostly not active. DELTEX1 expression was variable and, in most cases multiple times higher than that of controls. AIOLOS was elevated as we previously described in B-CLL samples. However, it could not be correlated with protein expression, suggesting posttranslational modifications. Conclusion: Based on the lack of expression of HES1 and the variability of DELTEX, in B-CLL samples we conclude that the Notch pathway is non-canonically activated. The coculture system could discriminate cell populations susceptible to apoptosis by Notch activation.
Some Biochemical Parameters and Their Relation with Tumor Marker Ca19.9 – Our Experience

Slobodan Dunjic¹, Dusan Vesovic², Jovana Pejic¹, Emilija Golubovic³, Milos Maletic¹, Petar Krakovic¹

¹Holistic Center, Center for Integrative procedures and Supplements “Dr Dunjić”, Serbia
²Preventive Medicine, VISAN -- Sanitary Medical School of Applied Sciences, Serbia
³Surgery Department, Institute for Radiology and Oncology of Serbia, Serbia

Background
CA 19-9 is used to help to monitor the efficacy of treatment response and/or recurrence of biliopancreatic malignancies. No every patient with pancreatic cancer will have an elevation of CA 19-9.

Objective
The aim of the paper is to present relation that we found between tumor marker Ca19.9 and some biochemical parameters.

Methodology
Laboratory analysis of both gender patients was statistically analyzed. Patients were divided into two groups: group with level of Ca19.9 within normal range (NR), and patients having elevated level of Ca19.9 (EL). None of patient was diagnosed with pancreatic tumor. All of patients were recruited from Center for Integrative Procedures and Supplements “Dr Dunjić”, Belgrade (the Center). Holistic-personalized approach in patients healing was performed in the Center. Statistical analysis was done by using Statistical Package for Social Sciences (SPSS).

Results
Total number of examines was 35. Mean age was 54,6+/-17,4yrs (minimum age was 25 yrs, maximum was 68 yrs). When gender is considered, mean age for females was 55,0+/-12,4 and for males was 53,1+/-8,6; no statistical significance related to age was found between groups. Numerous biochemical parameters have shown statistically significant difference between two groups examined – NR group vs. EL group.

The carcinoembryonic antigen – CEA: 1,78+/-0,8 vs. 24,8+/-3,6; p=0,026; df=1. Oxytocin level: 213,7+/-17,4 vs. 886,2+/-32,0; p=0,041; df=3. Level of 8-hydroxy-2`-deoxyguanosine (8-OHdG): 13,0+/-2,12 vs. 64,1+/-3,5; p=0,002; df=2. Vitamin D level was lower than recommended in both groups studied: 52,6+/-15,6 vs. 46,3+/-12,1. However, no statistically significant difference was found between groups.

Conclusion
These findings point that imbalance in human body, which is represented with Ca19.9 elevation, can be accompanied with numerous biochemical changes, such are CEA, oxytocin, and 8-OHdG. All parameters mentioned tend to be elevated, except for vitamin D. However, the clinical importance of these findings requires further research.
Predictive Personalized Monitoring of Tryptophan Metabolism and Neopterin in the Treatment of Cancer with Immune Checkpoint Inhibitors

Johanna Gostner¹, Simon Geisler², Marlies Stonig¹, Dietmar Fuchs²

¹Medical University of Innsbruck, Division of Medical Biochemistry, Biocenter, Austria
²Medical University of Innsbruck, Division of Biological Chemistry, Biocenter, Austria

Background

A subgroup of patients with cancer remains treatment refractory. This is likely due to the immunosuppressed status, which involves stromal cells, humoral mediators, and suppressive checkpoint molecules. Current clinical trials are focusing on how immunosuppressive conditions might be overcome using immune checkpoint inhibitors (ICI). ICI are likely key agents for cancer treatment in future. To reduce the number of treatment failures it will be important to define predictive biomarkers.

Objective

Degradation of the essential amino acid tryptophan via enzyme indoleamine 2,3-dioxygenase (IDO-1) represents an important strategy of immune response to limit growth of invading pathogens and tumor cells. IDO-1 is activated upon stimulation with T helper (Th) type 1 cytokine interferon-gamma (IFN-γ) in human monocytes/macrophages, dendritic cells (DCs), but also other cell types including tumor cells. In parallel, formation of the immune activation biomarker neopterin is induced upon IFN-γ-dependent mechanisms in human monocyte-derived macrophages and DC.

Methods

Neopterin levels can be assessed by ELISA. IDO-1 activity can be estimated by determining the kynurenine to tryptophan ratio (Kyn/Trp) in body fluids such as serum/plasma using HPLC or ELISA.

Results

Increased Kyn/Trp and neopterin concentrations parallel the course of malignant diseases and bear prognostic potential in patients suffering from gynecological cancer, colorectal cancer, malignant melanoma and hematological neoplasias.

Conclusion

Tryptophan deprivation can diminish T cell response, and thus, IDO-1 activity provides a negative feedback loop to control immune activation. Several immunosuppressive mechanism were reported to be associated with IDO-1 activation such as the induction of regulatory T-cells (Tregs) and of a regulatory DC phenotype in the presence of T-cells expressing cytotoxic T-lymphocyte associated antigen 4 (CTLA4). Development of immunodeficiency in cancer patients may emerge as a long-term side effect of anti-proliferative mechanisms during Th1-type immune response.

The combined determination of Kyn/Trp and neopterin is be able to identify the ICI status in cancer patients and should allow to identify treatment responsiveness of patients early.
Background

HNSCCs (Head-and-Neck-squamous-cell-carcinomas) are known to be among the most immunomodulating tumors, including possible effects on tumor growth through angiogenesis and cell migration. As we have previously shown the benefit of specific treatment of distant metastasis, we now aim to improve the diagnostic path regarding the metastasis detection through examining both the metastasis and primary tumor microenvironment. As the microenvironment is closely reviewed in leukemia we looked for cell markers already established in these tumors to find new diagnostic approaches.

Objective

By comparing the tumor microenvironment of patients with metastasis to patients without metastasis regarding their cell marker expression profiles we investigated possible microenvironmental influences on the formation of metastasis.

Methods

We analyzed tissue samples of 147 HNSCC patients, treated in the years 2000-2017 retrospectively with varying tumor locations and metastasis, reflecting the diversity of HNSCCs. We compared the patients with metastasis and those without. We used multiple immunohistochemically staining on tissue micro arrays including cell markers for T-, B- and Nk-cells to detect differences in the immunactive microenvironment.

Results

There was a marked difference in the expression of immunoactive cells between the different tumor localizations as well as between the degree of metastasis.

Conclusion

Our findings suggest a relevance of the tumor microenvironment in the formation of the metastasis. To use these differences in a diagnostic way, such as a immunohistochemically staining for staging, further research is required.
Unraveling the Tumor-Immune Microenvironment in Poor Outcome Pediatric Ependymoma

Andrea Griesinger, Andrew Donson¹,², Andrea Griesinger¹,², Davis Witt¹,², Lindsey Hoffman¹,², Ashley Yingst², Anandani Nellan¹,², Bridget Sanford², Jean Mulcahy Levy¹,², Kenneth Jones², Michael Verneris²,³, Nicholas Foreman, Nicholas Foreman¹,²

¹The Morgan Adams Foundation Pediatric Brain Tumor Research Program, Children's Hospital Colorado, USA
²Pediatrics, University of Colorado Denver Anschutz Medical Campus, USA
³Bone Marrow Transplant and Cellular Therapy, Children's Hospital Colorado, USA

Background

Ependymoma (EPN) is a brain tumor with poor survival in childhood. We previously demonstrated that immune factors are associated with survival. Most EPN occur in the posterior fossa and consist of two molecular subgroups (PFA and PFB) with distinct immune gene phenotype. We showed PFB patients develop an anti-tumor immune phenotype and are curable. In contrast, we found PFA have pro-tumor myeloid gene signature and have poor survival. As immune factors impact survival in PFA, immunotherapy development for this tumor subgroup is a rational strategy.

Objective

We hypothesize that a profound understanding of PFA immunobiology, viewed as a dynamic system, is needed for immunotherapy to truly be effective.

Methods

We used RNA-seq on snap frozen patient samples, flow cytometry characterization of immune populations, and co-culture experiments.

Results

Epigenetics of the PFA tumor has a profound effect on IL-6 secretion by the cell and this in turn has significant effects on the adverse myeloid phenotype. Myeloid cells exposed to media supernatant collected from PFA tumor cell lines, upregulated STAT3 signaling, inducing the secretion of IL-8. This was dependent on IL-6 as blocking with Tocilizumab attenuated this response. Lymphocytes exposed to the PFA myeloid microenvironment showed significant upregulation of PD1+TIM3+ surface markers suggesting exhaustion. Consistent with this, we found the T-cells were deficient in INFγ secretion following CD3/CD28 bead stimulation and the NK-cells had significant impairment in degranulation of tumor cells.

Discussion

Much immunobiology of pediatric brain tumors, including EPN, was inferred from adult glioblastoma. Our data shows that the immunobiology of EPN is very different from adult glioblastoma and tumor specific studies. Our studies have shown that the immunobiology of EPN is complex with interdependence between tumor cell, myeloid and lymphocyte populations and intervention at one point may have unpredictable effects at another likely leading to resistance to immunotherapy.
Immune Checkpoints in Metastatic Colorectal Cancer Prognosis and Prevention

Ehud Hauben¹, Kevin A Fenix¹, Chandra Kirana¹, Richard S Stubbs², Guy J Maddern¹

¹Department of Surgery, The University of Adelaide, Liver Metastasis Research Group, The Basil Hetzel Institute, Australia
²Department of Surgery, The Wakefield Clinic for Gastrointestinal Diseases, New Zealand

Colorectal cancer (CRC) is a major cause of morbidity and mortality throughout the world and is the second most common cancer in both men and women in Australia. The majority of CRC related deaths are attributable to liver metastasis - the most critical prognostic factor observed in CRC patients. Mounting evidence indicates that peripheral and local immune mechanisms can either support or prevent colorectal liver metastasis; however, to date there is no clinical test to predict metastatic risk in CRC patients and to allow informed selection of preventive treatment regimen. The challenge, therefore, is to identify the immune checkpoints controlling metastatic invasion, to enable individualized risk assessment and early therapeutic intervention. The aim of our research group is to utilise proteomic and immunological techniques to investigate the association between specific immune mediators and distinct CRC disease progression patterns, and to determine the functional role of these mediators in the control of colorectal liver metastasis. Indeed, we have previously identified prognostic biomarkers of metastatic progression in CRC patient blood and tissue samples. Here, using flow cytometric analysis of bowel and liver tumour biopsies collected from CRC patients, we found that both primary and secondary CRC tumours are significantly enriched with tissue-resident memory CD8+CD103+ T (Trm) cells. Notably, tissue microarray analysis of primary CRC tumour biopsies (n=300) found in TMN stage II CRC patients significant correlation between the presence of Trm cells and shorter liver metastasis free survival. We also observed poor cancerspecific overall survival in TMN stage II CRC patients with higher numbers of tumour infiltrating Trm cells. These data suggest that early exposure of primary tumour cells to active immunity results in selection of clones with resistant phenotype and higher metastatic potential.
Poster Board #7

**A Highly Sensitive Fluorescence Immunoassay for the Biomarker ASPORIN as Potential New Tool for Oncology-Research**

Gerhard Hawa, Teresa Jungwirth, Albert Missbichler

*R&D, FIANOSTICS GmbH, Austria*

ASPORIN, like decorin or biglycan is a member of the SLRP (small leucine-rich repeat proteoglycans /proteins) protein family. In addition to the well-known collagen binding properties of those SLRPs, ASPORIN is also a potent inhibitor of TGF-β. Several studies indicated that this protein may be a valuable biomarker for the progression and outcome of breast-, prostate- and colorectal cancer progression. However studying this biomarker so far was hindered by insufficient sensitivity of currently available assays. Therefore we decided to use our recently developed high sensitivity fluorescence immunoassay platform based on plasmonic micro titer plates (MEF-MTPs) to provide a reliable ASPORIN assay as new tool in oncology research. We hereby present a small pilot study in a blood donor cohort. Further studies will reveal the value of serum ASPORIN measurements in cancer patients.
C-reactive Protein Binds to Integrin α2 and Fcγ Receptor I, Leading to Breast Cell Adhesion and Breast Cancer Progression

Eun-Sook Kim¹, Sun Young Kim¹, Minsoo Koh¹, Hye-Min Lee¹, Kyoungmee Kim¹, Joohee Jung¹, Hoe Suk Kim², Woo Kyung Moon², Sejin Hwang³, Aree Moon¹

¹Duksung Innovative Drug Center, College of Pharmacy, Duksung Women’s University, South Korea
²Department of Radiology, Seoul National University Hospital, South Korea
³College of Medicine, Hanyang University, South Korea

Background

C-reactive protein (CRP) is an acute phase protein synthesized upon the inflammatory responses, associated with breast cancer. The process of tumor cell invasion and metastasis involves the adherence of cells to the extracellular matrix via integrin as a receptor for matrix molecules.

Objective

The present study investigated the role of CRP in the adhesive phenotype of breast cells and the underlying mechanisms.

Methods

Adhesion assay was used to assess the adhesive capacity of breast cells. The role of CRP in the in vivo tumor growth was determined using an orthotopic mouse tumor model with MDA-MB-231 cells.

Results

CRP induces adhesion of MCF10A breast epithelial cells through the activation of integrin α2 signaling. Expression of integrin α2 was induced by CRP in which transcription factors c-fos and SP1 may be involved. Binding of CRP with integrin α2 leads to the activation of focal adhesion kinase, paxillin and ERKs. CRP also binds to an Fcγ receptor I (FcγRI), and induces activation of paxillin, FAK and ERKs. Integrin α2 and FAK have crucial roles in the adhesive and invasive phenotypes as well as MMP-9 upregulation induced by CRP in MCF10A cells. Treatment with an inflammatory lipid sphingosine-1-phosphate induced CRP, which may be secreted and exert an autocrine effect by binding to FcγRI and integrin α2. Involvement of CRP in adhesion, invasion, anchorage-independent growth and upregulation of integrin α2, paxillin and FAK was observed in MDA-MB-231 triple-negative human breast cancer cells. We showed that CRP has an important role in intravasation and tumor growth in vivo, demonstrating the in vivo relevance of our in vitro results.

Conclusion

The present study elucidates a critical molecular basis between CRP, integrin α2 and FcγRI pathways in MCF10A and MDA-MB-231 TNBC cells, thereby providing useful information on CRP-induced aggressiveness of breast cells in the inflammatory microenvironment.
Poster Board #9

Changes of TCR Repertoire in Metastatic Renal Cell Carcinoma Patients Treated with Nivolumab

Martin Klabusay, Jan Skacel, Bohuslav Melichar

Department of Oncology, Palacky University, Czech Republic

Background

Renal cell carcinoma is the 10\textsuperscript{th} most common cancer with growing incidence rates. New drugs approved for treatment of metastatic renal cell carcinoma (mRCC) opened possibilities for management of these patients. Nivolumab, anti-programmed-death 1 protein (PD1) antibody selectively blocks the interaction between PD-1 and its ligand PD-L1 and enables a restart of the immune response against cancer cells, significantly improving survival in both treatment-naïve and pretreated patients with mRCC.

Objective

It was hypothesized that proliferation of specific T cell clones may be associated with response to anti-PD1 therapy. The aim of this study was to analyze T cell repertoire in mRCC patients treated with nivolumab.

Methods

Blood samples of 15 patients with mRCC were evaluated and compared to healthy controls. All patients were treated with nivolumab in 2\textsuperscript{nd} to 5\textsuperscript{th} line after prior interferon α, sunitinib, pazopanib, everolimus or axitinib. Mononuclear cells were isolated from the peripheral blood on Histopaque. Cells were stained with directly labeled anti-CD3 PerCP-Cy5.5, anti-CD4 APC, anti-CD8 APC-Cy7 and anti-TCR FITC and PE antibodies. In total, 24 Vβ TCR families were evaluated. Measurement was performed by multicolor flow cytometry. Data were analyzed with Wilcoxon non-parametric test.

Results

Significant changes in following TCR families (p<0.05) were confirmed: an increase of CD4\textsuperscript{+} cells Vβ3, Vβ12 and CD8\textsuperscript{+} cells Vβ8, a decrease of CD4\textsuperscript{+} cells Vβ1, Vβ13.6, Vβ18, Vβ22 and CD8\textsuperscript{+} cells Vβ1, Vβ7.1, Vβ13.2, Vβ14, Vβ21.3. In individual patients, increased populations of CD4\textsuperscript{+}Vβ9 (25.7\%) together with CD8\textsuperscript{+}Vβ3 cells (25.7\%) and CD4\textsuperscript{+}Vβ16 (20.1\%) together with CD8\textsuperscript{+}Vβ13.1 cells (29.6\%) were observed.

Conclusion

Changes in TCR repertoire were observed in patients treated with nivolumab with long-term stable mRCC. Very high levels of CD4 and CD8 lymphocytes with defined TCR Vβ families were identified in several patients. Further research is needed to clarify whether these observed changes are associated with outcome.
Patterns of TIGIT Expression in Normal Lymphatic Tissue, Inflammation and Cancer


1Department of Pathology, University Medical Center Hamburg-Eppendorf, Germany
2Oncology, Dianova GmbH, Germany
3Department of General, Visceral and Thoracic Surgery, University Medical Center Hamburg-Eppendorf, Germany

Background

TIGIT (T cell immunoreceptor with Ig and ITIM domains) is an inhibitory immune checkpoint receptor that was investigated as an immunotherapeutic target.

Objective

To study patterns of TIGIT expression in normal, inflammatory and cancerous human tissue samples.

Methods

Brightfield and multiplex fluorescence immunohistochemistry were employed to measure relative expression of TIGIT, PD-1 and standard lymphocyte markers in a “microenvironment tissue microarray” containing 4mm tissue spots from lymph nodes of healthy and HIV infected individuals, normal tonsils, Hashimoto thyroiditis, sarcoidosis, lichen sclerosis, IgG4-pancreatitis, rheumatoid arthritis colorectal and lung cancers.

Results

TIGIT expression was seen in CD8+ cytotoxic T cells, CD4+ T-helper cells, FOXP3+ regulatory T cells and in NK cells, but not in CD11c+ dendritic cells, CD68+ macrophages, and CD20+ B lymphocytes. TIGIT expression largely paralleled that of PD-1 expression. Both TIGIT and PD-1 showed marked location specific variations. For example, strongest expression was found in germinal centers of normal lymph nodes and Hashimoto thyroiditis. In general, TIGIT and PD-1 expression were higher in lymphocyte-dense compartments, such as areas of lymphocytic infiltration in sarcoidosis, IgG4 pancreatitis or rheumatoid arthritis, than in areas containing fewer and scattered lymphocytes. In lung and colorectal cancers, the density of TIGIT and PD-1 expressing T cells was highest at the invasion front. Also, the TIGIT and PD-1 expression levels were typically stronger in tumor adjacent stromal CD8+ cells than in tumor-infiltrating CD8+ cells. TIGIT is regularly expressed in a large subset of T cells.

Conclusion

The frequent co-expression of TIGIT and PD-1 may offer an opportunity for co-targeting these proteins with checkpoint inhibitor drugs.
Expression of the Immune Checkpoint Receptor TIGIT in Hodgkin's Lymphoma

Wenchao Li¹, Niclas Blessin¹, Ronald Simon¹, Martina Kluth¹, Kristine Fischer¹², Claudia Hube-Magg¹, Georgia Makrypidi-Fraune¹, Björn Wellge³, Tim Mandelkow¹, Nicolaus Debatin¹, Doris Höflmayer¹, Maximilian Lennartz¹, Guido Sauter¹, Jakob Izbicki³, Sarah Minner¹, Franziska Büscheck¹, Ria Uhlig¹, David Dum³, Till Krech¹, Andreas Lübcke², Corinna Wittmer¹, Frank Jacobsen¹, Eike-Christian Burandt¹, Stefan Steurer¹, Waldemar Wilczak¹, Andrea Hinsch¹

¹Department of Pathology, University Medical Center Hamburg-Eppendorf, Germany
²Oncology, Dianova GmbH, Germany
³Department of General, Visceral and Thoracic Surgery, University Medical Center Hamburg-Eppendorf, Germany

Background

Hodgkin's lymphoma (HL) is characterized by an environment of elevated inflammatory cells which play an important role for the pathogenesis of the disease. T cell immunoreceptor with Ig and ITIM domains (TIGIT) is an inhibitory immune checkpoint receptor and a putative target for novel immune therapies.

Objective

To study patterns of TIGIT expression in the T cell background surrounding malignant cells including Hodgkin cells, Reed-Sternberg cells, and histiocytic cells.

Methods

A microenvironment (ME) tissue microarray (TMA) was constructed from tissue punches measuring 2 mm in diameter obtained from formalin-fixed tissue samples of Hodgkin lymphoma lymph nodes (n=40) and normal human tonsil (n=2). The ME-TMA was stained by brightfield and multiplex fluorescence immunohistochemistry (IHC) and expression levels of TIGIT and PD-1, as well as standard lymphocyte markers (CD3, CD8, CD4, FOXP3), were evaluated in the lymphocytic background.

Results

TIGIT and PD-1 expression were found in all (100%) analyzed HL samples. In general, TIGIT was localized to the same cells as PD-1. Strikingly, expression levels of TIGIT and PD-1 were highly variable among the analyzed samples. Highest levels of TIGIT and PD were found in one sample of nodular lymphocytic-predominant HL (NLPHL). In conclusion, TIGIT expression is highly variable in patients with Hodgkin’s lymphoma.

Conclusion

Our results encourage further studies evaluating the role of TIGIT as a target for immunotherapies in Hodgkin’s lymphoma.
Expression of the Immune Checkpoint Receptor TIGIT in Seminoma

Wenchao Li¹, Niclas Blessin¹, Ronald Simon¹, Martina Kluth¹, Kristine Fischer¹², Claudia Hube-Magg¹, Georgia Makrypidi-Fraune¹, Björn Wellge³, Tim Mandelkow¹, Nicolaus Debatin¹, Doris Höflmayer¹, Maximilian Lennartz¹, Guido Sauter¹, Jakob Izbicki³, Sarah Minner¹, Franziska Büscheck¹, Ria Uhlig¹, David Dum¹, Till Krech¹, Andreas Lübcke², Corinna Wittmer¹, Frank Jacobsen¹, Eike-Christian Burandt¹, Stefan Steurer¹, Waldemar Wilczak¹, Andrea Hinsch¹

¹Department of Pathology, University Medical Center Hamburg-Eppendorf, Germany
²Oncology, Dianova GmbH, Germany
³Department of General, Visceral and Thoracic Surgery, University Medical Center Hamburg-Eppendorf, Germany

Background

Testicular seminoma is most the prevalent solid cancer type in young men. Because of their high density of immune cells, it is possible that immune checkpoint inhibitors could be a therapeutic option in these tumors. TIGIT (T cell immunoreceptor with Ig and ITIM domains) is an inhibitory immune checkpoint receptor and a potential immunotherapeutic target.

Objective

To investigate the relative expression of TIGIT, PD-1 and standard lymphocyte marker CD3+ in seminomas.

Methods

Immunohistochemistry was used to measure patterns of TIGIT, CD3+ and PD-1 expression of 79 seminomas in a tissue microarray (TMA) format.

Results

All tumors showed TIGIT and PD-1 staining in a variable fraction of the tumor infiltrating lymphocytes. The fraction of TIGIT positive T cells ranged from 2.3% to 69.4% and that of PD-1 from 0.8% to 56.5%. The staining pattern of TIGIT largely paralleled that of PD-1.

Conclusion

The results of our study demonstrate that the immune checkpoints receptors TIGIT and PD-1 are abundantly expressed in human seminomas. Once available, anti TIGIT therapies, possibly in combination with anti PD-1 drugs, may be effective in this tumor type.
Poster Board #13

Investigation of Biological Function of Costimulatory b7 Family Members in Endometrial Cancer

Oliviero Marinelli1,2, Massimo Nabissi1, Maria Beatrice Morelli1, Consuelo Amantini2, Giorgio Santoni1

1School of Pharmacy, Experimental Medicine Section, University of Camerino, Italy
2School of Biosciences and Veterinary Medicine, Experimental Medicine Section, University of Camerino, Italy

Background

Endometrial cancer (EC) is a gynecological malignancy classified into two clinicopathological types, endometroid type I and non-endometroid type II. Type I is characterized by slow growth and a good prognosis, while type II is very aggressive and with a poor survival. Recently, in immune-oncology there is a growing interest towards the costimulatory B7 family members as possible promising targets for immunotherapy. These proteins are cell-surface protein ligands, binding to receptors on lymphocytes to regulate immune responses, but previous evidence have demonstrated that inhibitory B7 molecules are frequently up-regulated in different tumors, which may contribute to immune evasion, invasiveness and chemoresistance. Up to now, very few information were provide about B7 members in EC.

Objective

Our objective was to characterize the expression profile of B7 members in EC.

Methods

B7 members were evaluated by RT-PCR and Western Blot analysis, in five different human EC cell lines and compared to normal endometrial samples.

Results

EC cell lines express several B7 members, such as, PD-L1, PD-L2, ICOS-L and B7-H3 with a significant difference compared with normal tissues. Specifically, PD-L2 is significantly over-expressed by an EC Type-II cell line, suggesting its correlation with tumour aggressiveness.

Conclusions

The role of inhibitory B7 molecules is not completely understood in cancer. Since no information are actually present in regard to B7 members and their roles in EC, we firstly evidenced that some B7 members were expressed in EC cell lines and their expression levels were different compared with normal tissues. These preliminary results are the basis for the development of a project focused in a better understanding of the B7 molecular role in EC.
Neutrophil to Lymphocyte Ratio as a Biomarker for Salivary Gland Cancer

Muhammad Masarwa¹, Alejandro Levoff², Boaz Forer¹

¹Department of Otolaryngology- Head and Neck Surgery, Barzilai University Medical Center, Israel
²Department of Pathology, Barzilai University Medical Center, Israel

Background and aims

Elevated blood neutrophil to lymphocyte ratio (NLR) and platelets to lymphocyte ratio (PLR) have been observed in some types of malignancies. The aim of this study is to test NLR and PLR potential in predicting salivary gland histology and treatment outcome.

Methods

Data from forty-six patients with salivary gland tumor, who underwent resection of salivary gland lesions in our institute during the last decade, was retrospectively reviewed. Twenty-four patients (52%) had benign tumor by histopathologic examination, while the rest (48%) had cancer. Complete blood count was performed within two months prior to surgery. Tissue leukocytes (both lymphocytes and granulocytes) infiltration was examined by light microscopy and immunohistochemistry assay (anti-CD15 and anti-CD45 antibodies).

Results

Calculated NLR values were higher in patients with malignancy in comparison to patients with benign tumor (2.7 vs 1.9, \( p = 0.02 \)). The NLR cutoff value was determined by ROC curve as 1.76 (AUC = 0.68; \( p < 0.05 \); CI-95%; sensitivity 73%, specificity 67%). Higher values of NLR have been observed in high-grade tumors compared to low-grade tumors (3.17 vs 2.03, \( p = 0.03 \)). No correlation was found between NLR and the stage of the cancer, nor with the overall survival (OS) rates. PLR values did not differ significantly between the tested groups. Examination of the excised tissues by light microscopy revealed leukocytic infiltration mainly inside the tumor and less commonly in the surrounding healthy tissue.

Conclusions

NLR, but not PLR, may serve as a biomarker for salivary gland cancer.
Poster Board #15

PD-L1 Expression in a Select Group of Tumours in North Indian Population; Exploring the Possible Use of PD-L1/PD-1 Targeted Therapy.

Vineet Talwar

*Medical Oncology, Rajiv Gandhi Cancer Institute and Research Centre, India*

**Background**

Programmed death receptor-1/programmed death ligand-1(PD-1/PD-L1) signalling pathway are important immune check point pathways which mediate self tolerance and control self damage. However sometimes these pathways are manipulated by cancer cells to evade immune surveillance. PD-L1 is an immunoinhibitory molecule that suppresses the activation of T cells, leading to the progression of tumors. Recent clinical trials further demonstrate the efficacy of PD-1/PD-L1 targeted therapy in various cancers and reveal a new era of cancer immunotherapy. The major role of PD-L1 determination as of now is to assess possible PD-1/PD-L1 targeted therapy.

**Objective**

To study PD-L1 expression in selected common tumours exploring the possible use of PD-1/PD-L1 targeted therapy in north Indian population.

**Methods**

Total 125 cases of selected common tumours were analyzed for PD-L1. IHC for PDL-1 was performed on Ventana Benchmark XT autostainer (Roche USA) as per the manufacture’s instruction with appropriate batch controls using PAL1 antibody (clone SP263, monoclonal, Ventana, Roche; CE marked).

**Results**

The distribution of cases by tumour type was non small cell carcinoma-adenocarcinoma -78, squamous cell carcinoma lung- 13, Small cell Carcinoma of Lung -2, melanoma -4,Breast carcinoma -3, Renal cell carcinoma -1, Hodgkins Lymphoms- 2, Urothelial Carcinoma-3.

Our analysis showed PD-L1 positivity in 41% cases. Among these non small cell carcinoma of Lung (Adenocarcinoma) showed 38.5%; Squamous cell carcinoma Lung showed 84.6 %; Miscellaneous Squamous carcinoma 57.1%; Miscellaneous Adenocarcinoma 25%; Melanoma 25%; Hodgkins Lymphoma 100% positivity while Small cell Lung Carcinoma, Breast Carcinoma, Renal Cell carcinoma, Urothelial Carcinoma did not show PD-L1 positivity in our study.

**Conclusion**

The PD-L1 positivity results indicate that there is a definite place for PD-1/PD-L1 targeted therapy in selected tumors especially non small cell carcinoma of Lung (Adenocarcinoma), squamous cell carcinoma lung, melanomas, bladder carcinomas and few other adenocarcinoma and squamous cell carcinomas.
Colon Cancer and Level of Some Biochemical Parameters – Our Experience

Dusan Vesovic1, Slobodan Dunjic2, Jovana Pejic2, Emilia Golubovic1, Milos Maletic2, Petar Krakovic2

1Preventive Medicine, VISAN -- Sanitary Medical School of Applied Sciences, Serbia
2Holistic Center, Center for Integrative procedures and Supplements “Dr Dunjić”, Serbia
3Surgery Department, Institute for Radiology and Oncology of Serbia, Serbia

Background
According to World Health Organization, cancer is the second leading cause of death globally, and was responsible for 8.8 million deaths in 2015. Globally, nearly 1 in 6 deaths is due to cancer. Aside conventional methods of cancer treatment, holistic approach also plays an important role.

Objective
The aim of the paper is to present relation that we found between presence of cancer and some biochemical parameters.

Methodology
Laboratory analysis of both, male and female patients was statistically analyzed. Patients were divided into two groups: group with no presence of colon cancer (noCC), and group with presence of colon cancer (CC). All of patients were recruited from Center for Integrative Procedures and Supplements “Dr Dunjić”, Belgrade (the Center). Holistic-personalized approach in patients healing was performed in the Center. Statistical analysis was done by using Statistical Package for Social Sciences (SPSS).

Results
Total number of patients was 217. There were 130 females (59.9%), and 87 males (40.1%). Mean age of all examinees was 44,97+13,6yrs (minimum 18; maximum 79). 194 patients (89,4%) belong to noCC group, while 23 (10,6%) belong to CC group. Numerous biochemical parameters have shown statistically significant difference between two groups examined – noCC vs. CC. Vitamin D: 55,18+23,6 vs. 42,7+22,3; p=0,022; df=22. Liver enzyme gama-GT: 45,83+6,39 vs. 107,67+10,3; p=0,043; df=51. Histamine blood level: 1,97+0,44 vs. 5,37+1,9; p=0,042; df=93. Noradrenalin: 2,83+0,97 vs. 27,2+4,24; p=0,000; df=21.

Conclusion
These findings point that colon cancer can be accompanied with numerous biochemical changes, such are vitamin D level, liver enzyme gama-GT, histamine, and noradrenalin. All of parameters mentioned tend to be significantly higher in patients with colon cancer, except for vitamin D level which was significantly higher in disease free group of subjects. However, the clinical importance of these findings requires further research which will clarify their impact on disease.
**Poster Board #17**

**BRCA1/2 and TP53 Mutation Status Associates with PD-1 and PD-L1 Expression in Ovarian Cancer**

**Verena Wieser**¹, Inge Gaugg¹, Martina Fleischer¹, Giridhar Shivalingaiah², Sören Wenzel², Susanne Sprung³, Sigurd Lax⁴, Alain G. Zeimet¹, Heidi Fiegl¹, Christian Marth¹

¹Department of Obstetrics and Gynecology, Medical University of Innsbruck, Austria  
²Division of Human Genetics, Medical University of Innsbruck, Austria  
³Institute of Pathology, Medical University of Innsbruck, Austria  
⁴Department of Pathology, Academic Teaching Hospital of the Medical University Graz, Austria

**Background**

Induction of checkpoint molecules such as programmed cell death protein (PD-1) and its ligand PD-L1 is an essential step in tumor immune escape which may be regulated by interferon gamma (IFNg, encoded by IFNG). Therapeutically, antibodies against PD-1 or PD-L1 restore T-cell immunogenicity and suppress tumor progression.

**Objectives**

We investigate the role of tumoral PD-1 and PD-L1 mRNA expression in ovarian cancer (OC) and explore its relation to IFNg and tumor protein 53 (TP53) and breast cancer gene 1/2 (BRCA1/2) mutation status.

**Methods**

We analyzed the mRNA expression of PD-1, PD-L1 and IFNg determined by quantitative real-time PCR in tissue of 171 patients with low grade serous (LGSOC; n=11), high grade serous (HGSOC; n=107), endometrioid (n=43) and clear cell (n=10) OC and compared it to each 14 normal ovaries and fallopian tubes.

**Results**

We observed an induction of the PD-1 pathway in OC tissue compared to healthy controls. Further, a significant correlation between PD-1, PD-L1 and IFNg expression was detected. PD-1 and PD-L1 mRNA expressions increased with tumor grade. However, only high PD-L1 mRNA expression was inversely associated with age. Notably, we further found that TP53 mutated tumors exhibited high PD-L1 levels and BRCA1/2 mutations were associated with both high PD-1 and PD-L1 levels. In the cohort of FIGO stage III/IV HGSOC, which represents the major subgroup, high PD-1 and high PD-L1 was associated with an adverse progression-free and overall survival, respectively.

**Conclusions:**

Our study suggests that PD-1/PD-L1 mRNA-expression is regulated by IFNg in OC and also influenced by TP53 and BRCA1/2 mutations. Therefore, these mutations might be served as potential predictive factors in guiding anti-PD1/PD-L1 immunotherapy.
AptaAnalyzer™- A New NGS Software Tool for Improved Acquisition and Analysis of Immune Signatures

Michael Blank, Christian Grohmann

Bioinformatics, AptaIT GmbH, Germany

Background
Next-generation sequencing (NGS)-based technologies are becoming a mainstay of modern immunoncology. However, the immune response on the level of T- and B-cell receptors derived from NGS data is not yet accessible for standard labs and generic methods and comfortable tools to process the vast amount of sequence reads are urgently needed.

Objective
AptaAnalyzer™ is an intuitive and user-friendly software tool enabling the improved identification of T- and BCRs on the basis of NGS data. Defined stages of the immune response, which are digitalized by NGS can be analyzed and compared at very high resolution.

Methods
The complete processing chain is supported: Raw NGS data are automatically pre-processed, structured and archived in form of collections in a data base. These collections store statistical values and sequence data in a form that allows its flexible fetching to finally generate results in the form of interactive tables and graphs on different levels of details. Individual as well comparative analysis of datasets (derived from individuals at different time points) enables a profound understanding of the immune response. Snapshots of the immune repertoire on the level of T- and BCRs can be captured very intuitively. Complete T/BCR chains or individual V-, CDR3- or J-regions can tracked over different time points of individuals to extract receptor patterns which are responsible for the advancement of a disease or which monitor drug response.

Conclusions
NGS data is systematically reduces down to a number of relevant receptors and thereby contributes to profound understanding of the immune response. AptaAnalyzer™ is very intuitive and at the same time very flexible, no bio-informatic skills are needed and scientists themselves can extract relevant information. AptaAnalyzer™ removes the bottleneck of NGS data analysis very efficiently. NGS data can be first time harnessed to leverage present experiments for a quick decision making.
Breast Cancer Tumour Infiltrating Lymphocytes – Expansion, phenotype and potential for therapy?

Vanessa Clay, Gray Kueberuwa, Anne Armstrong, Robert Clarke, Robert Hawkins

University of Manchester, Manchester Cancer Research Centre, UK

Background

The presence of tumour infiltrating lymphocytes (TIL) within the tumour microenvironment have been demonstrated to provide clinically relevant prognostic information in HER2 positive and triple negative breast cancers. However, little is understood about the phenotype of these T cells, their potential for \textit{ex vivo} expansion and subsequently their suitability as a novel adoptive T cell anti-cancer therapy.

Objective

We aim to isolate and expand TIL from primary breast carcinomas to determine whether \textit{ex vivo} expansion is possible. This expansion will be compared to PBMCs from matched patients. Additionally the phenotype of TIL and PBMCs will be explored to give further detail about the functional potential of these cells.

Methods

Tumours were enzymatically and mechanically disaggregated. Following this they were subjected to differential expansion methods varying culture conditions to determine the optimal expansion protocol. Alongside this, matched PBMCs were also expanded \textit{ex vivo}. The development of a 14-colour flow cytometry panel then allowed examination of the phenotype of these cells, specifically considering cytokine production and immune checkpoint markers.

Results

Optimisation of the TIL expansion protocol resulted in successful \textit{ex vivo} isolation and expansion of T cells from 85.7\% of breast carcinomas (n=14) vs 37.5\% (n=8) of TIL expanded using a protocol previously optimized in our laboratory for renal cell carcinoma (p=0.05). PBMCs were optimally expanded using the original protocol. Both cytokine and immune checkpoint expression differences were demonstrated by flow cytometry between T cells expanded using the two protocols.

Conclusion

Breast cancer TIL can be successfully expanded to clinically relevant numbers. The difference in \textit{ex vivo} culture behavior between different tumour types and PBMCs has also been demonstrated. Furthermore, the description of breast TIL phenotype is novel and informative when considering their potential as an adoptive cell therapy. Further work is planned to explore the anti-cancer potential of these cells.
Effectivity of Long Antigen Exposition Dendritic Cell Therapy (Lanex-DC®) in the Adjuvant Treatment of Rectal Cancer

Frank Gansauge

Centre of Oncologic, Endocrine and Minimalinvasive Surgery, GPS-Zentrum, Germany

Summary

PURPOSE: Despite adjuvant chemotherapy / radiotherapy relapse rates following curative resection of rectal cancer vary between 40 to 60% depending on the tumour stage. By introduction of neoadjuvant radio-chemotherapy the relapse rates have been reduced, anyhow the total outcome for patients suffering from this disease is still unsatisfactory. Here we retrospectively analyzed the outcome of immunotherapy in the additional adjuvant treatment of rectal cancer with long antigen exposition dendritic cell therapy (LANEX-DC®).

Patients

All patients (n=95) who underwent curative surgery for rectal cancer at our institution between March 2002 and June 2011 were included into this retrospective analysis. To all patient’s additional dendritic cell therapy was offered. 47 patients decided for an additional dendritic cell therapy together with the adjuvant treatment according to S3-quidelines (+DC), 48 patients decided only for an adjuvant treatment.

The whole procedure for gaining the mature dendritic cells was performed according to Good Manufacturing Practice Standards.

Results

Therapy with dendritic cells was well tolerated and no serious side effects were observed. In the +DC group 2 patients developed local relapse, 4 patients developed distant metastases. In the –DC group (n=48), 4 patients developed a local relapse and 15 patients developed distant metastases (total: 41,3%, p>0,001). The rectal cancer related survival rates (5-years) were as follows: all patients +DC 94,9%, -DC 73,9% (p < 0,001), Dukes C: +DC 83,3%, -DC 42,1% (p < 0,001).

Conclusion

We were able to demonstrate in a large retrospective analysis that additional treatment with dendritic cells (LANEX-DC®) significantly reduces relapse rates in the adjuvant treatment of rectal cancer.
Challenges of Tumor Associated Antigen Specific Chimeric Antigen Receptor Construction to Redirect Immune T Cells towards Solid Tumor Cancer Cells.

Beatrix Kotlan¹, Szabolcs Horvath², Eles Klara², Vanda Plotar², Katalin Czirbesz³, Emil Farkas⁴, Andras Szollar⁴, Istvan Vamosi-Nagy⁴, Mihaly Ujhelyi⁴, Akos Savolt⁴, Orsolya Csuka⁵, Laszlo Toth⁴, Gabriella Liszkay³

¹Molecular Immunology and Toxicology, National Institute of Oncology, Hungary
²Center of Surgical and Molecular Pathology, National Institute of Oncology, Hungary
³Oncodermatology Department, National Institute of Oncology, Hungary
⁴Center of Oncosurgery, National Institute of Oncology, Hungary
⁵Pathogenetics, National Institute of Oncology, Hungary

Background

There is a growing need to develop novel strategies against all types of cancer. Objectives: Targeting tumor associated antigens specifically would spare healthy normal cells.

Methods

Heavy (VH) and light chain (VL) Immunoglobulin (Ig) variable region-genes were amplified, using specific primers from breast cancer and melanoma. After constructed of single chain Fv (scFv) antibody fragments, scFv phage display libraries were generated and tested against preprepared cancer cell membrane preparations. Chimeric antigen receptor (CAR) constructs were designed using specific antibody fragments.

Results

Disialylated glycosphingolipid (GD3) targeting antibody fragments of human origin were defined and CAR was constructed, using Sleeping Beauty transposon system. HEK293 and immune T cells were then transduced with GD3 CAR constructs.

Conclusion

Uniquely tumorassociated disialylated glycosphingolipid for GD3- specific CAR–T gateway technology, T cell-engineering are highlighted cancer therapeutic.

Acknowledgements

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Poster Board #22

Investigating the Feasibility of Tumour Infiltrating Lymphocyte Therapy for Paediatric Malignancies with High Risk and Poor Prognosis

Gray Kueberuwa¹, Chitra Sethuraman², Edmund Cheesman², Ian Kamaly-Asl², John-Paul Kilday², Robert Hawkins¹

¹Cancer Sciences, University of Manchester, UK
²Paediatric Neuro-oncology, Royal Manchester Children's Hospital, UK

Background

Brain tumours are the most common solid malignancy of childhood, accounting for >20% of all paediatric cancers. Collectively, they remain the leading cause of cancer-related death and long-term morbidity in children. Infant lesions fare poorly since intensifying potentially effective conventional therapy causes overwhelming toxicity without conferring significant survival advantage. Tumour infiltrating lymphocyte (TIL) therapy consists of extracting immune cells from surgically removed tumours and growing them in the lab. This not only allows immune cells to be “switched back on”, but increases their total number. In this study we are investigating whether applying TIL therapy to paediatric brain tumours is feasible.

Objectives

We seek to assess

1. If there are significant T-cell infiltrates in high grade paediatric brain tumours
2. Whether these cells can be efficiently expanded ex vivo
3. The anti-tumour reactivity of expanded TILs against autologous tumour ex vivo

Methods

We report initial evidence that there is a significant presence of TILs in 4 high grade paediatric brain tumour patients with cell type and phenotype analysed by time of flight cytometry (cyTOF) upon dissociation after resection and after 3 weeks expansion in IL-2

Results

Initial samples have displayed up to 1300 fold expansion of TILs upon 3 weeks of culture and cyTOF analysis has shown that expanded cells have an increased capacity to secrete effector cytokines compared to peripheral blood lymphocytes cultured in the same conditions. Crucially, multiplex analysis of supernatant following co-culture of with autologous tumour and tumour lines shows that expanded cells possess anti-cancer activity.

Conclusions

These promising results suggest that TIL therapy for paediatric brain tumours may be feasible. Analysis of an increased number of samples will be required to substantiate this, along with optimisation of methodology to produce clinically relevant numbers of cells.
Silencing of Human Antigen R Protein Increases the Sensitivity of Cancer Cells to Combination Therapy

Yu-Li Lo¹, Vivian Juang¹, Chen-Shen Wang¹, Guan-Liang Lin², Huei-Ju Ting²

¹Institute of Pharmacology, National Yang-Ming University, Taiwan
²Department of Biological Sciences and Technology, National University of Tainan, Tainan, Taiwan

Human antigen R (HuR) plays an important role in posttranscriptional regulation of resistance-related genes, including galectin-3, c-Myc, P-glycoprotein (P-gp) and/or multidrug resistance-associated proteins (MRPs). In this study, we aim to verify if gene silencing by siRNA against HuR (siHuR) may regulate β-catenin signaling pathway via post-transcriptional modification for reversing chemotherapy resistance in colon cancer cells. Our results showed that siHuR decreased transcriptional expressions of galectin-3, β-catenin, cyclin D1, etc. in chemotherapy-treated colon cancer cells. Accordingly, the combination of chemotherapy and siHuR decreased the expressions of c-Myc, P-gp and MRP1, etc. HuR silencing enhanced the intracellular accumulation of chemotherapy in colon cancer cells. Furthermore, siHuR significantly enhanced chemotherapy-mediated apoptosis and thus intensified the cytotoxicity of chemotherapy. The combinatorial therapy significantly reduced the expression of Bcl-2, but increased the expression of Bax, as well as activity and expression levels of caspase-3 and -9. In conclusion, this is an innovative investigation linking gene silencing of human antigen R protein to oncogene down-regulation, survival signaling repression, efflux transporter reversal and apoptosis induction. Our study thus provides a powerful regimen for enhancing sensitivity of colon cancer cells to combination therapy.
APN401: Individual Cellular Immunotherapy Based on the Intracellular Master Checkpoint cbl-b

Hans Loibner\textsuperscript{1}, Guenther Lametschwandtner\textsuperscript{1}, Kerstin Westritschnig\textsuperscript{1}, Alexander Dohnal\textsuperscript{1}, Oliver Mutschlechner\textsuperscript{1}, Marc Salzberg\textsuperscript{1}, Pierre Triozzi\textsuperscript{2}

\textsuperscript{1}R&D, Apeiron Biologics, Austria
\textsuperscript{2}Oncology, Wake Forest University, USA

Background

The E3 ubiquitin ligase Casitas-B-lineage lymphoma–b (cbl-b) is an intracellular master checkpoint that negatively regulates activity of immune cells. Cbl-b\textsuperscript{−/−} mice show strong antitumor immunity. Cbl-b knock-down in human PBMC leads to distinct activation and may override sensitivity to suppressive mechanisms and control by CTLA-4 and PD-L1/PD-1. We have developed an individual cellular immunotherapy by ex-vivo silencing of PBMC for cbl-b by si-RNA and immediate re-infusion of silenced activated immune cells (APN401).

Objectives

APN401 was evaluated in a single dose, dose escalating Phase I study in patients with advanced solid tumors. Primary objectives were safety, evaluation of MTD and immunological parameters.

Methods

16 patients with advanced solid tumors not eligible for standard therapies were included. PBMC obtained by leukapheresis were transfected with cbl-b siRNA ex vivo by electroporation, and 5, 10 or 50 x 10\textsuperscript{5}/PBMCs/kg were re-infused i.v. once on the same day.

Results

Infusions were well tolerated, no dose-limiting toxicities were observed. Adverse events were transient fever and chills. There were no hypersensitivity reactions or evidence for autoimmune adverse effects. Silenced PBMC of all patients upon TCR stimulation produced enhanced amounts of IL-2 and IFN-\gamma in vitro. Following therapy, PBMC responses after in vitro stimulation with common tumor antigens were stronger, activation was seen up to 6 months. Four patients (2 pancreas, 1 colon, 1 renal cancer) had stable disease as best response. The strongest activation of PBMC was observed in the patient with the best clinical response (metastatic colon cancer; disease stabilization > 1 year).

Conclusions

Single dose infusion of PBMC ex-vivo silenced for cbl-b (up to 5 x 10\textsuperscript{6}/kg) was safe in solid cancer patients and led to immune activation. Disease stabilization was observed in 25% of patients. Currently three consecutive infusions of APN401 are tested; interim results indicate no safety issues.
**A Novel Mesenchymal Stem Cell Therapy in Steroid Refractory Graft-Versus-Host Disease**

**Emese Molnar¹, Aniko Barta², Arpad Batai², Zoltan Csukly², Lilla Lengyel², Gabor Kovacs², Tamas Masszi⁴, Gabor Mikala², Melinda Paksi², Marieni Reti², Eva Torbagyi², Hajnalka Andrikovics², Gergo Krivan³, Krisztian Kallay³, Halvard Bonig⁵, Peter Bader⁵, Peter Remenyi², Istvan Valyi-Nagy²**

¹Serology, Hungarian National Blood Transfusion Service, Hungary  
²Department of Hematology and HSCT, Central Hospital of Southern Pest National Institute of Hematology and Infectious Diseases, Hungary  
³Pediatric Hematology and Stem Cell Transplantation Department, Central Hospital of Southern Pest National Institute of Hematology and Infectious Diseases, Hungary  
⁴3rd Department Of Internal Medicine, Semmelweis University, Hungary  
⁵Division for Stem Cell Transplantation and Immunology, University Hospital, Germany

**Background**

Steroid refractory graft-versus-host disease (GvHD) is a life-threatening complication of allogeneic hematopoietic stem cell transplantation (HSCT). Growing experience accumulates about the immunomodulatory effect of mesenchymal stem cells (MSC) in numerous immunopathological disorders.

**Method**

We have evaluated the efficacy of a novel MSC product in 23 patients with steroid-refractory GvHD. Patients were treated with the licensed MSC-FFM /Kuci et al. Haematologica 2016/, four times per case weekly at a dose of 1 million cells/kg. Clinical response was assessed 28 days after the first dose.

**Results**

The patients’ median age was 22 (1-66) years with a male/female ratio of 1:1. Seventeen undergone HSCT with matched unrelated donors, the other six had stem cells derived from HLA-identical relatives. The first episode of GvHD after HSCT appeared on the median 48th day. Involved organs were skin, gut, and lungs. We applied an average of 3 lines of immunosuppressive therapy (1-5 lines) before the MSC treatment. The median time of first MSC-FFM infusions was 69 days after the onset of GvHD. Nine of the patients showed complete remission and nine resulted in partial remission. All of the patients GvHD NIH stage score was three before MSC-FFM infusions, and it decreased to a median of 1 after treatment. The overall survival on the 60th day after the first GvHD was 78.3%.

**Discussion**

According to our observation MSC-FFM-therapy is an effective treatment for GvHD in the majority of the observed cases. The application of MSC-FFMs offers a promising alternative in the therapy of GvHD.

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Poster Board #26

Tumor Regression Associated with Tissue Function Normalization by Ou MC Decrescendo Phenomenon – A Way for Cancer Prevention

Ming Cheh Ou¹, Dennis Ou², Chung Chu Pang³

¹Obstetrics & Gynecology, Taipei City Hospital, Taiwan
²Mechanical Engineering and Biomedical Engineering, Carnegie Mellon University, USA
³Occupational therapy, National Taiwan University, Taiwan

Background

Prompt remission of joint pain, edema of soft tissue by trauma, pain by infection or cessation of uterine bleeding in the studies with Ou MC decrescendo phenomenon treatment (OuDPT) indicate a restoration of normal tissue function.

Objective

This study is to observe the effect of OuDPT for human cancer.

Methods

OuDPT is performed by putting the contralateral hand over the lesion along human body anatomical axis (HBAAA) of left-right, dorsoventral or vertical axis by the patient.

Results

From 2011 to 2017, OuDPT showed therapeutic benefits for the cancer diseases of 10 patients. OuDPT resulted in an initial suppression or size-reduction of the tumor of all the 10 patients (including two-thirds size reduction of a pancreatic cancer with lymph node metastasis and partial regression of an endometrial cancer Ib in 2017). The neoplasm of 3 patients (endometrial cancer stage IIIb, Ovarian cancer stage IVa, suspected pancreatic cancer) with no other anti-cancer treatment reduced in size with OuDPT but resumed growth later despite continuing OuDPT.

Conclusions

When the functions of tumor tissues are normalized, tumor cells may conform to regulation by apoptosis, growth suppression, and metastasis suppression, which normal cells undergo. The normalization of tumor tissue function may involve not only tumor cells, but also the microenvironment in which the tumor cells are located. The normalization of the tumor microenvironment may subject tumor cells to an environment that suppresses metastasis, prevents uninhibited proliferation, minimizes angiogenesis, and eliminates abnormal cells via the normalized host immunological system. The probable cause of losing suppression of advanced cancer by OuDPT may be due to the effect incapable to fully reach the tumor cells or tumor adaption. However, OuDPT shows the ability to suppress cancer growth, which may be availed for cancer prevention.
Cytokine-Induced Killer Cells Combined with Different Immunotools Abrogate Triple Negative Breast Cancer Metastatization

Roberta Sommaggio¹, Elisa Cappuzzello², Anna Dalla Pietà², Matthias Peipp³, Antonio Rosato¹

¹Tumor Immunology Section, Istituto Oncologico Veneto (IOV)-IRCCS, Italy
²Department of Surgery, Oncology and Gastroenterology, University of Padova
³2° Department of Medicine, Christian-Albrechts-University, Division of Stem Cell Transplantation and Immunotherapy, Germany

Background

Cytokine-Induced Killer (CIK), CD3⁺CD56⁺ cells, are efficiently and rapidly expanded in vitro. They are capable of recognizing tumor cells without the need of antigen-specific priming and exert Antibody-dependent Cellular Cytotoxicity (ADCC) when combined with clinical-grade monoclonal.

Objectives

In this study, we evaluated the efficacy of CIK therapy in combination with different immunotools: the cetuximab (CTX), an EGFR-specific antibody, the bispecific antibody (BsAb) targeting EGFR and CD3 (EGFRxCD3) or the recombinant immunoligand which target EGFR and ULBP2 (EGFRxULBP2), in triple negative breast cancer (TNBC).

Material and methods

Immunotools-retargeted CIK cells from healthy donors, were tested for lytic activity against TNBC cell lines. In in vivo setting, different TNBC models were established in NSG mice, injecting MDA-MB-231 cells either i.v. or in mammary fat pad, or implanting Patient Derived tumor Xenografts, (PDX). The combined treatment consisted in i.v. injection of 1.5 mg CTX together with 10x10⁶ CIK cells. Tumor growth and metastases were monitored by bioluminescence or immunohistochemistry.

Results

CTX, EGFRxCD3 and the EGFRxULBP2, enhances CIK cells cytotoxicity in vitro. In vivo, the combined therapy induced a significant delay in tumor growth, compared to the control treatments, in all mouse models. Remarkably, in mice injected with the MDA-MB-231 cells in mammary fat pad, the combined treatment almost completely abolished metastatic spreading to the lungs. We confirmed this relevant finding in a mouse model where the primary tumor was surgically removed before the i.v. therapy, as it occurs for the treatment of patients, in order to allow the CIK cell transfer to act against distant metastases. The growth of metastases in lymph nodes and lungs was significantly delayed by the treatment with the combined therapy, and mice had a significant better survival.

Conclusion

The combination of CIK cells with specific immunotools represents a new perspective for adoptive immunotherapy to treat TNBC metastases.
Poster Board #28
Combining Oncolytic Virotherapy with PD-1 Immunotherapy in Pancreatic Cancer
Eunji Choi, Han-Jun Kim, Hyo Sung Kim, Sun Hee Do
Department of Veterinary Clinical Pathology, College of Veterinary Medicine, Konkuk University, South Korea

Pancreatic cancer is highly aggressive and metastatic, which usually expresses immunosuppressive microenvironment. Immunotherapy can be beneficial in preventing metastasis and local recurrence after removal of primary cancer. Direct oncolysis generated by oncolytic virotherapy can release tumor associated antigens and anti-PD-1 therapy can further enhance the immune response. On this basis, we aimed to characterize therapeutic effects and immune responses in systemic administration of oncolytic virus alone or combined with blockade of immune checkpoint receptor. C57BL/6 harboring pancreatic ductal adenocarcinoma (panc02), were treated with oncolytic vaccinia virus alone or combined with anti-programmed cell death-1 (PD-1) antibody. Our results showed that the combined treatment of oncolytic virus and anti-PD-1 antibody was most effective in tumor growth regression compared to treatment of oncolytic virus or anti-PD-1 antibody alone. The treatments also prevented metastasis and the growth pattern of tumors was less infiltrative compared to untreated tumors. The suppression of tumor growth correlated with the immune response as the level of tumor-infiltrating lymphocytes was increased following the treatment. Moreover, flow cytometry immunophenotyping of peripheral blood indicated that oncolytic virus has stimulated antitumor immunity, showing increase in level of cytotoxic T cells, NK cells, as well as NK T cells. Therefore, our study demonstrates that a balanced therapy combining oncolytic virus and anti-PD-1 antibody can lead to successful immunotherapy.
Poster Board #29

Resisting RECIST- NEW Methods of Assessing Tumour Response to Immunotherapy

Richard Gore, Robert Silvers, Kiran Thakrar, Daniel Wenzke, Gregory Jackson, David Rabin

*Department of Radiology, North Shore University Health System – The University of Chicago Pritzker School of Medicine, USA*

**Introduction**

Traditional chemotherapy is cytotoxic in nature and acts primarily by eliminating neoplastic cells. Change in tumour size, which is an indicator of change in the number of neoplastic cells, evolved into the radiologic biomarker of treatment response. Over the last decade, dramatic advances in understanding the genetics and molecular biology of tumours have revolutionized therapy for many neoplasms.

Molecular targeted therapy and immunotherapy have led to new, individualized tumour therapies. These interfere with signalling pathways and thereby inhibit cell growth but do not necessarily lead to cell death, unlike cytotoxic drugs. With targeted agents, lack of progression may be associated with a positive improvement in outcome, even in the absence of major shrinkage of tumours. Oncologists have become interested in the length of time that a cancer does not grow or metastasize. Progression free survival has become the preferred end point for many cancer therapy trials. In this presentation, new criteria for imaging tumour response in the era of personalized medicine are presented and guidelines for learning when it is appropriate to resist using RECIST are presented.

**Conventional Anatomic Criteria:**

- WHO
- RECIST
- RECIST 1.1

**Functional Criteria:**

- Choi
- Modified Choi
- MAST
- EASL
- mRECIST
- RECICL
- irRC
- SACT
**Metabolic Criteria:**

PERSIST

PET-CT

PET-MR

**Evolving Imaging Biomarkers:**

Perfusion

DWI

MRE

MRS

Volumetry

Growth kinetics

**Conclusion:**

RECIST 1.1 is the mainstay of evaluating tumour response to therapy for most cancers. These criteria depend primarily in assessing changes in tumour dimensions but they do not reflect other morphologic, functional, or metabolic changes that may occur with novel chemotherapy, targeted or immunotherapy. It is important for radiologists to integrate new concepts in the evaluation of tumour response in this era of personalized medicine and to learn when it is necessary to resist using RECIST criteria.
The Novel IDO Inhibitor BPRID0363 Exhibits Potent Antitumor Efficacy by Reverse of Immuno-Suppressive Microenvironment

Ching-Chuan Kuo, Chiung-Tong Chen, Shau-Hua Ueng, Teng-Kuang Yeh, Li-Mei Lin, Hsin-Huei Chang, Yi-Hsin Wang, Chin-Hsiang Huang, Ya-Chu Tan, Zih-Ting Huang, Manwu Sun, Tung-Wei Hsu, Shu-Ying Cheng, Ming-Shiu Hung, Su-Ying Wu, Jen-Shin Song

Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Taiwan

Immune suppression is one of the mechanisms in promoting tumor growth. Among many possible mediators involved in tumoral immune escape, indoleamine 2,3-dioxygenase (IDO), a heme-containing enzyme that catalyzes the initial and rate limiting step in the catabolism of tryptophan via the kynurenine pathway, is an important molecular target gaining considerable attention recently. Several pharmaceutical companies are known to be in pursuit of IDO inhibitors, and Incyte have reported good results in the phase II clinical trial of the IDO inhibitor Epacadostat. Recently, we reported that phenyl sulfonylhydrazides are a novel class of IDO inhibitor (J Med Chem 59:419-30, 2016). Among them, BPRID0363 showed potent effect toward tumor growth in many syngeneic tumor models. Of note, BPRID0363 was able to reduce the ratio of kynurenine to tryptophan of tumor by 80% and inhibited tumor growth with TGI of 70% in CT26 syngeneic tumor model. Given that IDO inhibition is expected to work by impacting the activity of lymphocytes, we noted that BPRID0363 significantly enhanced PMA and ionomycine-stimulated lymphocyte responsiveness. In addition, we also noted that BPRID0363 significantly increased CD8 infiltration, reduced Foxp3 and Ki67 of tumor tissues in tumor-bearing mice. Moreover, BPRID0363 increased the stimulatory factor levels, such as TNF-a, CD40, and GM-CSF (for cancer antigen presentation), IL-2 and IL-12 (for priming and activation), CCL5 (for trafficking of T cells to tumors), ICAM-1 (for infiltration of T cells into tumors), and IFN-g (for killing of cancer cells). Taken together, our results suggest BPRID0363 may have potential to serve as a therapeutic agent for cancer immunotherapy, and its pharmaceutical properties need to further pursuit.
Burden of Liver Metastases by Gene Expression and Immune Response in an Experimental Model of Breast Carcinoma in Mice

Dzeina Mezale¹, Ilze Strumfa¹, Andrejs Vanags², Ekaterina Pankova³,⁴ Stefan Petkov⁵, Philip Podshwadt⁵,⁶ Elizaveta Starodubova⁴, Juris Jansons⁷,⁸, Maria Isaguliants⁵,⁷

1 Department of Pathology, Riga Stradins University, Latvia
2 Department of Surgery, Riga Stradins University, Latvia
3 Gamaleya Research Center of Epidemiology and Microbiology, Russia
4 Englehardt Institute of Molecular Biology, Russia
5 Microbiology and Tumor Biology Center, Karolinska Institutet, Sweden
6 Ulm University Hospital, Germany
7 Kirchenstein Institute of Microbiology and Virology, Riga Stradins University, Latvia
8 Biomedical Research and Study Center, Latvia

Background

Although the prognosis of metastatic liver disease has recently improved, it remains a major treatment challenge. The 4T1_luc2 is a highly tumorigenic cell line which can spontaneously metastasize, thus, could be used as a relevant tumor model including the general field of immunization studies in oncology as well as liver metastases, in particular.

Objective

To characterize the burden of liver metastases in immunized and naïve individuals using experimental model of breast carcinoma in mice.

Methods

Liver samples (n=62) were analysed from mice transplanted with 4T1luc2 cells expressing variants of HIV-1 FSU_A enzyme. Fifteen mice were transplanted with 4T1luc2 expressing reverse transcriptase (RT-DNA immunized 9, naïve 6); 23, protease (PR-DNA immunized 15, naïve 8); 16, integrase (IN-DNA immunized 10, naïve 6). Controls (n=8) received parental 4T1luc2 cells. Metastases were evaluated in fifteen high power (400x) microscope fields of hematoxylin-eosin-stained slides by computer-assisted morphometry.

Results

Liver micrometastases were found in livers of 11/15 4T1luc2_RT; 9/16 of 4T1luc_IN; and 18/23 of 4T1luc2_PR implanted mice. RT immunized mice developed metastasis in 5/9; IN-immunized, in 3/10; PR-immunized, in 10/15 and naïve, in 8/8 examined cases. Metastases were found in 18/34 immunized vs 20/20 naïve animals (p=0.0003). The mean size of 4T1luc2_RT metastases was 878.7µm² (SD±557.4), 981.8µm² (SD±717.8) of RT-immunized and 787µm² (SD±387.8) of naïve; 4T1 luc2_IN, 808µm² (SD±798.2), 678.8µm² (SD±248.1) of IN-immunized and 845.5µm² (SD±895.8) of naïve mice metastases; 4T1luc2_PR, 735.2µm² (SD±508.7), 747.7µm² (SD±573.6) PR-immunized and 716.7µm² (SD±397.1) of naïve; and 594.6µm² (SD±415.3) in naïve 4T1luc2 implanted mice. Inflammatory infiltrates consisting of neutrophils
and lymphocytes were found in all groups. Occurrence of neutrophil-based inflammatory infiltrate was higher in metastases of 4T1Luc2_PR carcinomas compared to other groups.

Conclusions

Number of subjects with metastases among HIV_DNA-immunized mice implanted with HIV-expressing tumors was significantly lower than among naïve animals. However, the morphology suggests complex tumor-host interaction.
Tumor formation is a process that involves accumulation of genetic and epigenetic changes leading to the progressive transformation of normal, healthy cells into malignant. Tumor cells possess certain skills unknown to normal cells: survival, proliferation without dependence on growth factors, unlimited replication, avoidance of apoptosis, invasiveness, metastasis and angiogenesis. The development of tumorogenesis is closely associated with the influence of reactive oxygen species (ROS) at the variety of cellular functions. In the present study we investigated the effect of gallic acid on oxidative stress, tumor growth and angiogenesis in EAT (Ehrlich ascites tumor)-bearing mice. EAT cells (2.5 x 10^6) were implanted intraperitoneally (i.p.) in Swiss albino mice. After tumor inoculation, mice were injected i.p. with gallic acid (GA) at dose of 40 and 80 mg/kg bw in exponential growth phase from the 5 days after tumor cell injection (on day 5, 7, 9, 11). On day 13, ascites volume, the total number of cells, differential count of the cells present in the peritoneal cavity, functional activity of macrophages, anti-angiogenic and antioxidant parameters were determined. Results show that GA inhibited the growth of EAT cells and the formation of ascites in the peritoneal cavity of EAT-bearing mice. Further, results on decrease in the peritoneal angiogenesis and microvessel density show the anti-angiogenic potential of GA in vivo. Gallic acid also decreased nitric oxide (NO) level in tumor cells whereas NO level was increased in peritoneal macrophages. Decreased level of Arginase 1 (ARG 1) indicate that polarization is prevented in M2 macrophages. Based on the results we can conclude that the GA can activated macrophages and increase their cytotoxic activity through increased production of NO and prevent tumor growth and angiogenesis.
Durable Remissions Associated with Anti-CTLA-4 and Anti-PD1 Checkpoint Inhibitors in a Single Center

Bernardo Rapoport1,2, Teresa Smit1, Ronwyn Van Eeden1

1Medical Oncology, The Medical Oncology Centre of Rosebank, South Africa
2Department of Immunology, Faculty of Health Science, University of Pretoria, South Africa

Background

Treatment with the checkpoint inhibitors ipilimumab (IPI) and nivolumab (NIVO) are associated with durable remissions in patients (pts) with solid tumors. We describe the durable remissions associated with these agents. There were 19 pts treated with IPI and 25 pts treated with NIVO and 1 pt was treated with combination of IPI and NIVO.

Objective

The purpose of this analysis is to describe the durable remissions associated with IPI and NIVO on a variety of solid tumors treated at a single centre.

Method

This is a retrospective data analysis from 45 pts treated either in an expanded access programme, clinical trial setting or post-registration protocol.

Results

A total of 45 pts were analyzed. In total 167 cycles of NIVO (median = 4, range 1-16), and 64 cycles of IPI (median = 4 cycles, range 1-4) were administered. In the MMM group there were 5 responses out 20 pts (25%) treated with IPI including 3 pts with durable complete response (CR) of 74+, 46+ and 34+ months. One MMM pt treated with NIVO has ongoing partial response (PR) of 23 months. Among the pts with NSCLC 6 responses were documented among the 18 pts treated with NIVO (33%). Two of these pts had very good and durable PRs of 10+ and 18+ months. One RCC pt treated with NIVO has an ongoing PR of 13+ months. Two heavily pretreated pts with HD treated with NIVO have very good PRs of 14+ and 13+ months. Additionally, a very PR was documented in the NSCLC pt treated with the combination IPI and NIVO.

Conclusion

Anti-PD1 and anti-CTLA4 antibody treatment is associated with durable remissions in pts with a variety of solid tumours. Among our pts durable remissions and durable responses were documented in pretreated pts with RCC, NSCLC, HD and MMM.
Poster Board #34

Immunotherapy of Human and Murine Cancers by CpG and Photodynamic Vaccination in Mouse Models

Neng-Yao, Joesph SHIH, Kwang Poo Chang, Bala K. Kolli, Ko-Jiunn Liu, Kuan-Chung Hsiao, Chia-Hong Han

1National Institute of Cancer Research, National Health Research Institutes, Taiwan
2Department of Microbiology/Immunology, Chicago Medical School/RFUMS, USA

Alpha-Enolase (ENO1) has been depicted increasingly in the literature as a moonlighting protein involved in different ways in the pathogenesis of many diseases. It is known as a house-keeping glycolytic enzyme catalyzing the interconversion between 2-phosphoglycerate and phosphoenolpyruvate. Only recently was it detected on the cell surface and characterized as a plasminogen receptor, and thus scientists believe that it may have a crucial role in tissue invasion and tumor metastasis. An increase in the expression of cell-surface ENO1 was, indeed, found to markedly enhance the invasive capability of tumor cells, thereby promoting tumor metastasis in NSCLC and PDAC. Moreover, our previous report demonstrated that the invasiveness of tumor cells could be severely impaired by blocking the plasminogen-binding activity of surface ENO1 with specific antibodies. Together, data strongly suggest that ENO1 can be a potential therapeutic target.

Since the immune response against ENO1 is reversely correlated with the clinical status of NSCLC patients, we are going to demonstrate how to stimulate the ENO1 immune responses to re-build antitumor immunity, and how this action can bestow a survival benefit in preclinical mouse models. Two immunization strategies using CpG or photo-inactivated Leishmania as adjuvant to generate effective cell-mediated and humoral ENO1 responses leading to tumor growth suppression will be discussed.

Collectively, we like to show that ENO1 can be a potential good and safe target for cancer immunotherapy, and its cell-surface form may also provide a great opportunity for antibody-targeting therapy in the near future.
Acanthamoeba castellanii Induces the in Vitro Cytotoxicity and Cytokines Release in Human Corneal Epithelial Cells

Hae-Jin Sohn1, Ga-Eun Seo1-2, Heekyoung Kang1,2, Si-Eun Kim1,2, Jong-Hyun Kim3, Ho-Joon Shin1,2

1Department of Microbiology, Ajou University School of medicine, South Korea
2Department of Biomedical Science, Graduate School of Ajou University, South Korea
3Institute of Animal Medicine, College of Veterinary Medicine, Gyeongsang National University, South Korea

Acanthamoeba castellanii, a free-living amoeba that has two stages in trophozoite and cyst, and it causes primary acanthamoebic keratitis (AK). AK is a common disease in contact lens wearers and caused by contact lens wear and corneal trauma, and results in permanent visual impairment or blindness. In this study, we observed morphologic cytopathic effect and in vitro cytotoxicity in human corneal epithelial cells (HCECs) induced by trophozoites and/or cysts of A. castellanii, and the secretion pattern of cytokines from HCECs. A. castellanii trophozoites induced cyst formation in encystment buffer for 72 hours. HCECs (5x10^5 cells/ml/wells) were co-cultured with only trophozoites (5 x 10^5 cells/ml/wells) or only cysts (5 x 10^5 cells/ml/wells) or trophozoites (2.5 x 10^5 cells/ml/wells) and cysts (2.5 x 10^5 cells/ml/wells) for 3, 6, 9, 12 and 24 hours. The morphological changes of HCECs using a inverted microscope showed that HCECs changed into round shape, when cysts were co-cultured rather than only HCECs cultured. For cytotoxicity assay, high cytotoxicity against HCECs was observed in co-culture system with cysts. A. castellanii induced the expression of IL-1α, IL-6, IL-8 and CXCL1 in HCECs. The expression levels of IL-1α, IL-6, IL-8 were increased at the early incubation time (3, 6 hours) in HCECs co-cultured in trophozoites and cysts. The released cytokines may contribute to inflammatory responses in HCECs. These results suggest that the immune responses between A. castellanii and HCECs, especially the effect of A. castellanii cysts against target cells may be important for the elucidation of AK development.
Novel Self-Assembled RNA Origami Nanostructures for Anti-Cancer Immunotherapy

Yung Chang¹, Xiaodong Qi², Xiaowei Liu¹, Lawrence Matiski¹, Ryan Rodriguez Del Villar¹, Hao Yan²

¹Biodesign CIVV & School of Life Sciences, Arizona State University, United States Minor Outlying Islands
²Biodesign Center for Molecular Design and Biomimetics & School of Molecular Science, Arizona State University, USA

Nucleic acid sensing is an essential mechanism of the innate immunity. Microbial DNAs and RNAs and damaged cellular components are recognized by a diverse set of cellular receptors, known as pattern recognition receptors (PRRs). An engagement of PRRs with their ligands trigger a potent activation of the host defense signaling pathway, leading to rapid production of pro-inflammatory cytokines. Thus, DNA and RNA have been explored for the development of vaccines against cancer and infectious diseases. Recently, we created a novel self-assembled RNA-origami nanostructure that function as a Toll-like Receptor 3 ligand. Injections of this RNA structure into tumor-bearing mice resulted in a significant delay or even regression of the tumor growth and yet caused no apparent adversity to the animals. Moreover, this RNA nanostructure is highly stable and readily manufactured for a large quantity. Given their well-defined structure and configuration, as well as programmable nature, RNA-origami nanostructures represent a new line of vaccine platforms for rational design and construction of effective, safe and affordable immunotherapeutics.
Anti-Tumor Effect Induced in the MHC-I Deficient Tumor Model

Adrianna Grzelak, Ingrid Poláková, Julie Vacková, Michal Šmahel

Department of Genetics and Microbiology, Faculty of Science, Charles University, BIOCEV, Czech Republic

Background
Tumor cells can escape the host immune surveillance by downregulating major histocompatibility complex class I (MHC-I). Therefore, immunotherapy should induce both MHC-I-dependent and -independent immune reactions to maximize the anti-tumor effect.

Objective
The aim of this study was to design the effective combined cancer immunotherapy in the mouse TC-1/A9 tumor model characterized by down-regulated MHC-I expression.

Methods
Combined immunotherapy comprised three approaches. Innate immunity was activated with either the unmethylated oligodeoxynucleotide ODN1826 functioning as a TLR9 agonist, or α-galactosylceramide (α-GalCer) that activates invariant natural killer T cells (iNKT cells). DNA immunization against the papillomaviral E7 oncoprotein, that is produced in tumor cells, stimulated adaptive immunity and the blockade of immune checkpoint Tim-3 was used to inhibit immunosuppression. Different immunotherapeutic regimens were tested and tumor infiltrating cells were analyzed by flow cytometry. In vitro experiments on isolated tumor associated macrophages (TAMs) were performed to investigate their polarization state.

Results
ODN1826 and α-GalCer were effective vaccine adjuvants enhancing the poor efficacy of DNA immunization. Timing, but not number of adjuvant doses, was essential to achieve the significant tumor growth retardation. Flow cytometry analysis and in vivo depletion of cell subpopulations revealed that in response to the immunotherapy, various immune cells infiltrated tumors and contributed to the anti-tumor effect. In vitro tests on TAMs in co-culture with tumor cells showed a high modulation of iNOS and arginase activities, the hallmarks of M1 and M2 macrophages, respectively.

Conclusion
In comparison with single therapeutics, combined immunotherapy showed superior effect against tumor cells with reduced MHC-I expression. In this effect, CD8+ T lymphocytes, NK1.1+ cells, and TAMs were involved, but the contribution of Tim-3 blockade both in vivo and in vitro was marginal.

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Poster Board #38

Importance of Standardized Plant Immunomodulators in the Research of Biological Targeting Treatments in the Tumor Therapy

Tibor Hajto

Institute of Pharmaceutical Chemistry, Medical University Pécs, Hungary

There is growing evidence that the type-1 cells in innate immune system, which are able to kill tumor cells in MHC-unrestricted manner, exhibit more reversible escape mechanisms if it is compared with adoptive system. Similar to micro-organisms plants can also content Pathogenic Associated Molecular Pattern (PAMP)-like molecules, which are in contrast to bacteria, have no toxic side effects but are also able to activate natural antitumor mechanisms. Indeed, on top of conventional oncotherapy surprising clinical responses were observed in various tumor patients if rice bran preparation with fixed dose of arabinoxylan (12 to 45 mg/kg twice or three times a week), mistletoe extract injections with a fixed Viscum album lectin content (0.5-1.0 ng/kg twice a week) and wheat germ extract with a fixed dose of 2.6-dimethoxy-p-benzoquinone (50 to 80 mg/kg four times a week) were given. In addition, case reports were reported in that Growth Factor Receptor (GFR) cascade signaling pathway inhibitors were combined with standardized plant immunomodulators and astonishing remission of tumors were observed. These results may open new perspectives in the further tumor research since we must better learn to manipulate the regulatory axis of the neuroendocrine and immune system.
Novel Agonists of RORγ/RORγT Receptors as Potential Enhancers of Th17 Anti-Tumor Properties

Kaja Karaś¹, Anna Sałkowska¹, Aurelia Walczak-Drzewiecka³, Katarzyna Ryba³, Jarosław Dastych³, Rafał Bachorz², Marcin Ratajewski¹

¹Laboratory of Transcriptional Regulation, Institute of Medical Biology, Polish Academy of Sciences, Poland
²Laboratory of Molecular Modeling, Institute of Medical Biology, Polish Academy of Sciences, Poland
³Laboratory of Cellular Immunology, Institute of Medical Biology, Polish Academy of Sciences, Poland

Background

Adoptive cell therapy involving extraction, expansion, modification, and reinfusion of selected T cells, takes advantage of host endogenous immunity to fight cancer. Ex vivo cultivation protocols aim to create a sufficient number of long-term persistent and potent anti-tumor cells. A promising choice is Th17 lineage of CD4⁺ T helper cells possessing steam-like characteristics, which recently has been proven to directly lyse the tumors. Th17 cells’ differentiation and transcription of their signature cytokines (pro-inflammatory IL-17A and IL-17F) are controlled by master transcription factor RORγT, RAR-related orphan receptor C Th17-specific isoform. RORγ, longer isoform, possessing identical ligand binding domain (LBD) and recognizing analogous response elements within DNA sequence, occurs more versatile in the human body.

Objective

The aim of the research was to find novel ligands of RORγ/RORγT receptors allowing to improve Th17-mediated anti-cancer response.

Methods and Results

Screening of a chemical library using RORγ-HepG2 reporter cell line allowed to select activators of RORγ-dependent transcription. Plant-derived cardenolides: k-strophanthin, digoxigenin, and dihydroouabain were able to increase expression of RORγ-dependent genes and occupancy of the RORγ protein on their promoters in HepG2 cell line. Incubation of naive human CD4⁺ T cells differentiating into Th17 lymphocytes in the presence of selected cardenolides caused an increase in IL-17A and IL-17F mRNA levels and IL-17 secretion. Molecular docking of the compounds to the crystal structure of RORγ LBD provided additional evidence that these cardenolides act as RORγ/RORγT agonists and could be used to enhance receptors’ transactivatory properties.

Conclusion

Increasing the activation and persistence of effector immune cells used for immunotherapy is receiving growing attention. Proposed cardenolides might be applied during ex vivo expansion procedure to improve Th17 differentiation and maintenance of the anti-cancer response.

Acknowledgments

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SIRT2 as a Novel Molecular Target for Anti-Melanoma Therapy

Iwona Karwaciak¹, Anna Sałkowska¹, Marta Sobalska-Kwapis², Dominik Strapagiel¹, Marcin Ratajewski¹

¹Laboratory of Transcriptional Regulation, Institute of Medical Biology of Polish Academy of Sciences, Poland
²BiobankLab, Department of Molecular Biophysics, Faculty of Biology and Environmental Protection, University of Lodz, Poland

Background

Despite many treatment options, metastatic melanoma remained associated with extremely poor prognosis and only curable if detected at an early, pre-metastatic stage. According to the National Cancer Institute’s Surveillance, Epidemiology, and End Results (SEER) data, the 5-year survival rate for metastatic melanoma patients diagnosed between 2007 and 2013 was 19.9%. Melanoma cells often display intrinsic resistance to many therapeutic approaches including chemotherapy and radiotherapy, but the mechanisms involved in the resistance are largely unknown.

Objective

Previously, our screening studies linked the SIRT2, an NAD+-dependent deacetylase, with the phenotype of resistant melanoma cells with high proliferation ratio. This is why the decided to investigate deeper the role of SIRT2 in melanomas and their development.

Methods

We have generated melanoma cell lines (representing different stages of the disease: early stage and metastatic melanoma) with downregulated SIRT2 expression and control cell lines stably transfected with scrambled shRNA. Using different methodology including gene and protein expression analysis (including sequencing de novo), cell wound closure assay, and colony formation assay we have characterized newly established cell lines.

Results

We identified significantly lower migration activity in SIRT2-silenced cells as compared to that in control cells. Further, cells with the SIRT2 gene knockdown, had also the decreased colony forming ability. Analysis of the transcriptomes by de novo sequencing revealed that SIRT2 regulates many genes responsible for the migratory properties of the melanoma cells, and those suspected to be involved in multidrug resistance phenotype.

Conclusion

We have found that SIRT2 expression seems to be crucial for oncogenic potential of melanoma cells. Thus, our results shows for the first time that SIRT2 can be therapeutic target in melanoma therapy, and rational drug design targeting this deacetylase may improve efficiency of current regiments.

Acknowledgements

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Poster Board #41

Immunity on the Psychological Level?

Shulamith Kreitler

School of Psychological Sciences, Tel-Aviv University, Israel

Background

There is evidence that psychological factors affect the occurrence and course of physical diseases, including cancer, but it is mostly theoretically controversial. In order to promote defining the relation of psychological factors with immunity it is advisable to approach this issue with a well-grounded relevant theory.

Objective

It is our purpose to present a theoretical model for studying the involvement of psychological factors in immunity. It is based on the cognitive orientation (CO) theory that defines the major psychological factors functioning as psychological correlates of disease in addition to other risk factors and pathogens. These factors consist of specific beliefs defined formally and in contents. Combining the themes common to different diseases mainly cancer resulted in one concise questionnaire "The cognitive orientation questionnaire of health (COH)".

Methods

Administering COH to samples with different health conditions and comparing high to low scorers. Results. Previous studies showed that high scorers on COH who were healthy undergraduates had fewer episodes of sickness and of flu (Kreitler); men undergoing hernia surgery had post surgery fewer complications and shorter hospitalization (Bentwich); men who underwent coronary events had coronary events of lower severity (Greif et al.); women with breast cancer undergoing chemotherapy reported fewer and less symptoms after each phase (Richkov); men 50-60 yrs old had fewer risk factors for CHD i.e., they had a lower mean systolic blood pressure, higher HDL, lower LDL, lower triglycerides, etc (Brunner); healthy individuals 18-69 yrs old had lower levels of leukocyte adhesiveness (Berliner). COH was not related to health behaviors and adhering to medical instructions.

Conclusion

The findings support the conclusion that COH assesses a general tendency of health proneness that may be considered as immunity on the psychological level. It is advisable to assess its relation to physiological immunity indices.
Designed Peptide Therapeutics with Dual Intracellular Protein Target for the Treatment of Breast Cancer

Jue-Yeon Lee¹, Gook-Jin Yoon², Ji-Eun Choi¹, Kwang-Sook Park², Yoon Shin Park³, Chong-Pyoung Chung¹, Yoon-Jeong Park²

¹Research Institute, Nano Intelligent Biomedical Engineering Corporation (NIBEC), South Korea
²Dental Regenerative Biotechnology Major, Dental Research Institute, School of Dentistry, Seoul National University, South Korea
³Major in Microbiology, School of Biological Sciences, College of Natural Sciences, Chungbuk National University, South Korea

Background

Cancer stem cells (CSCs) represent a side-population of tumor cells with a high capacity to initiate tumor, metastasis and tumor relapse after therapy. Many cancer-and cancer stem cell-related proteins are controlled by composite post-translational modifications (PTMs), but available strategies only target one type of protein modification.

Objective

The purpose of this study is to demonstrate the anti-cancer activity of NIPEP-ACD-Tide series with dual target arrest function in human breast cancer stem cells (hBCSCs) and mouse xenograft model of hBCSCs.

Method

The effect of NIPEP-ACD-Tide series on hBCSCs and mouse xenograft of hBCSCs were investigated. The mode of action of NIPE-ACD-Tide series was demonstrated by the change of FER tyrosine kinase and HDAC protein expression level.

Results

The NIPEP-ACD-Tide reduced proliferation, spherical colonies formation, and migration of hBCSCs. In addition, significant suppression of tumor growth by NIPEP-ACD-Tide was demonstrated in hBCSCs xenograft model.

Here, we developed peptide (NIPEP-ACD-Tide) series that controls two types of modifications of the histone deacetylase (HDAC), based on the discovery of a protein complex that suppresses HDAC. In addition, we found that FER tyrosine kinase, a cancer-stem cell antigen, recruits different attachment enzymes to initiate cancer spheroids to confer tumor creation and this was suppressed by the peptide treatment.

Conclusion

NIPEP-ACD-Tide series showed significant anti-cancer activity in human breast cancer stem cells (hBCSCs). A rationally designed intracellular dual targeting FER tyrosine kinase and HDAC successfully suppressed tumor growth. These findings shed light on the regulation of protein intracellular CSCs target and present a strategy for targeting two modifications with one molecule.

This study was supported by the Bio and Medical Technology Development Program of the National Research Foundation funded by the Ministry of Science and ICT (NRF 2017M3A9B3063635).
Impact of Complementary Substances on Immune Cells and Cancer Cells Depends on Cell Stimulability

Martin Luzbetak¹, Nora Süßenguth¹, Elisabeth Kronemeyer¹, Jens Werner¹, Barbara Mayer¹,²

¹AVT Surgery, University of Munich, LMU, Germany
²R&D, SpheroTec GmbH, Germany

Background

Natural substances and micronutrients are more and more included in anti-cancer therapy. However, benefit and harm both are reported from one and the same substance. This emphasizes the urgent need for the systematic analysis of a personalized approach which patient will profit from which substance.

Methods

Risk analysis was performed using PBMCs isolated from patients diagnosed with advanced solid cancer. Benefit was analyzed using 3D-microtumors directly prepared from individual patient tumors. Blood cells and cancer cells were treated with different natural substances, namely Curcumin, Artesunat and Vitamin C, as single agents and in combination therapy with guideline-directed drugs for 72h. Impact on cell metabolic activity was measured with the CellTiter Glo luminescence assay. The cell phenotype was described by FACS analysis.

Results

In 80% of the patients natural substances induced a slight (mean: 10.7%, range: 2.3-17.7%) metabolic inhibition of the immune cells, which was minor in comparison to the strong immunotoxicity of chemotherapeutic drugs (e.g. 5-FU, mean: 33.5%; Gemcitabine: 67.2%). Contrary, 20% of the patients revealed a stimulatory effect on PBMC depending on the basic activity and the exhaustion of the immune cells. Combination therapy revealed that natural substances were able to reduce (mean: 16.4%, range: 5.2-42.8%) immunotoxicity mediated by chemotherapy. Analysis of the 3D-microtumors indicated that natural substances directly can mediate an anti-cancer effect, which was most obvious in relapsed tumors heavily pretreated with chemotherapeutic drugs. In addition, natural substances were identified as chemosensitizer. For example, Curcumin was found to increase efficacy of Mitomycin C in breast cancer, Bicalutamid in prostate cancer and 5-FU combined with Cisplatinum in gastric cancer.

Conclusion

Complementary substances have a different effect depending on dosing, timing, cell type and cell characteristics. Therefore preclinical testing is required to identify the most effective complementary substances for the individual cancer patient analyzing both immune cells and cancer cells.
Dual Effects of HDAC Inhibitors on Cancer-Associated Th17 Lymphocytes

Anna Sałkowska¹, Kaja Karaś¹, Aurelia Walczak-Drzewiecka², Jarosław Dastych², Marcin Ratajewski¹

¹Laboratory of Transcriptional Regulation, Institute of Medical Biology of Polish Academy of Sciences, Poland
²Laboratory of Cellular Immunology, Institute of Medical Biology of Polish Academy of Sciences, Poland

Background

Th17 cells play dynamic roles in inflammation and tumor immunity. While Th17 cells act as Th pathogenic cells in autoimmunity, their role in the tumor biology is still under debate. Th17 cells are characterized by their ability to secrete IL-17, and their generation is controlled by the RORγT transcription factor. HDAC inhibitors (HDACI) affect cancer cells and are promising drugs in some new anticancer therapies.

Objective

The aim of the project was to investigate the role of regulatory pathways involving histone acetylation in the regulation of human RORγT by analyzing the effect of HDAC inhibition on RORγT expression at different stages of Th17 lymphocyte differentiation.

Methods

Naive CD4+ cells were isolated from buffy coats and differentiated towards Th17 cells. Th17 cells were treated with HDAC inhibitors, for the analysis of gene expression of the RORγT, and IL17 genes. Binding of the ac-H3 and ac-H4 to the RORγT promoter in vivo was investigated using ChIP assays.

Results

CD4+ T cells cultured under Th17-polarizing conditions in the presence of HDACI showed a significant decrease in RORγT, IL17A and IL17F mRNA expression. Treatment of fully differentiated Th17 cells with HDACI led to induction of the RORγT, IL17A and IL17F expression. Chromatin immunoprecipitation analysis revealed that HDACI induced recruitment of the acetylated histone H4 to the RORγT promoter in both: differentiating and fully differentiated Th17 lymphocytes.

Conclusion

Role of Th17 lymphocytes in cancers is controversial, as they either promote or suppress tumor growth, depending on the stimuli they encounter within microenvironment. Thus, for the future expansion of HDACI-based anticancer treatments, the information that some HDACIs are able to stimulate or inhibit Th17 cells, depending on their differentiation stage, might be important.

Acknowledgements

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The Effect of Adipose-Derived Stem Cells on Immunoediting in HPV Positive and Negative Head and Neck Squamous Cell Carcinoma

David Utz¹, Mohamed Shamji², Guido Piontek¹, Maria Buchberger¹, Ulrich Zissler³, Anja Pickhard¹

¹Department of Otolaryngology Head and Neck Surgery, Technical University of Munich, Germany
²Allergy and Clinical Immunology, Inflammation, Repair and Development, National Heart and Lung Institute, Imperial College London, UK
³Center of Allergy & Environment, Technical University of Munich, Germany

Background

Head and neck squamous cells carcinoma (HNSCC) closely correlates with status the of the immune system: Patients with immunocompromising conditions have a higher risk for HNSCC however HNSCC patients with no other condition are found to be immunosuppressed. Therapeutics that target the suppression of the immune system show promising results.

Mesenchymal stem cells seem to play a central role in creating an immunosuppressive tumor microenvironment as they are found enriched in head and neck squamous cell carcinomas and inhibit T cell proliferation. Mesenchymal stem cell derived from adipose tissue (ASC) have various, often tumor promoting effects on several cancer entities.

Objective

Our objective was to assess the immunomodulatory properties of ASCs in the tumor microenvironment for HNSCC in vitro evaluating possible differences in between HPV positive and HPV negative cell lines.

Methods

We analyzed peripheral blood mononuclear cells for various subsets of B cell, T cells and NK cells with flow cytometry after exposure to conditioned medium from ASCs and HNSCC cells alone and in co-culture. Furthermore, we cultured ASCs and HNSCCs with PBMCs in a double as well as a triple co-culture.

Results

The attached preliminary results from four-day co-culture experiments show a higher fraction of CD8+ cells when cultured with tumor cells (Cal-27, SCC-UD-2) but a lower percentage of NK cells compared to co-culture with ASCs (ASC N10, ASC T 10). When co-cultured with a HPV+ cell line the fraction of B cells is higher.
Conclusion

ASC and HNSCC cells have a relevant effect on PBMCs. We aim to further investigate the certain phenotypes (e.g. Treg, Breg and TH17 cells) and analyse the soluble factors to reveal the mechanisms of these effects in the coming weeks.
Innate Immunity Based Therapy of Melanoma and Pancreatic Adenocarcinoma in Murine Model

Jan Zenka\textsuperscript{1}, Veronika Caisova\textsuperscript{1}, Ondrej Uher\textsuperscript{1}, Pavla Nedbalova\textsuperscript{1}, Karolina Kvardova\textsuperscript{1}, Kamila Masakova\textsuperscript{1}, Gabriela Krejcova\textsuperscript{1}, Lucie Padoukova\textsuperscript{1}, Ivana Jochmanova\textsuperscript{2}, Jindrich Chmelar\textsuperscript{1}, Jan Kopecky\textsuperscript{1}

\textsuperscript{1}Department of Medical Biology, Faculty of Science, University of South Bohemia, Czech Republic
\textsuperscript{2}1st Department of Internal Medicine, Medical Faculty of P. J. Safarik University in Kosice, Slovakia

Background

The original concept of the use of pathogen associated molecular patterns (PAMPs) in cancer management was improved. Our novel approach is based on the synergy between TLR agonists and tumor cell-bound phagocytosis stimulating ligand (mannan).

Objective

Our aim was to optimize the composition of therapeutic mixture and its administration scheme in order to maximize therapeutic effect on melanoma and pancreatic adenocarcinoma and to analyse underlying mechanisms.

Methods

We used mouse model of melanoma and pancreatic adenocarcinoma. Therapeutic mixture was injected intratumorally in indicated time points and its effect on tumor growth was measured. Cellular infiltration was evaluated using FACS. The interaction of tumor cells with cells of innate immunity was assessed \textit{in vitro}.

Results

Our therapy, based on three TLR agonists (R-848+poly(I:C)+LTA) working in synergy with phagocytosis stimulating mannan, resulted in complete eradication of advanced melanoma tumors in 83\% of treated mice. To achieve comparable results in the model of pancreatic adenocarcinoma, the addition of agonistic anti-CD40 antibody was necessary. Moreover, the activation of adaptive immunity following the innate immune attack was detected during the treatment.

Conclusion

Cancer immunotherapy based on the synergy of TLR agonists with stimulation of phagocytosis is very effective therapeutic tool that can be further enhanced by using checkpoint activators.
Analysing Intratumoral T Cell Receptor and Immunoglobulin Repertoires

Dmitriy Chudakov

Genomics of Antitumor Adaptive Immunity lab, Privolzhsky Research Medical University, Russia
Center for Data-Intensive Biomedicine and Biotechnology, Skolkovo Institute of Science and Technology, Russia
Genomics of Adaptive Immunity, Institute of Bioorganic Chemistry, Russia
Research Group Adaptive Immunity, CEITEC, Czech Republic
Molecular Technologies, Pirogov Russian National Research Medical University, Russia

T cell receptor (TCR) repertoire of tumor-infiltrating T cells, as well as repertoire of intratumorally produced immunoglobulins (IG), represent an important, yet poorly explored, source of information about tumor-immunity relations. This information could be helpful for patient stratification in immunotherapy clinical trials, as well as for individual design of cancer vaccines and T cell/antibody based therapies.

Depending on the quality and type of starting material (bulk tumor/sorted lymphocytes; RNA/DNA; fresh/frozen/paraffin-embedded samples) and high-throughput sequencing approach used (targeted amplification/RNA-Seq/Exome-Seq), the available data and methods of immune repertoires analysis should vary.

Based on our recent experience (including Ref. 1), I will describe possible approaches to the TCR/IG repertoires extraction from tumor-derived high-throughput sequencing data. I will also cover some basic principles of comparative post-analysis of immune receptor repertoires2, and indicate specific limitations related to scarce samples.


References:

**Poster Board #48**

**Predictor of Efficacy and Survival for PD1 Immune Checkpoint Inhibitors in Non-Small Cell Lung Carcinoma (NSCLC)**

Young-Chul Kim¹, Cheol Kyu Park¹, In-Jae Oh¹, Yoo-Duk Choi²

¹Lung Cancer Clinic, Chonnam National University Medical School, Hwasun Hospital, South Korea

²Pathology, Chonnam National University Medical School, Hwasun Hospital, South Korea

**Background and Objective**

Programmed cell death 1(PD-1) monoclonal antibodies are being used with different cut-off points of programmed cell death ligand 1(PD-L1) expression level. However, predictive role of extent (%) in PD-L1 expression above cut-off points need to be explored. In this study we correlated the extents of PD-L1 (SP263 or 22C3) expressions with progression free survival (PFS) and efficacy (disease control rate, DCR) to nivolumab and pembrolizumab.

**Methods**

Seventy-five case records (male 60, female 15, mean age 75 years) with lung cancer (adenocarcinoma 51, squamous 22, NSCLC 2) were reviewed. Efficacy was recorded as disease control (remission or stable disease, n=30), progression (PD, n=26), or not evaluable (NE, n=19). PD-L1 expressions were divided into High (SP263 >=30%, 22C3 >=80%) or Low groups (SP263 < 30%, 22C3 < 80%). PD-L1 group was defined as High, if any of the two stains were classified as High, and as Low for the rest of cases.

**Results**

Among patients treated with Nivolumab (n=38), SP263 High group showed significantly higher DCR compare with SP263 Low group (45.0% vs 11.1%, p=0.043), and longer progression free survival (PFS, p=0.047). In patients treated with Pembrolizumab (n=37), 22C3 High group had significantly higher DCR than Low group (68.2% vs 26.7%, p=0.018) and longer PFS (p=0.01). In combined analysis (n=75), patients with PD-L1 High group showed significantly high DCR than the PD-L1 Low group (54.3% vs 17.2%, p=0.001), and PFS was significantly longer in PD-L1 High group than Low group (median 9.0 vs 1.9 months, p<0.001).

**Conclusion**

High level expression of PD-L1 correlated with significantly higher response rate and longer progression free survival in NSCLC treated with nivolumab or pembrolizumab.
Lymphoid Interstitial Pneumonia – Therapeutic Options with Pleiotropic Effects of Rituximab Regimens.

Przemyslaw Zdziarski

Department of Immunology of Infectious Diseases, Hirszfeld Institute of Immunology and Experimental Therapy, Poland
Dept. Chemistry, Military Institute WITI, Poland
Dept. Oncology and Clinical Immunology, Lower Silesian Center, Poland

Lymphoid interstitial pneumonia (LIP) is a rare lymphoproliferative disease.

LIP in common variable immune deficiency (CVID) was observed during immunomodulatory therapy after progression of the disease. Due to humoral immunodeficiency and serious serum sickness (IgM paraproteinemia) rituximab was used initially in a low dose (150 mg/m² weekly). It resulted in temporary remission with the decrease of serum paraproteinemia, β2-microglobuline (β2M) and SUV decrease as well as increase of FVC. Owing to the relapse after six-month remission in the second cycle a standard dose of rituximab was used (375mg/m²). Therapeutic regimen with 375 mg/m² of Rtx in optimal schedule (i.e. every 3 weeks) resulted in no longer remission but higher incidence of opportunistic infections. In laboratory and immunological progress the increase of NK and NKT cells was observed after the initial dose but the standard one caused NK cell increase only. Unfortunately, the decrease of CD19+Bcells was comparable between both doses, as was the decline of FoxP3+ regulatory T cell. On the contrary, after the low dose absolute T cell (both CD4 and CD8) number decreased but after the standard one - it normalized. The rituximab therapy blocked previously reported vigorous CMV-specific immune response but Rtx (especially in low dose) brought further increase of persistent T cell activation (CD38+ T cells made up 79%).

In conclusion, low dose rituximab gives more pleiotropic effects than the standardized one. New observation concerns the increase in innate (NK and NKT) immune response that is disturbed in CVID: primary immunodeficiency require a different immunotherapeutic approach.
Treating Cancerous Leptomeningitis with Whole Brain Radiotherapy Combined with Immune Checkpoint Blockade

Jeng-Fong Chiou1,2, Lai-Lei Ting1, Long-Sheng Lu1,3

1Department of Radiation Oncology, Taipei Medical University Hospital, Taiwan
2Taipei Cancer Center, Taipei Medical University, Taiwan
3Graduate Institute of Biomedical Materials and Tissue Engineering, Taipei Medical University, Taiwan

Background

Cancerous leptomeningitis is a late manifestation in various cancers and is associated with poor survival outcome. Combining radiotherapy and immunotherapy is effective in treating brain metastases from solid tumors. Whether it is effective in treating leptomeningeal metastases is unknown.

Objective

To share an unusual treatment response of cancerous leptomeningitis with combined radiation therapy and immune checkpoint blockade.

Method

Retrospective medical chart review.

Result

We recently were referred for treating a 62-year-old woman with cancerous leptomeningitis from hepatic neuroendocrine carcinoma. The disease was diagnosed 2 years ago and she had been treated with three lines of chemotherapy. Leptomeningeal metastases was found as she developed severe dizziness followed by altered mental status. The patient received whole brain radiotherapy (30 Gy in 15 fractions) combined with two cycles of nivolumab at 200 mg per dose in 3 weeks. During the treatment, neutropenia, thrombopenia and seizures happened and was medically controlled. After treatment, she recovered consciousness gradually and brain MRI confirmed complete resolution of cancerous leptomeningitis. Unfortunately the primary hepatic tumors progressed and the patient passed away after 2 months.

Conclusion

Since cancerous leptomeningitis is limited in treatment options, our experience may contribute to further exploration on combining whole brain radiotherapy with immune checkpoint blockade as a new treatment direction.
Hu14.18–IL-2 (APN301) is an immunocytokine consisting of human IL-2 linked to hu14.18 mAb, which recognizes the disialoganglioside GD2. This antigen is expressed on the surface of a variety of tumors, in particular of neuroectodermal origin, e.g. melanomas, sarcomas, glioblastoma, breast cancer (stem cells), small cell lung cancer, melanoma, retinoblastoma. The intravenous (i.v.) application of hu14.18-IL2 (i.v.-APN301) has been tested in two Phase II clinical trials in neuroblastoma and melanoma and has already demonstrated anti-tumor effects. Subsequent preclinical development showed that intratumoral application of hu14.18–IL-2 (IT-APN301) results in enhanced antitumor activity in mouse models compared with i.v.-APN301. These studies in a murine melanoma (B78) model demonstrated induction of a tumor specific T-cell response destroying distant tumors due to an increased number of activated T and NK cells within the tumor. Also, a prolonged retention of hu14.18–IL-2 at the tumor site was seen with IT-APN301 compared with i.v.-APN301. Additional local radiation therapy and systemic checkpoint blockade improved the anti-tumor effect and led to a complete tumor regression in 73% of animals and a striking effect on survival. Therefore, clinical studies with IT application of hu14.18–IL-2 are in development for melanoma and GD2 positive pediatric cancers.
Complete Response of Stage IIIB Esophageal Cancer Combining Low-Dose Checkpoint Inhibitors with Interleukin-2 (IL-2) and Fever Range Hyperthermia

Ralf Kleef¹, Arthur Bohdjalian⁵, Viktor Bacher¹, Robert Nagy¹, Dwight McKee⁴, Dieter Schilling², Ralph Moss³

¹Immunology & Integrative Oncology, Ralf Kleef Hyperthermie
²Onkologie, Diakonissenkrankenhaus Mannheim
³Oncology, Cancer Decisions
⁴Oncology, Integrative Cancer Therapies
⁵Chirurgie, DrBoh

Patient was a 56-year-old male newly diagnosed with adenocarcinoma of the esophagus with mediastinal lymphadenopathy. Histology revealed adenocarcinoma stage UICC IIIB T4 N2 with disseminated mediastinal, para-esophageal and celiac lymph node metastasis measuring up to 2.2 cm. HER-2/new+.

Patient refused suggested neoadjuvant CHT with FLOT. He clinically presented with Karnofsky index of 90% with increasing difficulties swallowing food. Rapid weight loss of 6 kg in the last 2 months.

Therapy consisted of administration of the following combination protocol: Low-dose PD-1 immune checkpoint (IC) inhibitor nivolumab (0.5 mg/kg) with CTLA-4 IC inhibitor ipilimumab (0.3 mg/kg) administered weekly, over 3 weeks. Accompanied by loco regional hyperthermia with radiofrequency fields (13.56 MHz) using the Syncrotherm device 3 times per week (max output 400 w) over the tumor region in combination with high dose vitamin C (0.5 g/kg) and alpha lipoic acid (600mg) over three weeks. Followed by long duration fever range whole body hyperthermia (using Heckel device) in combination with low dose chemotherapy using cyclophosphamide 300 mg/m² to down modulate Treg cells. Moderate dose i.v. interleukin 2 (IL-2) under Taurolidine protection was administered for five days with careful titration to daily fever hyperthermia of max 39.5°C.

Results

Unexpectedly, restaging 8 weeks following initiation of therapy with CT and Gastroesophagoscopy (Upper GI Endoscopy) revealed complete response. Confirmed by histological analysis of multiple biopsies in the former tumour bed confirming complete pathological response (cPR). At that time the patient had started gaining weight again and was free of any cancer-related symptoms. Several months later, he has regained 6 kg of weight, feels good, no dysphagia, continues to be monitored. Follow-up time now is 1 ½ years.

Conclusion

This is one of several cases of advanced stage cancer patients having a complete response to primary immunotherapy treatment. Clearly, this combination immune treatment warrants further clinical studies.
Anti-tumor Effects of Newcastle Disease Virus in Mouse Mammary Carcinoma and Ehrlich Ascites Tumor

Lydia Gaćina, Dyana Odeh, Nada Oršolić, Marina Kukolj

Department of Animal Physiology, Faculty of Science, University of Zagreb, Croatia

Oncolytic viruses (OVs) selectively replicate in and kill cancer cells, spread within the tumor, while not harming normal tissue, and are also very effective at inducing immune responses to themselves and to the infected tumor cells.

This study investigated the anti-tumor effect of the lentogenic LaSota and B1 strains of Newcastle disease virus (NDV) against mouse mammary carcinoma (MCa) and Ehrlich ascites tumor (EAT). The aim was to determine the anti-tumor efficacy of these viruses themselves using in vitro cell culture models, or in combined effect with cytostatic cisplatin and hyperthermia using in vivo experimental model of lung metastases of MCa and EAT model. We injected Swiss albino mice with $2 \times 10^6$ EAT cells intraperitoneally (i.p.). The experimental groups were treated 48 hours after the introduction of EAT cells and after the spontaneous development of MCa i.p. and i.v. (intravenous) with $19 \times 10^5$ EID$_{50}$ virus strains LaSota or B1 alone or in combination with cytostatic cisplatin (10 mg/kg body weight) in physiological conditions (37 °C) or the hyperthermal conditions (43 °C).

The administered viruses showed a significant anti-tumor effect against MCa and the treated groups had significantly fewer lung metastases than the controls. The i.p. and i.v. administered viruses entered the bloodstream and produced viraemia, thus infecting the circulating tumor cells and tissue cells to achieve an oncolytic effect. At physiological temperature, both viruses achieved a strong anti-tumor effect, while in intraperitoneal hyperthermia they produced no effect. Cisplatin significantly increased the effect of the viruses both at the physiological temperature and in hyperthermia. The results of this study show that the NDV LaSota and B1 strains have a significant anti-tumor effect against MCa and EAT in vitro and in vivo. Cisplatin shows it can be an effective cytotoxic agent in combination with viruses against these tumors.
Poster Board #54

Tumor Angiogenesis-linked Targets Modulated by *Tinospora cordifolia*: An Ancient Weapon and Modern Targets

Rani Kumari, Kavita Rawat, Anju Shrivastava

*Department of Zoology, University of Delhi, India*

**Background**

Carcinogenesis is a multistep process where cancer cells modulate the stroma for their own benefit such as recruitment of new blood vessels by angiogenesis wherein endothelial cells play a crucial role. Recent studies show that the immune modulators from natural sources are more beneficial for prevention and treatment of several diseases. Therefore, in the present study we attempted to evaluate the anti-angiogenic potential of a nature’s elixir *Tinospora cordifolia* which has been used for centuries in ayurvedic medicine and is known for its potent anti-inflammatory activity.

**Methods**

To study anti-angiogenic potential of TCE we used *in vitro* and *in vivo* models. In *in vitro* study, the endothelial cells from main thoracic aorta of adult mice were cultured in presence or absence of Dalton’s Lymphoma Ascites (DLA) with or without TCE. For *in vivo* study, the DL bearing mice were treated with TCE, where peritoneum and mesenteries were observed for neo vascularization. Subsequently we monitored the expression of pro and anti-angiogenic genes - FGF, VEGF, VEGFR and TIMP3 in DL cells and mesentery by qPCR-RT. In order to ascertain the TCE effect on MMPs activity in mesentery tissue, gelatin zymography was performed.

**Results and Conclusion**

TCE inhibited DL cells and DLA-induced endothelial cells proliferation *in vitro* in a dose and time dependent manner. The *in vitro* observations were further corroborated *in vivo*, where TCE treatment significantly reduced DL-induced micro vascularization in peritoneum, peripheral sides of intestine and perivascular fatty tissue of mesentery. qPCR-RT analysis clearly demonstrated induction of anti-angiogenic genes and inhibition of pro-angiogenic genes in DL cells as well as mesentery tissue upon treatment. The reduction in MMPs activity in mesentery tissue further supported the anti-angiogenic potential of TCE. Thus our study strongly suggests the anti-tumor potential of *Tinospora cordifolia* via inhibition of tumor-induced angiogenesis.
Systemic Impact of Neutrophils during Tumor Progression: A New Exciting Target for Cancer Immunotherapy

Kavita Rawat, Rani Kumari, Anju Shrivastava

Department of Zoology, University of Delhi, India

Background

Neutrophils, the most abundant leukocytes in human circulation earlier considered having negligible role in cancer but growing evidences now strongly suggest their pro-tumoral role. Various contributions of neutrophils to tumor onset and progression are only beginning to emerge. Cancer chemoprevention with phytochemicals is an emerging strategy to prevent, delay or cure cancer. *Tinospora cordifolia* is known for its immense application in treatment of various diseases in the Ayurveda. In present study we first looked into the involvement of neutrophils in tumor progression and secondly whether *Tinospora cordifolia* extract (TCE) a potent immunomodulator has role in its regulation.

Method

Dalton’s lymphoma (DL) mice model was used for the study. Blood and tissue were collected to study neutrophils with respect to number, morphology and function at different time points of tumor progression. To characterize neutrophils, immunostaining with Ly6G antibody (neutrophil marker) was performed. For functional studies, ROS, elastase and MMPs were considered. ROS in tissue was assessed by DHE staining. Elastase in serum and tissue was monitored by ELISA and gelatin zymography was done for MMPs. For treatment with TCE, three time points i.e. simultaneous, early and late stage were selected.

Result and Conclusion

Blood profile and tissue histology showed an increase in neutrophils count with tumor progression. Alteration in biochemical parameters with damaged histo-architecture was also observed. Simultaneous TCE treatment prevented tumor growth and elevated life span. Blood parameters were found in normal range. Normal histo-architecture of tissue was maintained with low or no neutrophil infiltration. ROS, elastase and MMPs level were elevated in DL group which reduced upon treatment. TCE treatment in early and late stage slowed down tumor growth leading to increased survival. These findings indicate the immunotherapeutic potential of *Tinospora cordifolia* via regulating the systemic neutrophil infiltration.
INTRODUCTION

Over the last 10 years, the management of cancer patients has been revolutionized by the advances in immunotherapy with significant benefits for patient outcomes and comfort. These therapies however are associated with new toxicities and complications that: can be mild, moderate or life-threatening; may require alteration or cessation of therapy; or simulate disease progression. In this presentation the various classes of immunotherapy associated with pulmonary complications are reviewed and the drug-associated injuries and their differential diagnosis are presented.

PNEUMONITIS

Drug induced pneumonitis develops in up to 10% of patients on immunotherapy and remains a diagnosis of exclusion that must be differentiated from infection and malignant lung infiltration. Five different patterns have been described on CT: ground glass opacities with preserved bronchovascular markings; increased interstitial markings, interlobular septal thickening, peribronchovascular infiltration, subpleural reticulation, and honeycomb pattern in severe cases; cryptogenic organizing pneumonia-like, with discrete patchy or confluent consolidation with or without air bronchograms, predominantly peripheral or subpleural in location; non-specific, with a mixture of nodular and other subtypes, not clearly fitting into other subtype classifications.

BRONCHIOLITIS OBLITERANS

There is myxoid fibrous tissue filling the distal bronchioles and extending into alveolar ducts and associated with inflammatory cells. On CT imaging findings include: bilateral regions of patchy consolidation or small irregular nodular opacities, bronchial wall thickening and dilation, and small pleural effusions.

PSEUDEPROGRESSION

Immunotherapy often may initially provoke infiltration of cytotoxic T lymphocytes and other immune cells into the tumor bed. This may cause an increase in tumor size or the development of new lesions as an early response. Pseudoprogression is defined as ≥ 25% increase in tumor burden that is not seen on repeat imaging performed 4 weeks or more after the initial study. Mixed immune-related responses or pseudoprogression are quite problematic in assessing treatment response using RECIST criteria.
A Case Study of Testicular Seminoma Expressing SNPs and Mutations in PDGFRA, ESR1, and Other Genes: NGS Strategies in Liquid-biopsy

Emin Umit Bagriacik, Aytug Uner, Aysegul Atak Yucel, Resul Karakus, Gozde Tahtaci, Bediz Kurt Inci, Fatih Gurler, Melek Yaman

1Department of Immunology, Gazi University, Faculty of Medicine, Turkey
2Department of Medical Oncology, Gazi University, Faculty of Medicine, Turkey
3Life Sciences Research Center, Gazi University, Turkey

Background

Testicular seminoma is a subtype of testicular germ cell tumor (TGCT) that is the most common malignancy in young male adults. However, there are publications reporting cases in children and elderly. The patient in this case is a 62-year-old man with stage III testicular seminoma. He received carboplatin and cisplatin chemotherapy following orchiectomy. The patient completed a 44-month of progression-free survival.

Objective

To assess variant sequences of 12 genes related to human cancers by using next generation sequencing (NGS) analysis in the liquid biopsy sample of the TGCT patient who was treated with carboplatin and cisplatin.

Methods

Circulating DNA was isolated by using QIAamp® Circulating Nucleic Acid Kit (55114). The concentration of isolated DNA was measured by Qubit 2.0. Target enrichment was performed by using GeneRead OIAact Panels powered by QCI, Actionable Insight Tumor Panel (181910). Next generation sequencing was performed using a GeneReader Instrument (Qiagen). More than 750 variants for 12 genes were studied. Data was analyzed by a platform-specific pipeline software (Qiagen Clinical Insight-Analyse™ and Clinical Insight-Interpret™).

Results

In this particular case, we found several variants including gene polymorphisms (SNP) and mutations in the 12-gene panel tested. Particularly, SNPs and mutations for ALK (c.2535T>C), PIK3CA (c.-76-14537C>G), BRAF (c.1929A>G), PDGFRA, and NSF1 genes would be significantly related to the malignancy. We also detected variants with uncertain significance for KRAS and EGFR. Currently, these findings are still under further investigations.

Conclusion

Based on these findings, we concluded that NGS analysis in liquid biopsy of TGCT cases would have a great potential to find biomolecules for immunotherapy as well as for improved tools in diagnosis and prognosis of TGCT.