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President of the Management Board of the Termedia Publishing House
Janusz Michalak

Scientific Director of the Termedia Publishing House
Maciej Banach

Production Editor
Marzena Demska
e-mail: m.demska@termedia.pl

Editorial Office
Termedia sp. z o.o.
Kleeberga 2, 61-615 Poznań
tel./fax +48 61 822 77 81, ext. 604
e-mail: contemporaryoncology@termedia.pl

Marketing and Advertising
Małgorzata Prętka
tel./fax +48 61 822 77 81, ext. 507
e-mail: m.pretka@termedia.pl

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Genome based precision immuno- and targeted therapy

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Oral sessions

Session 2. Precision oncology

Chairs: Gabriela Kramer-Marek, Theresa Whiteside

KWO17-00037-2017-01

Camelid single domain antibody application in cell based therapies

Heman Chao[1], Marni Uger[1], Wah Wong[1], Baomin Tian[1], Pawel Wisniewski[2]

[1]Helix BioPharma Corp, Toronto, Canada
[2]Helix Immuno-Oncology Sp. z o.o., Warsaw, Poland

Camelids produce antibody that is devoid of lights chains. Their single domain *N*-terminal domain is fully capable of binding antigen with great affinity and specificity without requiring domain paring. The *N*-terminal domain can also be expressed in both bacterial and mammalian systems in high yield. These domains have been applied to research, diagnostic and even therapeutic applications. In this study, two camelid single domain antibodies have been applied to cell based therapies. One of the antibody targets VEGFR2 and the second antibody targets CEACAM6. Both antigens are solid tumors targets. Chimeric-Antigen Receptor (CAR) T cells were engineered to target CEACAM6 antigen or VEGFR2 antigen by transducing respective antibody coupled with appropriate intra-cellular signaling domains. CEACAM6-CAR-T and VEGFR2-CAR-T are cytotoxic to respective antigen carrying tumor cells relative to control T cells. *In vivo* xenograft studies also showed CEACAM6-CAR-T is very potent against BxPC3, a pancreatic cancer model. Given the simplicity of camelid single domain antibody relative to 'classical' antibody, these antibodies would be ideal candidates to be used in cell based therapies.

Key words: CAR-T, CEACAM6, VEGFR2, antibody.

Session 6. Cancer biology and novel therapeutic approaches II

Chairs: Magdalena Chechlińska, Andrzej Lange

KWO17-00016-2017-01

microRNAs as molecular markers in primary CNS lymphomas

Magdalena Chechlinska, Maria Cieslikowska, Grzegorz Rymkiewicz, Pawel Swoboda, Michalina Zajdel, Katarzyna Blachnio, Zbigniew Bystydzienski, Anita Jastrzebska, Krzysztof Goryca, Maria Sromek, Mariusz Kulinczak, Agnieszka Druzd-Sitek, Jan Walewski, Jan Konrad Siwicki

Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology, Warsaw, Poland

Differential diagnosis of central nervous system (CNS) tumors remains challenging, in spite of development of imaging techniques, cytological and flow cytometry examination of cerebrospinal fluid (CSF), and histological examination of stereotactic biopsy material. microRNAs are promising markers for faster and more reliable differential diagnosis of primary lymphomas and nonmalignant lesions in the CNS.

We aimed to assess the diagnostic value of miR-21, miR-19b and miR-92a, miR-155, miR-196b, miR-let-7b, miR-125b and miR-9 expression in CSF and brain biopsies (BB) from patients with primary CNS lymphomas (PCNSL) vs. neurological CNS lesions.

microRNA expression was assessed by RT-qPCR, with miR-24 as a reference, in CSF leftover samples and formalin-fixed paraffin-embedded stereotactic BB samples collected for the routine diagnostic purposes from patients suspected of PCNSL (n = 26) or neurological CNS lesions (n = 59), consulted at the M. Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology in Warsaw.

1. In the CSF, there were significantly higher levels of miR-21, miR-19b and miR-92a in patients with PCNSL than in patients with nonmalignant neurological lesions. CSF levels of the three miRNAs differentiated PCNSL from neurological lesions, with 54% sensitivity and 90% specificity.

2. In BB samples, miR-21, miR-19b and miR-92a expression did not differ between lymphomas and nonmalignant lesions.

3. In BB samples, miR-155 and miR-196b were significantly overexpressed and miR-let-7b, miR-125b and miR-9 were downregulated in PCNSL vs. nonmalignant neurological diseases.

In conclusion, miRs emerge as promising diagnostic markers that may support earlier PCNSL treatment decisions, thus improving patient outcome. Further development will include validation of miRs as markers on independent series of patients and NGS profiling of paired samples (CSF/brain biopsy) from PCNSL patients

Key words: microRNA, CNS lymhoma, differential diagnosis, cerebrospinal fluid.

Session 3. Tumor microenvironment

Chairs: Claudine Kieda, Viktor Umansky

KWO17-00030-2017-01

Advanced three-dimensional co-culture model to study tumor biology *in vitro*

Ewelina Dondajewska, Hanna Dams-Kozłowska, Andrzej Mackiewicz

Poznan University of Medical Sciences, Poznan, Poland
Greater Poland Cancer Center in Poznan, Poland

Recognizing the drawbacks of standard 2D cell culture methods, we constructed a 3D culture model to better represent cell morphology and function *in vitro*. Using silk extracted from Bombyx mori cocoons, a three dimensional, porous scaffolds for cell culture were produced. To further mimic tumor microenvironment, we included stromal cells cultured together with tumor cells to obtain a heterotypic co-culture model. Established cell lines: EMT6 murine breast cancer, and NIH3T3 murine normal fibroblasts were used in the studies and were transduced with lentivarial vectors to express fluorescent proteins. That enabled us to distinguish cells of both types in the co-culture while analyzing cells using CLSM or FACS. We optimized the methods of cell culture on the scaffolds. We compared cell growth, morphology, drug resistance and gene expression profiles of cell cultured as standard 2D monoculture, 3D monoculture and 3D co-culture. Using fluorescent activated cell sorting we separated cells from the co-culture and by RT-PCR we analyzed expression of genes responsible for the process of EMT, CAF transformation, ECM production and tumor aggressiveness. Results showed more epithelial phenotype of cancer cells while cultured on 3D silk scaffolds as shown by the drop in most common EMT markers. Furthermore, we observed significantly higher levels of mRNA for ECM proteins in fibroblasts cultured in 3D as well as some features characteristic for cancer associated phenotype of these cells while co-cultured with tumor cells in 3D. A general increase in MMP9, VEGFa and Vimentin was observed for both cell types. Using cytotoxic agent – doxorubicin we found cells cultured on 3D silk scaffolds to be much more resistant to the effects of the drug than cells cultured in standard monolayer.

In this study we have proven, that silk scaffold – based, three-dimensional culture model, constructed using both cancer cells and fibroblasts, is an advanced tool to study tumor biology *in vitro*. It provides a controlled environment, with the possibility to analyze cell-cell and cell-ECM interactions. Model can be easily expanded by the addition of endothelial cells, immune cells or adipocytes.

Key words: tumor microenvironment, co-culture, breast cancer, silk fibroin scaffold, 3D culture.

Session 6. Cancer biology and novel therapeutic approaches II

Chairs: Magdalena Chechlińska, Andrzej Lange

KWO17-00033-2017-01

Lymphocyte activation and exhaustion in the natural history of chronic lymphocytic leukemia

Ewelina Grywalska, Agata Surdacka, Michal Mielnik, Elzbieta Fitas, Jacek Rolinski

Department of Clinical Immunology and Immunotherapy, Medical University of Lublin, Poland

Introduction: Chronic lymphocytic leukemia (CLL) is a disease characterized by the accumulation of morphologically mature monoclonal lymphocytes B with CD19+/CD5+/CD23+ phenotype in lymphoid tissue, peripheral blood and bone marrow. The course of CLL is chronic by default. Of note, however, is its heterogeneity. Programmed cell death protein 1 and its ligand 1 (PD-1, PD-L1) are major inhibitory receptors regulating T cell exhaustion, i.e. a state of T cell dysfunction. The role of lymphocyte activation and exhaustion in the natural history of CLL is still a matter of discussion.

Aim of the study was to determine the percentages and absolute numbers of exhausted and activated lymphocytes B and T in peripheral blood and bone marrow of CLL patients. Moreover, we analyzed relationship between the number of PD-1-positive and PD-L1-positive lymphocytes and established prognostic factors in CLL.

Material and methods: The study included 40 untreated patients with CLL and 20 healthy subjects. The immunophenotype of peripheral blood mononuclear cells (in both groups) and bone marrow cells (solely in the CLL group) was determined by means of flow cytometry.

Results: Patients with CLL showed higher frequencies and absolute number of exhausted B lymphocytes CD19+PD-1+ ($p < 0.0001$), CD19+PD-L1+ ($p < 0.0001$), activated lymphocytes B with phenotypes CD19+CD25+ ($p < 0.0001$) and CD19+CD69+ ($p < 0.0001$), as well as higher frequencies and absolute number of exhausted T lymphocytes CD3+PD-1+ ($p = 0.0021$), CD3+PD-L1+ ($p = 0.0032$), and activated CD3+CD25+ ($p = 0.0027$), and CD3+CD69+ ($p = 0.0062$) lymphocytes T than the controls in the peripheral blood. Similar observations were done in the bone marrow samples ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$, $p < 0.0001$, $p = 0.0134$, $p = 0.0183$, $p = 0.0263$, and $p = 0.0169$, respectively). Enhanced exhaustion and activation of peripheral blood and bone marrow lymphocytes was associated with higher Rai stage, increased concentration of lactate dehydrogenase and beta-2 microglobulin and progression of the disease. The number of lymphocytes B CD19+ZAP-70+ correlated positively with the number of CD19+PD-1+ B cells and CD3+PD-1+ T cells.

Conclusions: The study confirmed the association between unfavorable prognosis and high expression of exhaustion and activation markers in CLL patients. Determination of PD-1+, PD-L1+, CD25+ and CD69+ lymphocytes T and B constitutes valuable diagnostic tool, completing cytometric evaluation of CLL.

Key words: programmed cell death protein 1, Rai stage, Prognostic factors, chronic lymphocytic leukemia.

Session 9: Optimizations in diagnostics and management of cancer family syndromes in Poland

Chairs: Bogdan Kałużewski, Cezary Cybulski

KWO17-00050-2017-01

DICER1 syndrome

Marek Niedziela

Department of Pediatric Endocrinology and Rheumatology, Poznan University of Medical Sciences Poznan, Poland

The DICER1 gene localized on the long (q) arm of chromosome 14 encodes a protein DICER1, an RNase III endoribonuclease, that plays a role in regulating the activity of other genes. The function of DICER1 protein is exerted via microRNA formation. MicroRNAs are involved in cell growth, proliferation and differentiation. Germline mutations in the DICER1 gene cause DICER1 syndrome. Abnormal DICER1 protein disregulates miRNAs production thus leading to uncontrolled tumor formation. Many types of tumors may develop in these patients such as tumors of the lungs (pleuropulmonary blastoma – PPB), kidneys (cystic nephroma), ovaries (Sertoli-Leydig tumors – SLCTs) and thyroid (multinodular goiter – MNG). Cystic and hyperplastic thyroid abnormalities are a common finding in the DICER1 syndrome, particularly prevalent in DICER1 carriers, likely higher than that for neoplasms. Rarely, individuals with DICER1 syndrome develop thyroid cancer. Moreover the other tumors such as embryonal rhabdomyosarcomas, Wilms tumors and other very rare entities, all comprise DICER1 syndrome. DICER1 syndrome is inherited in autosomal dominant pattern but with unknown penetrance. It is a rare condition and its prevalence is unknown. Affected individuals can develop one or more types of tumors, and members of the same family can have different types. Based on the literature it is hypothesized that second somatic "hit" in DICER1 is required in addition to a loss of-function germline DICER1 mutation in order to initiate tumor/cancer development. A genetic counseling and testing should be offered to the family of the affected child/adult.

Key words: DICER1, tumors, children, adults.

Lecturers abstract

KW017-00048-2017-01

Anti-programmed cell death protein 1 in cancer immunotherapy – what do we know so far?

Jacek Rolinski, Ewelina Grywalska

Department of Clinical Immunology and Immunotherapy, Medical University of Lublin, Poland

Programmed cell death protein 1, also known as PD-1 and CD279 is a protein expressed on T cells and pro-B cells in humans. It is a cell surface receptor that belongs to the immunoglobulin superfamily. PD-1 is known to be the major inhibitory receptor that functions as an immune checkpoint, playing an important role in down regulating the immune system; by preventing T cell activation it reduces autoimmunity and promotes self-tolerance. However, it has also been proven that T cells with high PD-1 expression lose the ability to eliminate cancer and infectious agents. Antigen presenting, infected by viral agents and cancer cells, have on their surface molecules PD-L1 (B7-H1) and PD-L2 (B7-DC) (programmed cell death ligand protein 1 and 2) are connected to the PD-1 antigen on the surface of cells results in inhibition of their activity. The concept of immunotherapy using antibodies against immune check points is based on their ability to reverse anergy of T cells in the tumor environment and inflammatory processes. Lymphocytes only receive then signals that activate and again therefore lymphocytes are able to act against tumor and infected cells. This strategy is proved to be very effective for some types of cancer and chronic viral infections, and it revolutionized existing immunotherapy methods. The administration of anti-PD-1 or anti-PD-L1 aims to abolish anergy of effector lymphocytes T caused by tumor and viral infected or antigen presenting cells. The anti-PD-1 and anti-PD-L1 therapies are widely used for the treatment or are undergoing the final stages of clinical trials in patients with different types of tumors. Therapies of this kind often provide long-term control of the disease and the development of specific immune response in large variety of tumors. Nivolumab (anti-PD-1) is already used in the treatment of melanoma, non-small cell lung cancer, and renal cancer. Pembrolizumab (anti-PD-1) is used in the treatment of melanoma and non-small cell lung cancer. Atezolizumab (anti-PD-L1) is used in the treatment of clear renal cancer, bladder cancer and non-small cell lung cancer. Moreover there are different case reports, describing positive clinical effect of the inhibitors of PD-1/PD-L1 pathway in the therapy of intracranial meningioma, colorectal cancer, gastric cancer or even penile cancer. They are generally less toxic than chemotherapy, but the treatment complications, i. e. autoimmune phenomena, are not uncommon.

Key words: cancer therapy, chronic viral infections, programmed cell death protein 1, nivolumab, pembrolizumab, atezolizumab.

Session 6. Cancer biology and novel therapeutic approaches II

Chairs: Magdalena Chechlińska, Andrzej Lange

KWO17-00002-2017-01

Adverse drug reactions of voriconazole in relation to CYP2C19 mutations among patients after allogenic hematopoietic stem cell transplantation

Beata Sienkiewicz[1], Donata Urbaniak-Kujda[2], Jarosław Dybko[2], Andrzej Dryś[3], Magdalena Hurkacz[1], Tomasz Wróbel[2], Anna Wiela-Hojeńska[1]

[1]Department of Clinical Pharmacology, Wroclaw Medical University, Wroclaw, Poland
[2]Department and Clinic of Haematology, Blood Neoplasms, and Bone Marrow Transplantation, Wroclaw Medical University, Wroclaw, Poland
[3]Department of Physical Chemistry, Wroclaw Medical University, Wroclaw, Poland

Voriconazole (VCZ) is indicated for treatment of invasive aspergillosis, candidemia, and fungal infections caused by Scedosporium spp. and Fusarium spp. It can also be an alternative for posaconazole in high risk hematological patients' prophylaxis. Pharmacokinetic properties of the drug are influenced by food intake, inter-individual variability and drug-drug interactions. The agent is metabolized by CYP2C19, CYP3A4 and CYP2C9. Only mutations of the first isoenzyme cause variability in voriconazole pharmacokinetics. VCZ treatment may lead to numerous side effects such as pyrexia, nervous system, respiratory, thoracic and mediastinal, gastrointestinal, hepatobiliary and skin disorders. The aim of our study was to determine the influence of CYP2C19 mutations on adverse drug reaction (ADR) occurrence during antifungal prophylaxis conducted with voriconazole in adult patients after allo-HSCT (allogenic hematopoietic stem cell transplantation).

We determined CYP2C19 genotypes in 30 patients after allo-HSCT using PCR-RFLP methods. Biometrical and biochemical data, information on the underlying disease, chemotherapy, prophylaxis failure, adverse drug reactions typical for the use of voriconazole, and probable drug interactions were collected. The observation and reporting of ADR took place from the -1 day before transplantation till the +20th day after transplantation. CYP2C19 genotypes were correlated with observed undesirable effects.

23 patients suffered from at least one side effect during therapy. Most frequent were gastrointestinal disturbances in 15, nervous system disorders in 11 and skin disorders in 7 cases. Patients with CYP2C19*1/*17 suffered mainly from skin, nervous system and gastrointestinal disturbances. CYP2C19*1/*2 and CYP2C19*2/*17 genotype adults showed mainly gastrointestinal, nervous system and skin disorders. Among CYP2C19*17/*17 patients skin disorders and vomiting were observed whereas wild type genotype was connected mainly with swelling. Statistical analysis showed a tendency for patients demonstrating the *2 allele, to experience ADR. No statistical significance was achieved, probably due to a limited number of patients.

In conclusion side effects are common during VCZ treatment. Patients with at least one loss of function allele are more likely to experience adverse drug reactions. Previous determination of CYP2C19 mutations may optimize antifungal treatment in high risk patients.

Key words: adverse drug reactions, voriconazole, CYP2C19, genotyping, hematology.