contemporary oncology

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Oral sessions

OPENING CEREMONY AND WELCOME ADDRESS

Adoptive T Cell Therapy of Cancer

Cassian Yee

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Adoptive cellular therapy involves the ex vivo enrichment and expansion of tumor-reactive T cells for transfer to patients with cancer. Three approaches have been developed to achieve this goal: the use of tumor infiltrating lymphocyte or TIL, extracted from patient biopsy material (TIL therapy), the engineering of lymphocytes using vectors expressing a chimeric antigen receptor (CAR) or T cell receptor (TCR) to redirect antigen specificity (CAR/TCR therapy), and third, the isolation and expansion of naturally-occurring antigen-specific endogenous T cells from the peripheral blood. This last form of ACT, known as endogenous T cell (ETC) therapy requires specialized methods to isolate and expand from peripheral blood, tumor-reactive T cells, often present at very low frequency, and, by sourcing effectors from the entire TCR repertoire, provide the greatest flexibility in delivering a T cell product of defined specificity and phenotype. Several first-in-human studies performed by our lab highlight the importance of antigen spreading in generating long-lasting clinical responses as well as identifying T cells with specialized memory properties. The ETC therapy approach allows for the greatest flexibility in targeting personalized and shared tumor-associated antigens and rapid implementation of adoptive cell therapy from epitope identification to T cell infusion. To broaden the pool of patients and extend the use of adoptive cell therapy to several solid tumor malignancies such as ovarian, lung, GI and breast cancers, we have validated several high value target tumor antigens that are expressed in significant fraction of these tumors. The use of memory-type T cells targeting tumor-associated antigens, provides a unique opportunity to evaluate the combination of adoptive cellular therapy together with immunomodulators such as immune checkpoint inhibitors and costimulatory agonists in a strategy that addresses both intrinsic and extrinsic tumor microenviornmental challenges to successful immune-based therapy.

SESSION 1. INTERACTIONS BETWEEN HOST AND CANCER

Chairs: Magdalena Chechlińska, Jan Lubiński

KWO18

Gene – environment interactions and cancer risk: environmental modifications of cancer risk and progression in hereditary breast tumours

Jan Lubiński

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Hereditary cancers development is strongly dominated by effects of inherited mutations in single genes. Carriers of such mutations have usually > 50% lifetime risk of developing cancers.

The latest advances in studies of hereditary breast cancers are suggesting that environmental modifiers can decrease enormously – to the level of a few percent, penetrance level of high risk mutations even in the case of BRCA1 pathogenic alterations.

The most probably such expected effects can be associated with oral contraceptives and/or microelements including selenium, cadmium, arsenic.

Results of breast cancer treatment can be significantly related to serum Se level and strongly improved by the use of cis-platinum.

SESSION 1. INTERACTIONS BETWEEN HOST AND CANCER

Chairs: Magdalena Chechlińska, Jan Lubiński

KWO18

Inherited mutations of *PALB2* gene and clinical characteristics of breast cancer

Cybulski C[1], Kluźniak W[1], Huzarski T[1], Wokołorczyk D[1], Kashyap A[1], Bogna R[1], Jakubowska A[1], Szwiec M[2], Byrski T[1], Dębniak T[1], Górski B[1], Sopik V[3], Akbari MR[3], Sun P[3], Gronwald J[1], Narod SA[3], Lubiński J[1]; Polish Hereditary Breast Cancer Consortium

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Mutations in PALB2 predispose to breast cancer. There are two founder mutations of PALB2 in the Polish population (509_510delGA and 172_175delTTGT) which are associated with 4 to 5-fold increased risk of breast cancer. We found that 10-year survival for women with breast cancer and a PALB2 mutation is worse than of patients with breast cancer without a mutation (48% vs. 75%, adjusted HR = 2.3; $p < 0.0001$). Given that women with a PALB2 mutation face a high risk of breast cancer and are at a higher risk of death, increased surveillance should be offered to PALB2 carriers. It should be established whether unaffected PALB2 carriers benefit from prophylactic mastectomy, and if PABL2 carriers with breast cancer benefit from specific treatment, in particular specific chemotherapy regimens.

SESSION 1. INTERACTIONS BETWEEN HOST AND CANCER

Chairs: Magdalena Chechlińska, Jan Lubiński

KWO18-00031-2018-01

Molecular changes in cancer-adjacent histologically normal epithelia

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Molecular alterations in histologically normal tissues in tumor proximity have recently been recognized. This has not been studied in endometrial cancer. We aimed to analyze the expression of selected genes associated with cancer progression mechanisms in tumor tissues from patients with endometrial cancer and in the matched, histologically normal endometria, as well as in normal endometrial epithelia from cancer-free patients.

Paired samples of primary tumors and histologically normal endometria from patients with endometrial cancer (59 pairs), samples of normal endometrium from cancer-free patients (n = 25), and endometrial cancer cell lines were examined by the RT-qPCR, for MYC, NR5A2, CXCR2, HMGA2, TWIST1, STK11 and SNAI1 expression. In statistical analyses, the Wilcoxon signed rank test were used for paired samples and the Mann-Whitney rank sum for groups of samples from endometrial cancer patients and from cancer-free patients.

The levels of MYC, NR5A2, CXCR2, TWIST1, STK11 and SNAI1 expression were significantly lower in tumor tissue than in the matched, histologically normal endometrium from the tumor proximity. Moreover, histologically normal endometria from the tumor proximity expressed significantly higher levels of MYC, NR5A2, CXCR2, and HMGA2 than normal endometria from cancer-free patients. Interestingly, MYC and TWIST1 expression did not differ between tumor tissue and endometrial samples from cancer-free patients. Endometrial cancer cell lines presented alterations in expressions similar to that in tumor tissues.

In conclusion, 1) histologically normal endometrium proximal to endometrial cancer presents significant disturbances in the expression of genes associated with "stemness" and/or EMT and metabolism; surprisingly, the examined genes associated with cancer progression mechanisms were often up-regulated in histologically normal cancer-adjacent tissues; 2) new anticancer therapies should take into account not only tumor characteristics, but also the molecular alterations in tumor-adjacent tissues, so far considered to be normal 3) cancer-adjacent histologically normal tissue samples are not representative of molecularly normal tissues.

Key words: endometrial cancer, gene expression, EMT, stemness, cancer-adjacent tissue.

SESSION 1. INTERACTIONS BETWEEN HOST AND CANCER

Chairs: Magdalena Chechlińska, Jan Lubiński

KWO18

The role of arginase-1 in antitumor immune response

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Depletion of essential (L-tryptophan) or semi-essential (L-arginine) amino acids has been shown to suppress antitumor immune responses. Arginase-1 (Arg-1) is a cytosolic enzyme catalyzing degradation of L-arginine to L-ornithine and urea, depleting tumor microenvironment of this compound. T cells need arginine to support their proliferation in the lymph nodes and to promote their ability to kill tumor cells. Arginine deprivation is associated with decreased proliferation potential of activated T cells as well as with down-regulation of CD3 zeta, a major signal transducer from the T cell receptor (TCR). Thus, arginine deprivation due to increased Arg-1 activity is a very smart strategy of the tumor to avoid T cell-mediated effector mechanisms and, at the same time, one of the potential targets of anti-tumor therapy. Arg-1 is overexpressed not only by cancer-associated fibroblasts (CAFs), myeloid-derived suppressory cells (MDSCs) but also numerous cancer cells such as renal cell carcinoma, breast carcinoma, prostate cancer and colorectal cancer. We have recently discovered that exosomes, a double-layered small vesicles produced by ovarian cancer cells, contain enzymatically active Arg-1. Exosome-derived Arg-1 suppresses proliferation of CD4 and CD8-positive T cells activated with anti-CD3/anti-CD28 antibodies as well as T cells activated in the antigen-specific manner. All these in vitro effects are reversed by addition of an arginase inhibitor. Arg-1 containing tumor-derived exosomes are efficiently being engulfed by the dendritic cells and transported to the draining lymph nodes to create immunosuppressive environment at the site of the development of the immune response. Moreover, tumor-derived Arg-1 is detectable in the blood of ovarian cancer-bearing animals. Arg-1-expressing ovarian cancer grows faster in vivo and its growth is slowed down by the treatment of animals with the arginase inhibitor. In vivo, in Arg-1-expressing ovarian cancer cells arginase inhibition results in maturation of the peritoneal dendritic cells and their enhanced ability to engulf and present tumor-derived proteins. Altogether, our findings provide the first evidence for the role of Arg-1 in the formation of an immunosuppressive microenvironment in ovarian cancer. We identify a novel mechanism of exosomal Arg-1 distribution from the tumor cells to antigen presenting cells. Moreover, inhibition of Arg-1 activity may be an attractive novel anti-cancer strategy. The latter idea will be further discussed in this presentation.

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SESSION 2. BIOMARKERS

Chairs: Joanna Chorostowska-Wynimko, Krzysztof Składowski

KWO18

Potentials and pitfalls of biomarker-based adaptive-randomization in cancer trials

Tomasz Burzykowski

Hasselt University, Belgium

Outcome-adaptive randomization (OAR) designs extend the traditional, fixed-ratio randomization (FRR) designs by allowing the randomization ratio to change continuously over time in function of the clinical outcome information collected during the trial. Since this generally results in more patients assigned to the 'more promising' treatment arm(s), OAR is suggested to increase patient-specific benefits in clinical trials (Lee *et al.*, 2012). In Bayesian biomarker-driven OAR designs, the adaptive randomization idea is combined with the targeted-design concept. In targeted designs, patients are pre-screened by using, e.g., biomarkers, before being randomized to treatments which would be deemed the 'most promising' based on patients' screening results (Simon *et al*., 2004). By combining OAR with the idea of targeted designs it is possible to change the ratio of assigning patients within particular biomarker strata to the 'most promising' treatment arms during the course of the trial. Such combined designs have also been proposed to test the efficacy of a novel targeted treatment while simultaneously identifying predictive markers for the treatment (Gu *et al*., 2016).

Advantages of Bayesian biomarker-driven OAR designs, as compared to the FRR designs, in terms of, for instance, a reduced total target sample size or a decrease in the variation of the accrued sample size have been reported (Zhou *et al*., 2008; Barry *et al*., 2015; Gu *et al*., 2016). However, several issues with OAR designs have been identified, including potential bias in the results due to time trends in the prognostic characteristics of the patient population (Korn *et al*., 2017), statistical inefficiency due to imbalance in the number of patients assigned to different treatment arms (Korn *et al*., 2015), and a non-trivial probability of ending up with a substantially larger number of patients included in the worse-treatment arm (Thall *et al*., 2015). It also appears that using an imperfect biomarker-assay results in a reduced power of a Bayesian biomarker-driven OAR design for efficacious stratum-treatment combinations and an increased type-I error probability for inefficacious combinations. Thus, the apparent promise of OAR designs may require careful reflection, as the designs may not be as advantageous as advocated.

SESSION 2. BIOMARKERS

Chairs: Joanna Chorostowska-Wynimko, Krzysztof Składowski

KW018-00029-2018-01

The role of the CRNDE oncogene in cancer development and screening

Lukasz Szafron, Anna Balcerak, Monika Jackiewicz, Agnieszka Dansonka-Mieszkowska, Ewa Grzybowska, Magdalena Chechlinska, Jolanta Kupryjanczyk

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Until recently, the CRNDE transcripts were regarded as long non-coding RNAs (lncRNAs). These transcripts are highly overexpressed in many types of cancer. Despite its negative impact on cancer prognosis, the function of the CRNDE gene is still poorly understood. It seems to play its oncogenic role via the PI3K/AKT/mTOR, Raf/MAPK and Wnt/B-catenin signaling pathways and by interacting with several microRNAs. Furthermore, the levels of exosomal CRNDE transcripts in blood plasma were recently reported as a novel, sensitive and specific biomarker for diagnosis and prognosis of colorectal cancer. Our earlier results showed that elevated expression of CRNDE is correlated with a poor prognosis in ovarian cancer patients. We also discovered that the CRNDE protein product (CRNDEP) was upregulated in cells characterized by a high proliferation rate in both cancer and normal tissues. Moreover, its artificial, induced overexpression in HeLa cells resulted in the formation of stress granules, and CRNDEP localized within these structures. Endogenous CRNDEP was found predominantly in the nucleus, and in dividing cells it became a component of centrosomes. In our recent study, we analyzed a DNA sequence that separates the CRNDE and IRX5 genes (which share the same bidirectional promoter). The region of 232 bp with the highest concentration of CpG islands was searched for methylated cytosines using bisulfite conversion of unmethylated cytosines to uracils followed by PCR and Sanger sequencing. We analyzed 134 human ovarian carcinomas treated with platinum/cyclophosphamide (PC, $n = 32$) or taxanes/platinum (TP, $n = 102$), and 20 normal ovaries/fallopian tubes. The study revealed hypomethylation of this region in cancers compared to normal tissues (Fisher's exact test: $p = 2.529e\text{-}05$). Noteworthy, the methylation pattern was distinct between ovarian cancers and normal samples (Pearson's correlation: R2= -0.239, $p = 0.095$). As to the clinical aspects of the tumors, we proved that the methylation adversely influenced cancer outcome, increasing the risk of death (HR 1,983, $p = 0{,}013$) in all patients, and the chance for complete remission (HR 0.023, $p = 0.033$) in patients treated with TP, without TP53 accumulation. The results are promising, CRNDE may prove to be new diagnostic and prognostic marker, or even a target for a molecular therapy, but further evaluation of the clinical significance of the CRNDE gene products is necessary.

Key words: CRNDE, promoter methylation, ovarian cancer, biomarker, lncRNA.

SESSION 2. BIOMARKERS

Chairs: Joanna Chorostowska-Wynimko, Krzysztof Składowski

KW018

Circulating tumor DNA in lung cancer – clinical potential and challenges

Joanna Chorostowska-Wynimko

Department of Genetics and Clinical Immunology, National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland

Circulating tumor DNA (ctDNA) is emerging in lung cancer as a key potential biomarker/diagnostic material for informed clinical decision-making process, post-diagnosis surveillance but it may also play a crucial role in the detection of pre-clinical cancer.

1/ ctDNA allows precise detection of predictive biomarkers for targeted therapies, mainly activating or resistance mutations (e.g., T790M) in EGFR gene with sensitivity ~60-80% and specificity ~99%. Tumor tissue is still considered the preferred definitive standard sample type; however, for many patients, this sample type is not available (~10–25%). Recent guidelines recommend ctDNA analysis as first-choice method for detection of resistance mutations in patients experiencing disease progression after first-line treatment with EGFR tyrosine kinase inhibitors (TKIs).

2/ It has been suggested and subsequently demonstrated that monitoring EGFR mutations with plasma DNA reflects the clinical course of lung cancer patients treated with EGFR-TKI. Detection of T790M resistance mutation with plasma DNA was correlated with activating EGFR mutation type, exon 19 deletions and tumor progression. Since at this stage re-biopsy is successful only in minority of NSCLC patients, T790M surveillance in ct DNA might prove useful not only in monitoring EGFR TKI therapy effectiveness but also in designing strategies for subsequent treatment.

3/ The source of free circulating DNA (cfDNA)appears to be mainly the result of cell death – either by necrosis or apoptosis but also active DNA shedding. A raised ctDNA level is therefore non-specific for cancer, but may indicate the presence of number of pathologies. Moreover, in blood, cfDNA is always present as small fragments, which makes reliable assessment challenging. In a group of 50 resectable NSCLC patients, 101 subjects with chronic respiratory inflammation (chronic obstructive pulmonary disease, sarcoidosis, or asthma) and 40 healthy volunteers, we demonstrated that elevated plasma cfDNA levels in NSCLC resulted primarily from tumor development rather than inflammatory response, raising the potential clinical implications for lung cancer screening and early diagnosis. Assessment of plasma cfDNA levels provided 90% sensitivity and 80.5% specificity in discriminating NSCLC from healthy individuals (area under the curve (AUC) = 0.90). Likewise, the plasma cfDNA integrity in healthy individuals proved significantly different than that found in patients with NSCLC or benign lung tumours ($p < 0.0003$) and provided promising discriminatory power (91% sensitivity, 68.2% specificity). These data demonstrate that the quantitative and qualitative plasma cfDNA analysis offers promising diagnostic capacity comparable to the values presented by conventional imaging modalities used in clinical practice.

SESSION 2. BIOMARKERS

Chairs: Joanna Chorostowska-Wynimko, Krzysztof Składowski

KWO18

Serum metabolomics – new approach to support early detection of lung cancer

Piotr Widłak, Małgorzata Roś-Mazurczyk, Karol Jelonek, Anna Wojakowska, Monika Pietrowska, Łukasz Marczak, Krzysztof Polański, Michał Marczyk, Joanna Polańska, Rafal Dziadziuszko, Jacek Jassem, Witold Rzyman

Objectives: The role of a low-dose computed tomography (LD-CT) lung cancer screening remains a matter of controversy due to its low specificity and high cost. Screening complementation with blood-based biomarkers may allow a more efficient pre-selection of candidates for imaging tests or discrimination between benign and malignant chest abnormalities detected by LD-CT. We searched for a molecular signature based on a serum metabolome profile distinguishing individuals with early lung cancer from healthy participants of the lung cancer screening program.

Material and methods: Blood samples were collected during a LD-CT screening program performed in the Gdansk district (Northern Poland). The analysis involved 100 patients with early stage lung cancer (including 31 screen-detected cases) and the pair-matched group of 300 healthy participants of the screening program. MALDI-ToF mass spectrometry was used to analyze the molecular profile of lipid-containing fraction of serum samples in the 320-1000 Da range. The GC/MS approach was used to identify and quantify small metabolites present in serum.

Results: Several components of the serum lipidome were detected, with abundances discriminating patients with early lung cancer from high-risk smokers. An effective cancer classifier was built with an area under the curve of 0.79 and 0.72 in the training and test groups, respectively. Corresponding negative predictive values were 100% and 92%, and a positive predictive value was 28% each. The downregulation of a few lysophosphatidylcholines (LPC18:2 and LPC18:1) in samples from cancer patients was confirmed using a complementary LC-MS approach. Moreover, several metabolites were detected in the sera which abundances discriminated patients with lung cancer (31 screen-detected cases) from matched controls (92 healthy individuals). Majority of differentiating components were downregulated in cancer samples, including amino acids, carboxylic acids and tocopherols, whereas benzaldehyde was the only compound significantly upregulated. A classifier including nine serum metabolites allowed separation of cancer and control samples with 100% sensitivity and 95% specificity.

Conclusions: Metabolome-based serum signatures showed potential usefulness in discriminating early lung cancer patients from healthy individuals. These signatures, though not validated in an independent dataset, deserves further investigation in a larger cohort study.

SESSION 2. BIOMARKERS

Chairs: Joanna Chorostowska-Wynimko, Krzysztof Składowski

KW018-00058-2018-01

Serum microRNAs as biomarkers of high-dose irradiation

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Effective planning for the medical response to a radiologic or nuclear accident is complex. Due to limited resources for medical countermeasures, the key would be to accurately triage and identify victims most likely to benefit from treatment. Similarly, predicting the occurrence of radiation toxicity during radiotherapy is a field of intensive studies in oncology. Our recent works performed in animal models and during clinical studies have advanced the field by formulating an efficient algorithm for the detection of high-dose, potentially lethal irradiation.

Our tool relies on microRNAs – an emergent class of serum biomarkers that show remarkable stability, ease of quantification and good replicability. The diagnostic model was devised by a series of irradiation experiments on mice and non-human primates (Macaca mulatta). The diagnostic model itself is a two-tiered classifier that uses miR-133b, miR-215, miR-375 expression to identify exposed individuals (Area under the ROC curve 0.99 (95% CI: 0.98-1.00)) and, by using sex-adjusted expression levels of miR-30a and miR-126 provides a for irradiation and an AUC of 0.79 (95% CI: 0.56-1.00) to identify NHPs that would die after the exposure to doses within the LD30-LD70 range. Furthermore, by overlapping these results we identified a subset of miRNAs that after bioinformatic analyses revealed evolutionary conservation of miRNAs themselves and their promoter regions, evidencing mechanistic involvement in the mitigation of radiation exposure's effects.

In conclusion, measurement of serum miRNA expression may be used as an efficient diagnostic tool in mass exposure scenarios to unknown doses of radiation, in order to facilitate screening for patients that will require bone marrow transplantation in order to survive.

Key words: biomarker, radiation, biostatistics.

SESSION 2. BIOMARKERS

Chairs: Joanna Chorostowska-Wynimko, Krzysztof Składowski

KW018-00028-2018-01

SNAIL-microRNA axis is a key regulator of alveolar rhabdomyosarcoma progression

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Rhabdomyosarcoma (RMS) is a frequent non-epithelial tumor of soft tissue that causes death and morbidity of children and adolescents. RMS originates from an impaired myogenic differentiation of satellite cells or mesenchymal stem cells. Despite numerous efforts, the precise mechanism of RMS development is unknown.

SNAIL is a zinc finger transcription factor that plays an eminent role in the epithelial to mesenchymal transition (EMT), the main mechanism responsible for the invasiveness and metastasis of neoplasms. Nevertheless, the role of SNAIL in non-epithelial tumors is poorly understood. We hypothesized that SNAIL-microRNA axis may be a crucial pathway affecting development of metastatic tumor of non-epithelial origin, such as RMS.

We discovered that expression of SNAIL is elevated in the aggressive alveolar subtype of RMS, characterized by a low myogenic differentiation status. Interestingly, differentiation of RMS cells downregulates SNAIL level. Moreover, SNAIL silencing completely abolishes the growth of human RMS xenotransplants in mice. SNAIL affects also RMS metastasis by reorganization of actin cytoskeleton.

To investigate the effect of SNAIL silencing in RMS on microRNA and small RNA transcriptome, next generation sequencing on the Illumina NextSeq 500 system was performed. The differential expression analysis was done using the EdgeR software package and TMM normalization. We discovered that SNAIL inhibits myogenic differentiation and metastasis by regulation of microRNAs expression. Sequencing and gene ontology results revealed that SNAIL silencing downregulated and upregulated many different microRNAs that were associated with muscle and actin cytoskeleton structure. For example myomiRs (myogenic microRNAs), such as miR-206, miR-1, miR-133b and miR-378a-3p were upregulated in RMS cells with downregulated SNAIL level. Interestingly, we discovered not only miRNAs, but also other small RNAs associated with SNAIL expression in RMS. What is more, we have also identified putative novel miRNAs, which are predicted from the sequences that do not map to any organism found in miRbase, or to other known RNA sequences.

To conclude, our data suggest that deregulation of SNAIL-microRNA axis might be responsible for RMS development and progression. New microRNA candidates need to be validated in further studies in vitro and in vivo.

Key words: rhabdomyosarcoma, microRNA, SNAIL transcription factor, tumor progression.

The project was supported by the grant from the National Science Centre in Poland to KS: 2015/17/D/NZ5/02202.

SESSION 3. TUMOR MICROENVIRONMENT AND ITS TARGETING

Chairs: Claudine Kieda, Sergio Abrignani

KWO18

Overcoming immunosuppression in tumor microenvironment

Viktor Umansky

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Melanoma microenvironment is characterized by a strong immunosuppressive network, where myeloid-derived suppressor cells (MDSC) induced by chronic inflammation play a major role. Most factors involved in chronic inflammatory reactions are produced during acute inflammation, inducing the T-cell stimulation. However, a long-term secretion and maintenance of these mediators in the process of tumor development stimulates an enrichment and activation of myeloid regulatory cells such as MDSC. Using a *ret* transgenic mouse melanoma model that closely resembles human melanoma, we found in melanoma lesions, increased concentrations of chronic inflammatory factors (IL-1beta, IL-6, IFN-gamma etc.) associated with the MDSC accumulation that inhibit anti-tumor reactivity of T cells. Moreover, $CCR5^+$ MDSC displayed a stronger immunosuppressive phenotype and activity. In addition, an enrichment of $CCR5^+$ MDSC in melanoma lesions was associated with an increase in the concentration of CCR5 ligands in tumor microenvironment. The accumulation and activation of MDSC could be mediated not only by soluble inflammatory factors but also by tumor-derived exosomes. These microvesicles were shown to convert immature myeloid cells into immunosuppressive MDSC. Targeting of MDSC migration and functions by ultra-low dose paclitaxel or blocking CCR5-CCR5 ligand interactions in tumor-bearing mice significantly prolonged their survival associated with the restoration of T cell anti-tumor reactivity.

In advanced melanoma patients that were resistant to immunotherapy with checkpoint inhibitors, MDSC targeting induced a beneficial therapeutic effect. We suggest that chronic inflammatory mediators and MDSC are of critical importance for melanoma pathogenesis and their neutralization should be included in combined melanoma immunotherapies to increase their efficiency.

SESSION 3. TUMOR MICROENVIRONMENT AND ITS TARGETING

Chairs: Claudine Kieda, Sergio Abrignani

KWO18

Tumor and its hypoxic microenvironment, what should be the target?

Claudine Kieda

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Killing specifically cancer cells is the ultimate and constant aim. It has led to large improvement of the therapeutic approaches and their outcome. But this has also led to uncover the mechanisms of encountered pitfalls. Those are highly informative and are mainly pointing out the part played by the microenvironment.

Tumor development occurs and goes along with the setting of a favorable microenvironment which gets started by the angiogenic switch then is propagated and maintained due to the constant hypoxic condition of the tumor site.

Hypoxia, the common parameter in tumor development, selects aggressive cells in niches and rules the angiogenesis. Tumor angiogenesis is pathologic and appears as a critical hallmark of cancer for its phenotype and consequences on tumor growth, resistance and immune suppression. The extent of consequences that the pathologic angiogenesis exerts in cancer sums up in the microenvironment properties.

Targeting tumor cells in tumors cannot be considered separately from the whole microenvironment, on the other hand, the modulation of the microenvironment conditions opens new means to target the tumor cells themselves.

To exemplify the intercellular cross-talk regulating tumor cells development in relation to the microenvironment, the endothelial cells particular properties provide a new insight with deep consequences on the potential tumor treatment approaches. Normal endothelial cells ability to use the glycolytic pathway in normoxic conditions, as cancers cells do, makes them particularly able to survive in harsh hypoxic conditions in the tumor site and to develop a growing angiogenesis in response to hypoxia. The control of angiogenesis in the tumor context appears to be a strategy allowing the microenvironment targeting as the first necessary condition to achieve tumor cell targeting.

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SESSION 3. TUMOR MICROENVIRONMENT AND ITS TARGETING

Chairs: Claudine Kieda, Sergio Abrignani

KWO18

Repolarizing tumor infiltrating immune cells to enhance antitumor therapy

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Cancer immunotherapies exploit various approaches from stimulating effector mechanisms to counteracting inhibitory and suppressive mechanisms. High grade gliomas are rapidly progressing brain tumors with a very poor prognosis despite extensive resection combined with radiation and/or chemotherapy. Histopathological and flow cytometry analyses of human and rodent experimental gliomas revealed heterogeneity of immune cells infiltrating tumor and its niche. Intratumoral density of glioma-associated microglia/macrophages (GAMs) and myeloid derived suppressor cells (MDSCs) is highest in malignant gliomas and inversely correlates with patient survival. Tumor-reprogrammed GAMs release immunosuppressive cytokines and chemokines blocking antitumor responses. Pharmacological reprograming of innate immunity is considered to be a new approach in tumor immunotherapy. Several approaches aimed at repolarizing innate immune cells have been developed, i.e. using oligodeoxynucleotides or siRNA inhibitory molecules conjugated to CpG oligonucleotides. We have identified new tumor-derived signals and mechanisms driving myeloid cell accumulation and their pro-tumorigenic polarization. We demonstrated that granulocyte macrophage colony stimulating factor-GMCSF/CSF2 which interacts with its receptor on microglia was essential for microglia infiltration and tumor growth *in vivo*. The expression of *CSF2* is upregulated in human malignant gliomas and correlates inversely with survival. Down-regulation of *CSF2* in human glioma cells reduced GAMs accumulation, impaired tumor growth in nude mice and prolonged survival. To translate those results into clinically relevant setting, we designed and tested *in vitro* a series of humanized short peptides interfering with binding of CSF2 to cognate receptors. We selected non-cytotoxic peptides with potent activity in blocking microglia-dependent glioma invasion in cell co-cultures. Moreover, we provide an evidence for anti-tumor activity of the water soluble, human CSF2 targeting peptide delivered intra-cranially to human U87 gliomas growing in nude mice. *In vivo* imaging and histological evaluation showed reduction of tumor growth. Our results show that targeting glioma-microglia interactions with short interfering peptides could be a novel therapeutic strategy.

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SESSION 3. TUMOR MICROENVIRONMENT AND ITS TARGETING

Chairs: Claudine Kieda, Sergio Abrignani

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Functionalized bioengineered spider silk spheres for siRNA delivery to the tumor microenvironment

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The limited serum stability and the cell-specific delivery limit a clinical application of siRNA molecules. An efficient system for siRNA therapeutics delivery is needed, and silk as a biocompatible and biodegradable material can be used for developing a drug delivery system. Thanks to the technology of synthetic gene construction, the bioengineered silk can be functionalized by adding peptide of the desired properties. Silk spheres made of silk functionalized with the nucleic acid binding peptide can be loaded with therapeutic CpG-siRNA to target the macrophages in the tumor microenvironment.

We constructed, produced and purified the bioengineered silks: i) MS2 and ii) MS2KN a hybrid variant that contained a nucleic acid binding domain (KN). The therapeutic molecule CpG-siRNA efficiently bound to MS2KN in contrary to control silk MS2. The complexes and spheres formed by MS2KN silk protected siRNA from degradation in serum. The MS2KN spheres were loaded with CpG-siRNA constructs, internalized and processed by target macrophages in the absence of transfection reagents. The CpG-STAT3siRNA molecules delivered in silk spheres were observed inside the cells for the more extended period than naked nucleic acid construct. Moreover, the silencing activity of CpG-STAT3siRNA provided by silk nanoparticles was delayed and extended compared with the activity of the naked oligonucleotides. The prolonged Stat3 silencing resulted in the more pronounced downregulation of interleukin 6 (IL-6).

The application of silk spheres not only protected siRNA therapeutics from degradation in serum but also modified the kinetics of their action thus it can elicit a different effect in vivo. Since STAT3 and IL-6 drive growth and progression of various tumors, the application of CpG-STAT3siRNA loaded into MS2KN silk spheres may be of great importance for cancer immunotherapy.

Key words: drug delivery system, cancer therapy, silk spheres.

SESSION 3. TUMOR MICROENVIRONMENT AND ITS TARGETING

Chairs: Claudine Kieda, Sergio Abrignani

KWO18-00014-2018-01

Short peptide binding GM-CSF interfers with glioma-microglia environmnent and inhibits glioblastoma progression

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Glioblastoma (WHO grade IV, GBM) is a malignant, very aggressive, primary brain tumor which due to lack of efficient therapy caused by the heterogeneity of genetic abnormalities of tumor cells remains incurable. Tumor microenvironment plays an important role in growth, metastasis and response to treatment in many tumors, also GBM. Therefore, an approach to target tumor microenvironmet gained recently an increased attention. Brain resident immune cells – microglia and peripheral macrophages accumulate in malignant glioma and constitute 30-50% of the tumor mass. Glioma cells overexpress and secrete proteins that reprogram microglia and peripheral macrophages into cells which potentiate tumor invasion and growth, furthermore suppress antitumor immunity. Glioma-derived granulocyte macrophage colony-stimulating factor – GM-CSF (Csf-2) induces accumulation and protumorigenic activation of microglia/macrophages. We designed a humanized peptide that selectively binds to GM-CSF, blocks its binding to respective receptors on microglia, and inhibits activation of the receptors and downstream signaling pathways resulting in inhibition of glioma invasiveness. First, we designed a peptide library containing 26 peptides 14-residue long each. Next, we identified the peptides binding GM-CSF using peptide microarrays, enzyme-linked immunosorbent assay (ELISA) and a technique based on surface plasmon resonance (SPR). Subsequently, we selected peptide (G7) with the most potent capacity for inhibition of U87 MG and LN18 glioma cell invasiveness in the presence of human and mouse microglia cell line using the Matrigel Matrix cell invasion assay. We also confirmed that this peptide blocks binding of GM-CSF to its receptor using a method based on SPR technique and LigandTracer. Antitumor activity of G7 peptide in vivo was confirmed in orthotopic xenograft mouse model.

Key words: melanoma, Hippo pathway, LATS1, EMT.

SESSION 4. CANCER IMMUNOTHERAPY – NEW APPROACHES

Chairs: Paweł Kalinski, Helen Gogas

KW018

Combinatorial Adjuvants and Dendritic Cell Therapies Sensitize "Cold" Tumors to Checkpoint Blockade

Pawel Kalinski

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The presence of $CD8^+$ cytotoxic T cells (CTLs) in the tumor microenvironment (TME) is required for the clinical effectiveness of PD-1 blockers and other therapies of solid tumors. In contrast, local prevalence of regulatory T cells (T*regs*) and myeloid-derived suppressor cells (MDSCs) predicts poor outcomes. We observed that intratumoral or systemic delivery of type-1-polarized dendritic cells (αDC1s) or combinatorial chemokine-modulating [CKM] regimen composed of TLR3 ligands, IFNα and COX2 blockade can abrogate the baseline heterogeneity of TMEs of different types of human cancers, uniformly enhancing their ability to attract CTLs. Interestingly, CKM preferentially induces CTL attractants (CXCL9, CXCL10 and CCL5) in tumor lesions, rather than healthy tissues, allowing local intratumoral impact upon systemic CKM application. The CKM-driven intratumoral accumulation of spontaneously-arising or vaccination-induced CTLs shows strong therapeutic synergy with PD-1/PD-L1 blockade in mouse models of ovarian and colorectal cancers, with similar results achieved by type-1-polarized DCs. Ongoing clinical trials NCT01545141 (metastatic colon cancer; systemic CKM involving rintatolimod, IFNα and celecoxib) and NCT02432378 (ovarian; local CKM) provide early indication of the safety and local effectiveness of CKM in enhancing intratumoral CTL accumulation and modulating PD-1/PD-L1/PD-L2 system in the TME of human cancers.

SESSION 4. CANCER IMMUNOTHERAPY – NEW APPROACHES

Chairs: Paweł Kalinski, Helen Gogas

KW018

New old approaches for melanoma therapeutic vaccination

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Immune checkpoint inhibitors (IChI)have changed the landscape of cancer therapy including melanoma. The treatment is not cancer specific, is clinically beneficial only in fraction of patients, however, when various inhibitors are combined the clinical effects are excellent but toxic. Accordingly, the intensive research is carried out to delineate the mechanisms of resistance and transient effects in some patients. The clinical responses are related to the immune-status of the patient and failures are due to the specific lymphocytes exhaustion.

Discovery of immune checkpoint synapses and better understanding of local tumor immunosuppression mechanisms partially explain the failure of clinical effectiveness of number of therapeutic cancer vaccines. It is becoming generally accepted that it is not enough to inhibit immune checkpoint synapse but boosting of specific anti-cancer vaccination is required. Accordingly, cancer vaccines are intensively being tested in combination with these inhibitors.

We have developed therapeutic genetically modified whole cell allogeneic melanoma vaccine (AGI-101H). Genetic modification have changed the vaccine cells phenotype into melanoma stem cells-like. This led to generation of T cells responses and specific antibodies directed to stem cell markers in patients. Number of phase II clinical trials has demonstrated the highest clinical efficacy of AGI-101H in the treatment of advanced melanoma patients (stage IIIB-IV) with resected disease in adjuvant setting. The vaccination comprised of two phases induction and maintenance. In case of recurrence re-induction with or without surgery was applied and followed by maintenance. Combination of re-induction with surgery was statistically more effective than re-induction alone. More than 33% of patients never progressed, 40% progressed and had re-induction, while the rest did not respond. The liquid biopsy predictive of response pre-treatment biomarker(s) was identified. High expressing patients (80%) survived over 15 years, while all non-progressing patients displayed high expression. Resection of returning metastases followed by vaccination continuation confirms immunosuppressive status of formed tumors and supports combinational approach of cancer vaccination with IChC or other approaches affecting tumor microenvironment such as hypoxia normalization.

Enrichment of whole cell murine melanoma vaccine with melanoma stem cells or murine inducible pluripotent stem cells (miPS) in model studies confirmed our clinical results. Addition of stem cells into vaccine resulted in significantly longer disease free survival and overall survival (OS) of immunized mice. The most effective was addition of miPS resulting in over 70% of mice surviving experiment limits (120 days). However, vaccine composed only of irradiated (adjuvant less) miPC had no clinical effect.

Finally, our mechanistic studies demonstrated that AGI-101H vaccine mode of action may be related to driving residual melanoma cells into dormancy.

The study was supported by a grant INNOMED/I/6/NCBR/2014 (PerMel) "Personalizacja leczenia czerniaka przy użyciu szczepionek terapeutycznych".

SESSION 4. CANCER IMMUNOTHERAPY – NEW APPROACHES

Chairs: Paweł Kalinski, Helen Gogas

KWo18

Chimeric antigen receptor-based therapies against solid tumors – work in progress

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Among the immunotherapies, adoptive anticancer strategies, including T cells expressing engineered, chimeric antigen receptors (CAR-T cells), are an exciting and rapidly growing field that involves extracting the patient's own immune cells, expanding and modifying them ex vivo to recognize tumor associated antigens, before transferring them back to the patient. CAR molecules are synthetic proteins, which provide immune effector cells with the ability of highly precise and effective recognition of the target cancer cells combined with the immediate stimulation to kill these cells. These abilities make the CAR-based technology applicable, in theory, to almost all types of cancer. And yet, despite impressive results achieved with CAR-based therapies against some hematologic malignancies, CAR-based strategies are most often far less effective in combat against solid tumors.

The main obstacles in development successful CAR-based therapies against solid tumors are those related to balancing between efficacy and safety. Indeed, rarity of targets with exclusive solid tumor specificity poses a risk of frequent "on-target, off-tumor" adverse effects. Additional issues relate to trafficking and efficient penetration of CAR-T cells to the tumor sites, and also survival and functionality of CAR-T cells under hostile, immunosuppressive microenvironment of tumor mass, with elevated immune and metabolic checkpoints. Other challenges are common with those in CAR-T therapies against hematologic tumors, i.e. seeking for efficient and long-lasting expression of CAR molecules in the effector cells, curtailing the cytokine release syndrome, or heterogeneity of tumors causing escape with the loss of target.

Researches have been proposing a range of strategies to address the challenges present in CAR-T-mediated treatment of solid tumors. These are for instance: replacing T lymphocytes with natural killer (NK) cells, utilizing a range of gene delivery systems, more precise targeting of CARs, arming the CAR-harboring cells with additional secretory agents, such as stimulatory cytokines (e.g. IL-12), or combinatory therapies, e.g. with immune or metabolic checkpoint inhibitors. There is also a constant search for generating a universal, "off-the-shelf" CAR-based strategy that could be used in patients faster and with increased flexibility.

Despite some obstacles, one should expect that in a foreseeable time the scale, ambition and innovative character of CAR-based research will provide more successful therapies against a range of solid tumors.

SESSION 4. CANCER IMMUNOTHERAPY – NEW APPROACHES

Chairs: Paweł Kalinski, Helen Gogas

KW018-00038-2018-01

Immune regulatory cells in tumour progression

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Tumour progression has been linked to the activity of immune regulatory cells which suppress immune surveillance. On the other side, different subsets of immune regulatory cells are considered a viable option in the immunosuppressive treatment with first clinical trials testing them completed already. It is therefore mandatory to take into account a risk of oncogenesis when the preparations of these cells are used in the clinic. The experience from completed trials as well as from preclinical studies with immune regulatory cells can give insight into a real influence of these cells on the tumour induction and progression. In this lecture we will show our preclinical and clinical experience with different subsets of immune regulatory cells with special attention to their role in oncogenesis.

Key words: regulatory cells, immune tolerance, tumour progression.

SESSION 5. TARGETED CANCER THERAPIES

Chairs: Piotr Rutkowski, Piotr Radziszewski

KW018

How to optimize targeted therapy in gastrointestinal stromal tumors?

Piotr Rutkowski

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Gastrointestinal stromal tumor (GIST) became a model for targeted therapy. The majority of GIST are associated with activating, constitutive, mutually exclusive mutations of two genes: KIT and PDGFRA (PDGF receptor-alpha). Molecular diagnostics is currently an essential part of GIST management. The first effective systemic therapy introduced into clinical practice in GIST and DFSP, was imatinib - tyrosine kinase inhibitor acting on, e.g. KIT and PDGFR alpha/beta. Use of the drug revolutionized treatment of inoperable and/or metastatic cases and demonstrated dramatic activity in locally advanced cases. Adjuvant treatment of GIST patients with high risk of recurrence with imatinib for 3 years increases relapse-free survival (RFS) and overall survival (OS) compared with one-year therapy [SSGXVIII study; 5-year RFS 71.1% vs. 52.3% for 36-month and 12-month therapy with imatinib respectively; and 5-year OS, 91.9% vs. 85.3% respectively]. Adjuvant treatment in a group with a high risk of recurrence after resection of the primary tumor should last 3 years but still the optimal duration of treatment and imatinib dosage (for exon 9 *KIT*) is not determined. At patient qualification for adjuvant treatment it is mandatory to assess the mutation status of the GIST while the use of adjuvant treatment with imatinib in GIST genotypes with low sensitivity to imatinib (PDGFRA p.D842V or wild-type) remains questionable. The greatest benefit of adjuvant therapy is achieved in patients with the highest risk of relapse (> 5 mitoses/50 HPF and/or tumor size > 5 cm; location of the primary tumor in other parts of the gastrointestinal tract than the stomach; resection within microscopically confirmed infiltrated surgical margins – R1 or rupture of the tumor during surgery) and with tumors harboring mutations in the exon 11 KIT gene.

The results of prospective phase I-III clinical trials for the treatment of inoperable or metastatic GIST with imatinib demonstrated the median OS of about 5 years which is about a 4-fold increase when compared with historical data (median survival: 12-15 months). The median PFS of patients treated with imatinib is 2-3 years. The best long-term activity of imatinib is seen in patients with mutations in exon 11 KIT. The other predictive factors include e.g. performance status, neutrophil-lymphocyte ratio, tumor burden, etc. Moreover, predictive nomograms have been recently built. Personalization of therapy is related also to specific populations, as in older patients, patients with *KIT/PDGFRA* wild-type GIST etc. During the treatment with imatinib some patients experience disease progression associated with drug resistance. A small portion of patients (10-15%) – (GIST CD117+) show primary and early resistance during the initial six months of treatment. In patients responding to the treatment, along with extending the treatment duration, can occur secondary (acquired) resistance to imatinib. It is estimated that during 2-3 years of treatment with imatinib 40-50% of patients show evidence of progressive disease. Imaging studies can present limited (focal) form of progression where surgery can be utilized. Generally however, multifocal progression is more often. It was found that likely different mechanisms accompany primary and secondary resistance to imatinib therapy. The most common secondary resistance is a result of the acquisition of additional mutation or mutations in KIT or PDGFRA which lead to changes in receptor conformation and the inability to bind to imatinib. Secondary resistance, observed in patients who initially responded well to treatment, is frequently associated with the selection of polyclonal cells harboring an additional KIT gene mutations (approx. 60–80% of tumors). The most common secondary mutations are located in the kinase domain of the KIT receptor encoded by exons 13, 14 and 17. Use of second line tyrosine kinase inhibitors should be considered in case of progression after the use of increasing imatinib dose. The use of other inhibitors, acting on different points in metabolic pathway than the associated with mutation in *KIT* exon 11, can help overcome resistance to imatinib. Currently, the only approved drug in the second line treatment in case of resistance to imatinib or intolerance to treatment is sunitinib. Longer OS and higher overall response rate were observed in patients with a primary *KIT* mutation in exon 9 versus exon 11. Prospective, randomized, placebo-controlled clinical study with regorafenib showed prolongation of in patients with GIST resistant to imatinib and sunitinib. The drug has been registered as therapeutic option in the third line of treatment. I Poland we use sorafenib based on the results of phase II study. Over the last few years major progress has been made in elucidating the mechanism of disease progression (as secondary mutations in KIT and/or PDGFRA kinase domains) and resistance to imatinib in GIST and some other therapies were introduced into clinical practice. Several trials of new agents (mostly new tyrosine kinase inhibitors) designed to overcome resistance are underway. Some of the molecules show promising activity in *PDGFRA D842V* mutant cases (BLU-285 or crenolanib) and also in *KIT* genotype cases with secondary mutations (BLU-285, ponatinib, DCC-2618, PLX-948).

SESSION 5. TARGETED CANCER THERAPIES

Chairs: Piotr Rutkowski, Piotr Radziszewski

KW018

Is personalized treatment of prostate cancer based on biomarkers possible?

Piotr Radziszewski

Warsaw Medical University, Warsaw, Poland

Along with significant advances in prostate cancer biology research, we also observe the rapid development of modern diagnostic tests. New biomarkers are derived to detect disease while it is organ-confined to stratify the risk and to aid clinical decision-making. Majority of these tools have already been validated clinically, but only a few have received premarket clearance and administration approval (Table).

Superiority of novel tests is visible not only in improved detection accuracy but predominantly in the assessment of tumour aggressiveness and selection of patients eligible for conservative management. Together with emerging prospective updates, more often, the question about PSA replacement or its supplementation is being raised. Most of the tests presented here aim at outperforming markers, nomograms and calculators used to date or supply additional value to them. Based on current knowledge, not every described assay is eligible for population screening, but most show at least interesting potential as stratifying, differentiating and prognostic tools. There are also factors that are limiting further progress. Price and availability are some such factors. With an average cost of 500 USD per measurement, now they do not meet one of the most important conditions of a screening test. Utility of biomarkers as decision-supporting tools often seems to be limited by lack of clear indications on further clinical proceeding. Defining cut-offs in large validation cohorts and calibrating test-based scores are not helpful in solving the issue. Similar problems are faced by clinicians when using nomograms – stratifying patients as exhibiting high risk of reaching a particular end-point is not equal to diagnosing this end-point. At the same time, there is also growing competition from multiparametric MRI, which provides binary and usually straightforward detection of PCa both in primary and rebiopsy settings. Having considered the above factors, it seems that PSA will stay with us in the immediate future, but the future of these new technologies remains unclear.

Biomarker	Brand name	Material	Primary indication	FDA-approved	Recommended by EAU guidelines	External validation studies available	Cost per patient
PSA, fPSA and pro2PSA – protein-based test	PHI	Blood	First biopsy decision	Yes	Yes	Yes	USD 500
tPSA, fPSA, iPSA and hK2 – protein-based test	4K-score	Blood	First biopsy/ rebiopsy decision	No	Yes	Yes	USD 1,200
PCA3 – qRT-PCR	Progensa	Urine (post DRE)	First biopsy/ rebiopsy decision	Yes	Yes	Yes	USD 300-500
8 proteins prostate expression	ProMark	Biopsy specimen (FFPE)	Eligibility for AS	No	No	No	USD 3,900
17 (12 + 5) genes – qRT-PCR	Oncotype Dx	Biopsy specimen (FFPE)	Eligibility for AS	Yes	Yes	Yes	USD 4,200
46 (31+15) genes – qRT-PCR	Prolaris	Biopsy specimen (FFPE)	Eligibility for AS	Yes	No	Yes	USD 3,400
22 RNA biomarkers prostate expression	Decipher	Tisse (post RP specimen)	Eligibillity for post-RP RT	No	No	Yes	USD 3,400
GSTP1, APC and RASSF1 – methylation assay	ConfirmMDx	Biopsy specimen (FFPE)	Rebiopsy decision	No	Yes	Yes	USD 3,300
4 metabolites (aminoacids) LC-MS assay	Prostarix	Urine (post DRE)	First biopsy/ rebiopsy decision	No	No	No	Discontinued
Immuno-PCR (IPCR) for pg/mL PSA levels	NADiA ProsVue	Blood	Eligibillity for post-RP secondary treatment	Yes	No	Yes	No data obtained
NGS in cfDNA	Developed by chronix biomedical	Blood	First biopsy decision	No	No	Recruiting participants for clinical trial (NCT02771769)	1,300 USD
2 mRNA biomarkers combined with clinical variables	SelectMDx	Urine	Eligibility for AS	No	No	No	300 USD
SChLAP1 expression (ISH or qRT-PCR)	Developed by genomeDx biosciences	Tisse (post RP specimen) or Urine (post DRE)	Risk stratification in patients after RP	No	No	Yes	No data obtained
PCA3 and ERG exosome-derived gene expression (qRT – PCR)	ExoDx prostate intelliscore	Urine	First biopsy/ rebiopsy decision	Yes	No	Yes	600 USD

FDA – Food and Drug Administration; EAU – European Association of Urology; tPSA – total PSA; fPSA – free PSA; iPSA – intact PSA; PHI – Prostate Health Index; qRT-PCR – quantitative reverse transcription polymerase chaining reaction; DRE – digital rectal examination; FFPE – formalin-fixed paraffin-embedded; AS – active surveillance; LC-MS – liquid chromatography-mass spectrometry; NGS – next generation sequencing; cfDNA – cell-free DNA; ISH – in situ hybridization.

SESSION 5. TARGETED CANCER THERAPIES

Chairs: Piotr Rutkowski, Piotr Radziszewski

KWO18

Targeted sequencing of cancer- and epigenetic-related genes in glioblastoma reveals a deep deregulation of epigenetic mechanisms

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Recent whole genome studies demonstrated that epigenetic enzymes, histones and chaperone proteins harbor mutations that may result in gross alterations of the epigenome leading to genome instability. Such mutations are common in pediatric hematopoietic and solid tumors, and are targets of innovative treatments with epigenetic enzyme inhibitors. Glioblastoma (GBM, WHO grade IV) is a common and most lethal primary brain tumor in adults, and remains incurable by conventional therapies. Greater understanding of GBM genetics may lead to more targeted and effective treatments. Here we report the results of targeted next-generation sequencing of cancer- and epigenetics-related genes in 118 fresh frozen glioma samples of grade II, III, and IV collected from Polish (n = 97) and Canadian (n = 21) populations. We employed a second generation DNA sequencing target enrichment panel comprising 600 cancer-related genes and 100 epigenetic-related genes. The target region spanning 7 MB (1 MB = 1 x 10^6 base pairs) was designed to cover meaningful portion of genomic, cancer-related sites with a strong emphasis on epigenetic regulators (histone modifiers, chromatin modelers, histone chaperons). Several filtering steps were used to eliminate variant calling errors: mapping quality > 35, each variant coverage > 20x, the penetration of each variant > 20%. Targeted sequencing of GBMs demonstrated mutations in different genetic drivers (including well known *EGFR, TP53, PDGFR* and *PTEN* mutations) and numerous genetic alterations in genes responsible for histone and chromatin modifications, chromatin remodeling and DNA methylation. Newly discovered variants were confirmed by ultra-deep sequencing.

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SESSION 6. CANCER CELL

Chair: Pravin Seghal

KWO18

The MxA reticulum is a novel cytoplasmic organelle in cancer cells distinct from the standard reticulon-4-based endoplasmic reticulum (ER)

Pravin Seghal

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Type I Interferons (IFNs α and β) are used extensively today in the treatment of human cancer, leukemia, viral infections and multiple sclerosis. This IFN exposure strongly (>100-fold) induces expression in human cells of a dynamin-family large GTPase called the "myxovirus resistance protein A" (MxA). Human MxA is a cytoplasmic protein long established to have a broad-spectrum antiviral effect, and to bind to and tubulate lipid membranes. Deletions of *MxA* occur in prostate cancer, and wt MxA inhibits cancer cell motility, invasiveness and metastasis. We have previously shown that MxA enhanced endosome-associated BMP4 and BMP9 signaling in a kidney cancer cell line (HEK 293T). In addition to development of variably-sized MxA endosomes, MxA expression from exogenously introduced MxA vectors or IFN-induced endogenous MxA in liver (Huh7) and kidney (HEK293T) cancer cells leads to development of novel tubuloreticular membrane organelle (the MxA reticulum; "MxA-R") in the cytoplasm. Previous investigators over the last 15 years have claimed that such structures are "a subcompartment of the smooth endoplasmic reticulum (ER)". Our new data show that this long-standing representation is incorrect. The novel MxA reticulum is distinct from but located alongside the standard reticulon-4 (RTN4) based endoplasmic reticulum (ER). Live-cell and high-resolution imaging studies using various ER markers, single and double-immuno EM studies, as well as correlated light and electron microscopy studies (CLEM) help characterize the MxA reticulum as a new cytoplasmic organelle in cancer cells. Functional studies in the literature show that MxA enhances IFN signaling in a positive feed-forward loop to increase IFN effectiveness. We suggest that the novel MxA reticulum participates to enhance novel membrane-associated pathways of anti-cancer signaling elicited by Type I IFNs.

SESSION 6. CANCER CELL

Chair: Pravin Seghal

KWO18-00039-2018-01

Significance and role of pattern recognition receptors in malignancy

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Pattern recognition receptors (PRRs) are key sensors of innate Immunity. They are expressed on the surface and/or in cytoplasm of several cell types of most eukariota. They are able to recognize various microbes, owing to so called pathogen-associated molecular patterns of these microorganisms (PAMPs) and also sense unwanted products of own metabolism, so called danger associated molecular patterns (DAMPs). The effect of PRRs recognition is the activation of cell genome and gene transcription manifested by secretion of several proinflammatory cytokines, up-regulation of MHC molecules and other. Moreover, boosting of PRRs is not limited to activation of innate immunity. Conversely, induced cytokine signals activate acquired immunity of both, humoral and cell-mediated immune responses. The immune mechanisms mediated by PRRs have been well documented in various branches of microbiology and in particular, in viral diseases. They also became subject of interest of cancer immunologists, but were mostly neglected, until recently. Out of three main types of PRRs, Toll-like receptors (TLR), NOD-like receptors (NLR) and RIG-1 receptors (RLR), the first ones, well characterized, were thoroughly studied in various cancers. TLR expression was shown in several tumor cells and in tumor infiltrating lymphocytes (TILs). Their role turned out to be dichotomous, either promoting or suppressing tumor growth and proliferation. Application of man made agonists of some cancers was found useful in the treatment of tumors. Imiquimod, agonist of TLR7 is a well known example used in the therapy of basal cell and other skin carcinomas. Agonist stimulation of TLRs and simultaneous chemo-radiotherapy were found to collaborate for cancer patient health improvement. In some cancers expression of TLRs on PBMC turned out to have prognostic value. Novel approach in tumor immunity appears the detection of cytosolic DNA sensors. This so called STING (stimulator of interferon genes) have emerged as pivotal regulator of innate immune responses to both, exogenous and endogenous DNA. STING may act in concert with RIG-1 receptors well known to induce antiviral RNA. Promising results in the therapy of cancer in animal models raised hopes for the application of joint STIG-RLR stimulation in the treatment of human cancers.

Key words: pattern recognition receptors, cancer promotion, cancer suppression, receptor agonist, cytosolic DNA sensors (STING).

SESSION 6. CANCER CELL

Chair: Pravin Seghal

KWO18-00046-2018-01

Identification of cancer-associated KRAB-ZNFs

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The exact mechanisms driving the carcinogenesis are intensively studied nowadays. In this work, we aimed to characterize factors involved in the regulation of epigenetic landscape in cancer cells. We focused on KRAB-ZNFs family of transcription factors that act as epigenetic suppressors contributing to the deposition of chromatin repressive marks and DNA methylation. Due to its high complexity, KRAB-ZNF family has not been studied in sufficient details and the involvement of its members in carcinogenesis remains largely unexplored. In this study, we identified cancer-related KRAB-ZNFs based on gene expression analysis of pan-cancer dataset deposited in The Cancer Genome Atlas. We analyzed 6272 tumor and normal tissue samples from 16 different cancer types. We showed that some of the KRAB-ZNF genes are commonly upregulated in the majority of the analyzed cancer types, which suggests that they act through similar molecular mechanisms. This upregulation is also true for most of the KRAB-ZNF splicing variants, whose expression rises in parallel in tumors compared to normal tissues. We confirmed our observations in a panel of cell lines and tissues for the two most common cancer types: lung and breast cancer. Immunohistochemistry staining on tissue microarrays from The Human Protein Atlas confirms the presence of selected KRAB-ZNF in lung and breast cancer tissue samples. Altogether, we identified and characterized cancer-related KRAB-ZNF factors that may have an important function in cancer initiation and progression. These factors may have a potential to become targets for novel oncotherapy or serve as oncologic biomarkers.

Key words: cancer, TCGA, epigenetics, KRAB-ZNFs.

SESSION 6. CANCER CELL

Chair: Pravin Seghal

KW018-00057-2018-01

TRIM28 protein domains in maintenance of pluripotency state in human inducet pluripotent stem cells

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Triptate motif protein 28 (TRIM28) function is crucial in early stages of development of mammals and according to our results, is responsible for maintaining self-renewal capabilities of iPS cells. TRIM28 plays role in regulation of cancer stem cells (CSC) populations and tumorigenesis.

TRIM28 is a multidomain protein and displays diversified biological fuctions in cells as a result of its structural complexity and enzymatic activity. TRIM28 triggers the formation of heterochromatin by recruiting nucleosome remodeling complexes, heterochromatin protein 1 (HP1), histone modifying enzymes: histone deacetylase-containing complex NuRD and histone methyltransferase SETDB1. This multidomain protein may also contribute to maintenace of pluripotency state in phosphorylation-dependent manner.

Here we evaluate the role of particualr domains and a few crucial phosphorylation sites in process of self-renewal of human induced pluripotent stem cells.

We replaced the expression of endogenous TRIM28 protein by exogenous structural mutants in RING domain (C91A), PHD domain (C628R), and Bromodomain (N773G). We have also examined three different phosphorylation mutants: Ser 437 located near HP1-binding domain, C-terminal serine – Ser824, located near Bromodomain and triple tyrosine phosphorylation mutant which ablates tyrosine phosphorylation Y449F/Y458F/Y517F (3YF).

Key words: TRIM28, iPSC, pluripotency, self-renewal.

CLINICAL SESSION 7: HAEMATOLOGICAL MALIGNANCES AS CANCER MODELS

Chairs: Lidia Gil, Waldemar Priebe

KWO18

Innovative therapies for lymphoma

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Lymphomas are a heterogeneous group of lymphoproliferative malignancies with differing patterns of behavior and responses to treatment reflecting their clinical, biological and pathological diversity. Two main types of lymphoma are Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) with B-cell NHL constituting > 90% of all NHL. During the last two decades, significant progress has been made in the management and treatment of lymphoma. First, antibody therapies revolutionized lymphoma treatment with rituximab a monoclonal antibody (MoAb) for CD20 antigen greatly improving patients' outcomes when added to chemotherapy. Immunochemotherapy with anti-CD20 MoAbs is current standard of care for many subtypes of B-NHL. Nowadays, novel antibody therapies, like antibody-drug conjugates and bispecific antibodies have been developed and already entered clinical practice. Brentuximab vedotin is an antibody-drug conjugate composed of an anti-CD30 monoclonal antibody conjugated by a protease-cleavable linker to the microtubule-disrupting agent monomethyl auristatin E approved for the use in patients with relapsed/refractory HL. Blinatumomab, a bispecific monoclonal antibody construct that enables CD3-positive T cells to recognize and eliminate CD19-positive acute lymphoblastic leukemia (ALL) blasts is approved for use in patients with relapsed or refractory B-cell precursor ALL. A novel class of immunomodulatory antibodies that produce tumor regression by inhibiting immune checkpoints and activating T cells have been shown to induce durable remissions as well as prolong survival of patients with refractory classical Hodgkin's lymphoma. Two antibodies blocking the programmed death 1 receptor (PD-1) on T cells (nivolumab and pembrolizumab) have been approved for the use in patients with refractory/relapsed cHL. Adoptive cell therapy with chimeric antigen receptor (CAR) engineered T cells (CAR-T) have lately shown unprecedented efficacy in B-cell malignancies. CAR-T cell-based therapies targeting CD19 can induce durable remissions as well as prolong disease-free survival in heavily pretreated patients. However, toxicities associated with CAR-T administration remain a significant concern. In 2017, two CAR-T cell therapies have been approved by FDA: tisagenlecleucel in certain patients with relapsed/refractory B-cell ALL and axicabtagene ciloleucel in patients with large B-cell lymphoma after at least two other kinds of treatment failed.

Except new immunotherapies, another breakthrough approach is targeted therapy with small molecule agents like inhibitors of the B-cell receptor (BCR) signaling pathway and B-cell lymphoma-2 (Bcl-2) family of regulatory proteins (venetoclax) that show substantial benefit in patients suffering from certain types of B-cell malignancies.

CLINICAL SESSION 7: HAEMATOLOGICAL MALIGNANCES AS CANCER MODELS

Chairs: Lidia Gil, Waldemar Priebe

KWO18

Targeting Therapeutically Resistant Leukemias

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Adult acute myeloid leukemia (AML) is the common acute type of leukemia in adults with a 5-year survival rate of approximately 26%. The prognosis is especially poor for patients who are older than 60 years of age, with a median survival of only 5 to 10 months.

Currently, a wide range of drugs is used to treat AML and, consistently, these various treatments lead to the development of different forms of resistance. The identification of the mechanisms responsible for specific drug resistance can lead to new therapeutic approaches to overcome drug resistance in AML.

Anthracyclines are one of the most effective antitumor agents against hematologic malignancies, including AML. However, their use is limited by de novo or acquired drug resistance. In addition, their efficacy is often limited by cumulative, dose-dependent cardiotoxicity.

Annamycin is an anthracycline antibiotic, discovered and developed by us and formulated in liposomes. The development of Annamycin was guided by selection of structural modifications leading to the its lack of cross-resistance to other anthracyclines, high affinity for lipid membranes, reduced cardiotoxicity, and potent inhibition of topoisomerase-II. Preclinical studies of liposomal Annamycin confirmed, in vitro and in vivo, the intrinsic nature of favorable properties such as lack of cross-resistance, the ability to overcome multidrug resistance in vitro and in vivo, and dramatically reduced cardiotoxicity. The results of the preclinical studies that warranted clinical development of Annamycin will be discussed.

More effective treatment of high-risk AML requires not only development of novel cytotoxic agents that are safer and more efficacious, but also development of unique, novel strategies that go beyond previously explored targeted molecular therapies; strategies that lead to drugs working in combination with cytotoxic agents as well as immune-based therapeutics. In our approach, we considered as potential targets key oncogenic transcription factors that are generally considered as non-druggable. One of the important targets in hematologic malignancies, including AML, is signal transducer and activator of transcription 3 (STAT3). We will present our efforts directed towards the development of a unique class of inhibitors of activated forms of STAT3 (p-STAT3) that can potentially be explored for the treatment of AML patients both as single agents and in combination with other therapeutic approaches.

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CLINICAL SESSION 7: HAEMATOLOGICAL MALIGNANCES AS CANCER MODELS

Chairs: Lidia Gil, Waldemar Priebe

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Is Immunotherapy a Game Changer in Multiple Myeloma?

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Over the past decade, the introduction of proteasome inhibitors (PIs) and immunomodulatory drugs (IMiD and their combinations has transformed the treatment of patients with multiple myeloma (MM). With significant improvement of response rates, progression-free-survival (PFS), and overall survival (OS), treatment aims now also include the possibility of achieving a "cure" for an increasing proportion of patients. In pursuit of improved outcomes, immunotherapies have emerged as the next important tool for treatment of multiple myeloma. The most advanced is the development of monoclonal antibodies (mABs), of which elotuzumab for SLAM family member 7 (SLAMF7) and daratumumab for CD38 have been already approved for the treatment of relapsed and refractory multiple myeloma (RRMM), with several other mABs and their combinations in earlier phases of development. The magnitude of the impact of an addition of mAbs to the backbone of established regimens approaches a level of significance previously not seen in RRMM. Based on results from the first of several ongoing phase III trials in newly diagnosed MM (NDMM), a similar phenomenon is also observed in this patient population, with anticipation of impending approval of the first mABs in NDMM. Having already the "game changing" impact of mABs, even more promising appear to be the first results of treatment of MM with chimeric antigen receptor (CAR) T cells. With a number of CART cell studies in progress, the first two most advanced bb2121 and LCAR-B38M studies with CARTs targeting BCMA show previously unseen levels of overall response rates approaching 100% and complete response (CR) rates > 70% in heavily pretreated RRMM. Newer generation of CARTs show a promise of not only higher activity but also reduced risk of cytokine release syndrome and neurotoxicity associated with CARTs. These early results are already generating interest in evaluating CARTs as a replacement for autologous transplantation in MM and incorporating CARTs into more definite cure strategies. Along with the development of CARTs, other immunotherapies are making headways into MM therapy. The most promising appear BCMA-targeting antibody drug conjugates and bi-specific mABs (also called BiTEs) and MM-targeting vaccines. The clinical benefits of these treatment strategies will need to be confirmed in well-designed phase III trials and optimized using established and novel prognostic tools, including testing of minimal residual disease (MRD).

Key words: cellular therapy, antibodies.

CLINICAL SESSION 8. CANCER IMMUNOTHERAPY AND COMBINATIONAL THERAPIES

Chairs: Iwona Ługowska, Jacek Mackiewicz

KWO18

How to increase immunotherapy efficacy of renal cell carcinoma

Jacek Mackiewicz

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The development of immune check-point inhibitors changes the treatment landscape in many malignancies. The approval of anti-PD1 (nivolumab, pembrolizumab) and anti PD-L1 (durvalumab, atezolizumab, avelumab) improved cancer patient outcome. Currently nivolumab is approved in the second line treatment in advanced renal cell carcinoma (RCC). To enhance the efficacy of immune check-point inhibitors, these drugs can be combined with immune check-point inhibitors with different mode of action, co-stimulatory molecules, targeted therapy, cancer vaccines, antimetabolites, chemotherapy, radiotherapy or various bacterial species. The combination of nivolumab with ipilimumab (anti-CTLA4) in intermediate and poor risk RCC patients showed higher efficacy then standard sunitinib in a phase 3 study. Also high activity is observed when anti-PD1/PD-L1 are combined with anti-angiogenic agents. These combinations demonstrated acceptable toxicity and response rate in 60-70% of advanced RCC patients treated in early phase studies. Currently many phase 3 studies are ongoing evaluating immune check-point inhibitors with targeted therapy. To overcome the primary and secondary resistance to immune check-point inhibitors these drugs can be combined with antimetabolites. Early phase studies demonstrated high activity of the combination of anti-PD1 with IDO (indolamine 2,3-dioxygenase) inhibitors in many cancer types including RCC. Phase 3 studies are ongoing evaluating these combinations. Adenosine receptor blockers combined with immune check-point inhibitors showed also some activity in preclinical models and early phase studies. Further development of these combinations is also ongoing.

CLINICAL SESSION 8. CANCER IMMUNOTHERAPY AND COMBINATIONAL THERAPIES

Chairs: Iwona Ługowska, Jacek Mackiewicz

KWO18

Real-world treatment and outcomes of metastatic cutaneous melanoma patients treated with immunotherapy in Poland

Iwona Ługowska

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The clinical trials with PD-1 pathway–blocking agents have produced exciting clinical responses in cancer patients (specially with melanoma). However, there is a limited real-world data on treatment outcomes among advanced melanoma patients treated with immunotherapies including anti-PD1 antibody.

The objective of this study is to show anti-PD1 treatment patterns and outcomes among patients with advanced melanoma treated in two oncological centres of the Maria Skłodowska Curie Institute (in Warsaw and Cracow). In this retrospective multicentre, observational chart review study, we included 244 patients who received pembrolizumab or nivolumab therapy (mean age: 63 years (36% of them were > 70 years old); 58% were male, 33% had *BRAF* positive mutation, in 65% cases – LDH was above normal range, and 16% of patients had CNS metastases present at the time of decision). In 55% cases, anti-PD1 antibody was initially received as first-line, in 32% as a second line and in 12% of patients as a third line of therapy. There were statistically significant difference in 12 months Progression Free Survival depending on line of anti-PD1 therapy, and it was 39%, 26%, 20%, respectively. When *BRAF* mutation was positive, *BRAF* inhibitors in monotherapy or in doublet with *MEK* inhibitor were administered. The age, sex, type of anti-PD1 antibody, or *BRAF* status were not statistically significant factors on survival, in contrary to presence of CNS metastases, or LDH elevation. During the time observed, the toxicity was similar as reported in clinical trials. In real world there are slightly worse outcomes of checkpoint inhibitors therapy than reported in clinical trials, which is linked to more advanced underlying comorbidities, different treatment lines prior anti-PD1 therapy, and older population included.

Conclusions: The overall survival and safety issue are comparable as reported in clinical trials. The is still pressing clinical need for research to develop more effective end-of-life option following progression on immunotherapy, especially in *BRAF* negative population.

CLINICAL SESSION 8. CANCER IMMUNOTHERAPY AND COMBINATIONAL THERAPIES

Chairs: Iwona Ługowska, Jacek Mackiewicz

KWO18

Treatment strategy in patients with metastatic colorectal cancer – based on new perspectives

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The modern approach to the treatment of colorectal cancer is closely related to personalized medicine, which is associated with adaptation of the therapeutic procedure and methods of prevention to each patient. An individual approach contributes to the effectiveness and safety of the therapy used and increases the likelihood of complete cure. Therefore, treatment of colorectal cancer is undoubtedly personalized medicine.

Current treatment of metastatic colorectal should be based on 3 steps:

- STEP 1 - stratification patients according to patients are „fit" or „unfit";
- STEP 2 - assessment patients for potential surgical treatment;
- STEP 3 – first line: qualifying patients to the appropriate systemic treatment regimen.

"Fit" patients should be treated with all available therapeutic methods, while the best supportive care remains for patients "unfit". 30% of patients after metastazectomy R0 have a chance to be cure. And the next important stage of treatment is qualifying patients to the appropriate first line systemic treatment, because only ~45% patients received second line. Nowdays, the standard of systemic treatment of colorectal cancer is mainly molecular-targeted therapies, most of which are available in Poland (e.g. anty-EGFR, anty-VEGF). Unfortunately, despite aggressive oncological treatment, most patients develop disease progression and treatment resistance. Hence searching for new therapeutic options. The closest to everyday clinical practice seem to be monoclonal antibodies against immune checkpoints (like pembrolizumab, nivolumab, ipilimumab) in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high. Other molecules in clinical trials include: famitinib in refractory mCRC patients, linifanib in KRAS mutant metastatic and refractory CRC or dabrafenib and trametinib, in patients with BRAF*V600E* mutation metastatic CRC.

SESSION 9. CLINICAL

Chairs: Rafał Stec, Andrzej Jakubowiak

KWO18

Redox, oximetric and vascular imaging provide insight into tumor microenvironment

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Tumor microenvironment may determine tumor cell evolution, tumor phenotype and its aggressiveness. We have used non-invasive imaging in preclinical models to characterize tumor oxygen level, redox state and vascular structure.

DCE-MRI, as well as TOF angiography was used to characterize changes in tumor permeability and vasculature during tumor growth. Results from EPR oximetry, EPR redox imaging and Doppler ultrasonography show significant differences between metastatic and non-metastatic tumors. These findings are in agreement with immunohistochemistry and Western blot data showing enhanced oxidative stress, microvascularization and EMT markers in more invasive tumors. More aggressive tumors were characterized by a slower growth rate, higher vascularization, and indications of oxidative stress.

SESSION 9. CLINICAL

Chairs: Rafał Stec, Andrzej Jakubowiak

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Aromatic iodo-lactones induce apoptosis in canine lymphoma cell lines

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Introduction: Many natural and synthetic compounds with a lactone moiety and an aromatic ring exhibit cytotoxic activity against different cancer cell lines. An interesting group of such compounds seem to be those with both lactone moiety and an aromatic ring which, in addition to antimicrobial or antiviral activity, also exhibit antitumor properties. Although canine hematopoietic neoplasms are highly chemosensitive, we still do not have an effective therapeutic regimen that provides a long-term remission in the majority of the patients. Therefore, based on earlier observations of the pro-apoptotic potency of chiral β-aryl-δ-iodo-γ-lactones, we decided to evaluate their activity also in canine lymphoma/leukemia cells. AIM OF THE STUDY: In the present study, ability to induce apoptosis of two enantiomeric trans isomers of 5-(1-iodoethyl)-4-(2',5'-dimethylphenyl)dihydrofuran-2-one was assessed against selected canine lymphoma and leukemia cell lines.

Material and methods: CLBL-1 (canine lymphoma), GL-1 and CLB70 (canine leukemias) cell lines were used in the study. At the same time, the tests were performed on the human Jurkat cell line (acute leukemia). Apoptosis was determined using annexin V/PI staining and western blot after 24h of incubation with a tested compounds. RESULTS: The enantiomers (+)-(4R,5S,6R)-1 and (–) (4S,5R,6S)-2 were found to induce classical caspase-dependent apoptosis through downregulation of the expression of anti-apoptotic proteins Bcl-xL and Bcl-2. Although the mechanism of apoptosis induction was the same for both enantiomers, they differed in their strength, as stronger antineoplastic activity in vitro was exhibited by isomer (+)-(4R,5S,6R)-1.

Conclusions: Enantiomers (+)-(4R,5S,6R)-1 and (–)-(4S,5R,6S)-2 induced classical caspase-dependent apoptosis. However, in case of canine lymphoma and leukemia cells, the tested compounds also caused a fragmentation of the pro-apoptotic Bid protein, which indicated the involvement of the apoptotic receptor pathway that strengthened the mitochondrial pathway.

Key words: canine lymphoma, canine leukemia, apoptosis, iodolactones.

SESSION 9. CLINICAL

Chairs: Rafał Stec, Andrzej Jakubowiak

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Optimization of antifungal prophylaxis with posaconazole in paediatric oncology – concepts and strategies

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Posaconazole (PCZ) is a new generation triazol antifungal agent. It is primarily used for the prophylaxis of invasive fungal infections (IFI) in hematopoietic stem cell transplant (HSCT) recipients, also children although the safety and efficiency profile has not been established in this group. The pediatric onco-hematology population is particularly exposed to adverse drug reactions (ADR).

The aim of our study was to determine the impact of ABCB1 polymorphism on the safety and effectiveness of posaconazole treatment in children. We also took into consideration other agents potentially influencing antifungal prophylaxis (BMI, age, drug co-administration).

70 children aged from 2 months to 17 years were recruited for the study. The IFI prophylaxis was conducted before and after allogeneic HSCT with a posaconazole oral formulation. ABCB1 polymorphism genotyping using a PCR-RFLP method by Siegmund et al. was performed.

We established that the ABCB1 genotype has impact on the PTT (partial thromboplastin time) parameter but not on the efficiency and safety profile of PCZ. During antifungal prophylaxis most frequent ADR are liver function abnormalities (n = 52), gastrointestinal disturbances (n = 50), skin disorders (n = 28), and neurological symptoms (n = 26). High BMI values are connected with increased risk of visual disturbances and skin reactions. The co-administration of PCZ and proton pump inhibitors leads to raised liver function parameters, cardiac failure and vascular disorders.

In conclusion although ABCB1 polymorphism has no impact on efficiency and safety of PCZ treatment, it may influence the time of bleeding in children with hematological malignances. The careful evaluation of drug co-administration needs to be performed to ensure proper treatment and its safety.

Key words: posaconazole, ABCB1, ADR, hematology.