

VIROLOGY AND MOLECULAR MEDICINE SESSION
Bucharest, March 28, 2012

SPECIAL CONFERENCES ABSTRACTS

**ABERRANT CYTOKINE RECEPTOR SIGNALING DRIVING ACUTE
LYMPHOBLASTIC LEUKEMIA**

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Mutational activation of cytokine receptors has been described in solid cancers but has not been generally known to drive acute leukemia. We have recently described a subgroup of acute lymphoblastic leukemia characterized by an aberrant expression of the receptor to the cytokine thymic stromal lymphopoietin (TSLP). This receptor is a heterodimer of CRLF2 and IL7RA. In addition to its aberrant expression we discovered a series of activating mutations either in the receptor

components themselves or in the signaling JAK1 or JAK2 enzymes. These mutations cause constitutive, ligand independent, activation of JAK-STAT pathway. We have also described the same mutational activation of the interleukin 7 receptor in T-ALL. Inhibitors of these signaling pathways, for example novel JAK inhibitors, could serve as targeted therapies of these bad prognosis leukemias

**THEILER'S VIRUS: FIGHTING AGAINST INNATE IMMUNITY TO ESTABLISH
PERSISTENT INFECTIONS OF THE NERVOUS SYSTEM**

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Theiler's virus (TMEV) is a neurotropic murine picornavirus. This virus has a striking ability to persist in the central nervous system of infected mice, in front of a strong and specific host immune response. Chronic infection of the nervous system and the associated inflammatory response trigger demyelinating lesions reminiscent of those of multiple sclerosis.

Two proteins encoded by Theiler's virus, L and L*, are instrumental in the establishment of persistent infections. Mutations affecting either of these proteins do not affect virus replication in BHK-21 cells but dramatically decreases the viral load *in vivo*.

L is a fascinating peptide (76 aminoacid-long) endowed with multiple activities. It interferes with nucleocytoplasmic trafficking of cellular components

by triggering hyperphosphorylation of nucleoporins and blocks the expression of type-I interferon by infected cells.

L* is unique among picornaviruses in that it is translated from an alternative open reading frame. In infected cells, this protein is partitioned between the cytoplasm and the mitochondrial outer membrane. We show that the cytoplasmic form of L* inhibits the IFN-induced RNaseL, through direct protein-protein interaction. These results demonstrate a novel viral mechanism evolved to elude the antiviral OAS/RNase L pathway. Interestingly, L* was found to act in a species-specific fashion as Theiler's virus L* protein blocked murine RNase L but not human RNase L or RNase L of other mammals or birds.

Taken together, our results show that L and L* proteins act in concert to antagonize innate

immune responses and to allow the establishment of persistent infections *in vivo*.

INITIATING MYELOID CANCERS: JANUS KINASE AND AND CYTOKINE RECEPTOR MUTANTS COOPERATING WITH CHROMATIN MODIFIERS

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Human myeloproliferative neoplasms are largely associated with activating mutations on JAK2 (V617F) and the thrombopoietin receptor (TpoR)., the TpoR W515 mutants Three related but distinct diseases are induced by these mutants, especially JAK2 V617F, namely Polycythemia Vera, Essential Thrombocythemia and Primary Myelofibrosis. While ATP-competitive inhibitors of JAK2 are entering clinical practice for myelofibrosis, with a clear benefit, more specific inhibitors are required for targeting only the disease clone and spare normal hematopoiesis. Our structure function studies, coupled with *in vivo* mouse adoptive transfer experiments identify several specific structural targets in JAK2 V617F and TpoR W515 mutants, which could be employed screening assays. The levels of signaling by TpoR are essential for establishing ET, PV and PMF phenotype by JAK2 V617F. During disease progression the TpoR is down-modulated via enhanced ubiquitination and degradation, involving a block in recycling. Inhibition of JAK2 or of proteasomes induce a recovery of platelet TpoR in knock-in JAK2 V617F mice. Importantly, treatment with JAK2 inhibitor restore TpoR levels

in JAK2 V617F-positive myelofibrosis patients. We show that at high levels of JAK2 expression, TpoR induces anti-proliferative and senescence signaling, and down-modulation of TpoR is associated to progression of ET to PV and myelofibrosis. TpoR down-modulation will also explain why at late stages of differentiation, megakaryocytes from MPN patients continue to proliferate, unlike megakaryocytes from healthy controls, which arrest proliferation in the presence of Tpo. Continuous proliferation of late megakaryocytes contributes to marrow fibrosis by shedding of TGF-beta and PDGF containing granules. We identified constitutive activation of STAT5 as a major event in several MPN patients, with expression of novel genes, such as Lipoma Preferred Partner gene (LPP), which normally is not a target of STAT5. Chromatin immunoprecipitation with anti-STAT5 followed by global hybridization with chips that captured virtually all human promoters allowed us to identify novel biomarkers of ET and myelofibrosis and novel cross-talks between constitutively active STAT5 and several tumor suppressors in myeloproliferative neoplasms.

THE SIGNIFICANCE OF HIV RNA LEVELS IN THE CEREBROSPINAL FLUID AS BIOMARKER OF HIV ENCEPHALOPATHY

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Background. The measurement of HIV RNA from cerebrospinal fluid (CSF) might provide

useful information about the HIV-associated neurocognitive disorders. We evaluated the

usefulness of measurement of HIV RNA in paired plasma-CSF samples from children adolescents and young adults diagnosed with HIV encephalopathy (HIVE).

Methods. The HIV viral load (VL) from 129 subjects was quantified in paired CSF-plasma samples (using Roche Amplicor and TaqMan assays with detection limits between 20 and 400 copies/ml). Data from 38 patients diagnosed with HIVE, were compared with those from 60 patients with various neurological opportunistic infections and from 31 subjects with HIV but without neurological complications. Intact blood-brain barrier (BBB) function was defined as an albumin index <9.

Results. Overall the CSF VL were lower compared to plasma (3.44 vs. 4.20 log₁₀ copies/ml, p<0.001). Patients diagnosed with HIVE had mean CSF loads values (4.39±1.49) similar to plasma values (4.50±1.36). Sixteen of the patients with HIVE had higher CSF HIV RNA

values compared to plasma. In patients with HIVE, CSF HIV RNA levels were positively correlated with CSF albumin levels (0.45, p<0.05) and pleocytosis (0.56, p<0.05). Nine of 16 patients with HIVE had intact BBB. Naïve patients had higher HIV loads in both plasma and CSF regardless their neurological condition compared to antiretroviral-experienced patients. HIV RNA CSF values from patients with HIVE were higher than from patients with neurological opportunistic infections (4.39 vs 3.31 p<0.001) and from neurologically asymptomatic patients (4.39 vs. 2.57, p<0.001).

Conclusions. In our group of children and adolescents HIV RNA levels in the CSF similar or higher compared to plasma were a good indicator of HIVE. Patients with HIVE had the highest HIV RNA values in CSF by comparison with patients with neurological opportunistic infections or neurological asymptomatic patients.

TRANSMITTED DRUG RESISTANCE IN TREATMENT- NAÏVE HIV-1 INFECTED ROMANIAN PATIENTS

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Introduction. Transmitted drug resistance (TDR) represents a public health concern, leading to a delay in virologic suppression and to an increased risk of earlier virologic failure. Considering the fact that data on non-B subtypes are limited, and subtype F is predominant in Romania, our goal was to assess the prevalence of TDR mutations in the pol gene of HIV-1 isolates from drug-naïve Romanian patients.

Material. Twenty HIV-1 strains from untreated individuals, newly diagnosed (n = 10) and chronically infected (n = 10), with detectable HIV RNA viral load were investigated.

Methods. Resistance genotyping was performed using the ViroSeq HIV-1 Genotyping System (Celera Diagnostics, Alameda, USA). For subtyping purposes all sequences were submitted to the REGA database and TDR were defined according to Stanford University HIVdatabase. Among newly diagnosed patients, recent infections

(acquired in the previous 6 months) were detected by BED-CEIA in 2/10 cases.

Results. Although F subtype remained prevalent, we observed an increase of HIV-1 infections which involved other clades, especially in newly diagnosed individuals; HIV-1 subtype F1 was recognized in 75% of cases, followed by subtypes C (10%) and B (10%), while circulating recombinant form CRF06_CPX was found in 1 patient. All HIV-1 isolates harbored multiple minor mutations in the protease gene often detected at polymorphic positions. Accessory mutations and several atypical substitutions at key positions known to be linked with drug resistance were found within the RT gene (M41W; T69S/N, V106IV, E138G). No major transmitted drug resistance mutations were identified in treatment-naïve HIV-1 infected Romanian patients.

Conclusion. Minor mutations, detected with high frequency in the protease and RT genes of all

studied HIV-1 isolates, can affect the susceptibility of HIV-1 to different antiretroviral drugs, the magnitude of resistance conferred by major

mutations, and the capacity of acquiring some resistance mutations.

EPIGENETIC SILENCING OF GNMT GENE IN PANCREATIC ADENOCARCINOMA

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Pancreatic cancer remains a major challenge for therapy and biomarkers identification. Many epidemiological and clinical studies had proved the importance of early detection to decrease the mortality caused by pancreatic cancer.

Epigenetic silencing of tumor suppressor genes is a major contributor to neoplastic transformation and is an area of intense research. Identification of genes which undergo cancer-specific CpG island hypermethylation and correlation of these data with tumor stage, progression, and long-term prognosis are becoming increasingly common.

The aim of this study was to identify new factors involved in pancreas oncogenesis.

The results of bioinformatic analysis of microarray data found a down regulation of GNMT gene (glycine N-methyl transferase) in pancreatic adenocarcinoma. GNMT posses CpG islands in the promoter and is an important gene involved in methyl group metabolism and in maintaining a normal methylation status of the genome. In order to verify our hypothesis of GNMT epigenetic regulation, we evaluated the GNMT gene expression and promoter methylation

status in 30 paired samples (normal/pancreatic adenocarcinoma).

Promoter methylation status was quantified in qMS-PCR using bisulphite treated DNA samples (EpiTect Bisulfite Kit – Qiagen) while GNMT gene expression was determined in qRT-PCR.

We found significantly higher methylation frequencies ($p < 0.001$) in adenocarcinomas (2.82–100%, median=36.05%) than in controls (0.28–14.02%, median=4.39%). GNMT gene expression was decreased in adenocarcinomas in contrast with normal controls. Only in 4 cases (13.33%) GNMT expression levels were similar with normal while 26 cases (86.67%) presented a decreased expression. Fold change expression of investigated gene was between -0.02- (-4.55), median = -2,42, suggesting an important downregulation of this gene.

These results sustain the involvement of epigenetic alterations in pancreatic oncogenesis, We consider that GNMT promoter gene hypermethylation is a factor for GNMT gene inactivation.

This work was supported by **POSDRU/89/1.5/S/60746**

THE ROLE OF HUMAN PAPILLOMAVIRUS E7 ONCOGENE IN EPIGENETIC ALTERATIONS

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High risk human papillomaviruses (hrHPVs) are considered the etiologic agents of cervical

carcinoma, a leading cause of cancer death in women, worldwide. Our studies on E7 HPV

silencing by siRNA technique revealed a histone methyl transferases and demethylases modulation. E7 HPV is one of the major viral oncoproteins highly expressed in cervical carcinomas.

Aim. To evaluate the expression of epigenetic factors involved in cell reprogramming in HPV oncogenic transformation in biological samples.

Material and methods. Cervical specimens from women (31-49 years old) with hrHPV-induced cervical lesions as well as samples from normal cervix were included into the study. E7 HPV levels were quantified using a standard curve. Histone methyl transferases (HMT) and demethylases (KDM) expression levels were investigated by qRT-PCR. Cellular status assessing the hrHPV infection severity and epigenetic status

were confirmed by p16 and MBDs quantification respectively.

Results. While HMTs expression levels were found to be less correlated with cytology severity, KDMs correlates positive with cytology degree. Among demethylases, KADM1B and KADM6B pattern seems to have important consequences for epigenetic reprogramming. Moreover, the trend of MBD4 expression levels (as sign of DNA hypomethylation) is similar with KADM1B and 6B. KADMs expression pattern is similar with p16 which is linked to oncogenic transformation in HPV-induced lesions. Our results underline E7 oncogene role in epigenetic mechanisms involved in tumour development.

RECENT SHIFTS IN TRANSMISSION PATHWAYS AND INFECTING HCV GENOTYPES IN ROMANIA

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Introduction. Viral genotype is the most important factor in assessing the optimal treatment duration for hepatitis C virus (HCV) infection; HCV genotypes can be ranked, in a decreasing order of susceptibility to interferon-based treatment, as follows: genotypes 2, 3, 4 and 1. Genotype 1 or 1b are almost exclusively reported in Romanian patients with chronic hepatitis C, but due to the increasing number of infections related to injecting drug use (IDU), both the pattern of hepatitis C virus (HCV) transmission, and the circulating genotypes in Europe have changed. As there are little available data in this respect for Romania, the aim of our study is a preliminary analysis of the distribution of HCV genotypes circulating among IDUs compared to those circulating in general population.

Methods. 300 hepatitis C infected patients with active viral replication were enrolled in a retrospective study, 62 of whom reported injecting drug use as risk factor for HCV infection acquisition. Genotyping using commercial Line Probe Assay (Innogenetics) was confirmed by sequencing of Core PCR products followed by phylogenetic analysis.

Results. In general population reporting a history of blood transfusions received during surgical or obstetrical interventions (61.9% females, mean age-42.6±14.9 years), HCV subtype 1b was found in 92.6% of the samples, subtype 1a in 5.4% of the samples, subtype 4a in 1.2%, and subtype 3a in 0.8% of the samples. No geographical clustering was evident for HCV 1b sequences. In IDUs (86.7% men, mean age - 27.6±3.7 years, mean age at first drug use- 17.5±3.9 years) HCV subtype 1b is still prevalent, but lower than in general population (50% of the subjects), and other subtypes begin to emerge, especially in younger patients (1a-in 23.1%, 4-in 11.5%, 3a-in 7.7%). The genetic distances among the HCV 1a strains are very homogenous and small, with high sequence identity with other European strains, suggesting the recent entrance of this subtype in Romania from singular or limited sources of infection. These data indicate the possibility of major shifts in the distribution of the dominant subtype, underlining the need for a continuous epidemiological surveillance of HCV infections in IDUs, who can act as a bridging group toward the general population.

ANTIVIRAL TREATMENT OF CHRONIC HEPATITIS C IN ROMANIA: IS HIGHER RATE OF SUSTAINED VIRAL RESPONSE RELATED TO A HIGHER GENERAL INCIDENCE OF IL28B RS12979860 CC GENOTYPE?

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Background. Patients infected with HCV genotype-1 undergoing standard of care treatment with pegylated interferon (PEG-INF) and ribavirin (RBV) achieve sustained viral response (SVR) at a rate of approximately 45% internationally and of approximately 56% in Romania. Genetic markers are currently associated with SVR and suggested to aid setting up the length and composition of combined antiviral therapy in chronic hepatitis C, alongside the on-treatment viral response. IL28B rs12979860 CC genotype is considered a predictive factor for achieving SVR in 55-80% of HCV genotype-1 patients.

Objective, methods and patients. We investigated the frequency of IL28B rs12979860 CC genotype in 11 HCV infected patients with completed standard treatment and in 11 HCV infected intravenous drug users (IDUs), untreated for HCV infection, all of Romanian origin. The method used was based on melting temperature analysis of real-time PCR products upstream IL28B gene, with Light Cycler 2.0.

Results. Overall, the rs12979860 C allele frequency was 86.4%, with the following genotype distribution: CC=50.0%, CT= 36.4%, TT=13.6%. Important differences were noted between the two groups we studied: only 2 (18.2%) IDUs had a CT genotype, the remaining 9 (81.8%) having a CC genotype, while HCV-infected and treated patients showed the following genotype distribution: CC=18.2%, CT=54.5%, TT=27.7%.

Conclusions. The 50% occurrence of the CC genotype in our Romanian study population suggests that other factors might additionally act in support of SVR occurrence. C allele frequency observed in our patients is closer to Irish (73.9%) and Danish (76.5%) populations than to Russians (61.4-64.1%) or Hungarians (65.1%), among other Caucasian ethnic groups. Risk populations might harbor important rs12979860 genotype distribution variation, but the small number of cases in each group analyzed here could have biased our preliminary results.

DEGRADATION PATHWAYS OF HEPATITIS B VIRUS ENVELOPE GLYCOPROTEINS

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Hepatitis B virus (HBV) belongs to the *Hepadnaviridae* family of enveloped DNA viruses. It was previously shown that HBV can induce endoplasmic reticulum (ER) stress and activate the IRE1-XBP1 pathway of the unfolded protein response (UPR), through the expression of the viral regulatory protein X (HBx). However, it remained obscure whether or not this activation had any functional consequences on the target genes of the UPR pathway. Of these targets, the ER degradation-enhancing, mannosidase-like proteins

(EDEM)s are thought to play an important role in relieving the ER stress during UPR, by recognizing terminally misfolded glycoproteins and delivering them to the ER-associated degradation (ERAD).

In this study, we investigated the role of EDEM)s in the HBV life-cycle. We found that synthesis of EDEM)s (EDEM1 and its homologues, EDEM2 and EDEM3) is significantly up-regulated in cells with persistent or transient HBV replication. Co-expression of the wild-type HBV envelope proteins with EDEM1 resulted in their

massive degradation, a process reversed by EDEM1 silencing. Surprisingly, the autophagy/lysosomes, rather than the proteasome were involved in disposal of the HBV envelope proteins. Importantly, inhibition of the endogenous EDEM1 expression in HBV replicating cells significantly increased secretion of both, enveloped virus and subviral particles. This is the first report

showing that HBV activates the ERAD pathway, which, in turn, reduces the amount of envelope proteins, possibly as a mechanism to control the level of virus particles in infected cells and facilitate the establishment of chronic infections.

This work was supported by POSDRU/89/1.5/S/60746 grant.

POST-THAW MOLECULAR BEHAVIOR OF MESENCHYMAL STEM CELLS FROM HUMAN BONE MARROW

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Importance of bone marrow-derived mesenchymal stem cells (BMSC) has increased due to their efficiency in transplant medicine and immunomodulatory properties. BMSC are multipotent and non-immunogenic and can differentiate into mesenchymal origin tissues. They can escape recognition by natural killer cells, also being immunosuppressive and inhibiting the proliferation of alloreactive T cells. HLA-identical mesenchymal stem cells expanded *ex vivo* have been infused to promote haemopoietic recovery after haemopoietic-stem-cell transplantation. We have performed a complex evaluation in dynamic of human BMSCs after cryopreservation in a medium containing 10% dimethyl sulfoxide. Evaluation and characterization: BMSC markers expression, morphology, differentiation capacity,

cell recovery, proliferation rate, viability, apoptosis, karyotype, telomerase activity, GSH levels, PCNA expression at different time points. Cryopreservation did not alter the expression of MSC markers, differentiation capacity and karyotype. Telomerase activity revealed a maximum activity at 24 hours post-thaw, which co-occurred with lowest GSH levels. Telomerase might have a role in oxidative stress protection. Maximum metabolic activity and cell viability was found at 5 days post-thaw. A single post-thaw evaluation of BMSC units before transplantation is not sufficient and a rigorous evaluation in dynamic must be assessed; the time interval between thawing and infusion might influence the transplant outcome.

ACCURATE PREDICTION OF STRUCTURE AND GATING MECHANISM OF TWO-PORE-DOMAIN CHANNELS

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Mechanosensitive TREK channels belong to the family of K2P channels, a family of widely

distributed, well modulated channels that uniquely have two similar or identical subunits, each with

two TM1-P-TM2 motifs. Our goal is to build viable structural models of TREK channels, as representatives of K2P channels family. The structures available to be used as templates belong to the 2TM channels superfamily. These have low sequence similarity and different structural features: four symmetrically arranged subunits, each having one TM1-P-TM2 motif. Our model building strategy used two subunits of the template (KcsA) to build one subunit of the target (TREK-1). Our models of the closed channel were adjusted to differ substantially from those of the template, *e.g.*, TM2 of the 2nd repeat is near the axis of the pore whereas TM2 of the 1st repeat is far from the axis. Segments linking the two repeats and immediately following the last TM segment were modeled *ab initio* as α -helices based on helical periodicities of hydrophobic and hydrophilic residues, highly conserved and poorly conserved residues, and statistically related positions from

multiple sequence alignments. The models were further refined by two-fold symmetry-constrained MD simulations. We also built models of the Open state and suggest a possible tension-activated gating mechanism. Seeking experimental support to the models, we accomplished thermodynamic analysis of mouse TREK-1 gating and functional testing of several deletion mutants. TREK-1 motifs not present in canonical K channels include divergent cytoplasmic N- and C-termini, and a characteristic 50-residue extracellular loop in the first homologous repeat. Deletion of extracellular loop ($\Delta 76-124$) reduced the average current density in patches, increased spontaneous activity and generated a larger sub-population of high-conductance channels. Subsequently determined X-ray structure of TRAAK channel indicates a remarkable agreement with previously developed computational models for TREK channels encourage modelling other channels in the K2P family.