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ABSTRACT BOOK

Translational research in viral hepatitis: Addressing the gaps for cure

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Scientific Organising Committee

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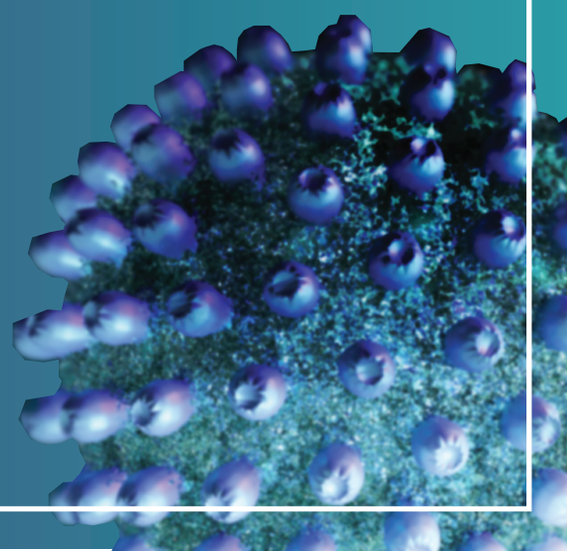

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ORAL ePOSTER ABSTRACT PRESENTATIONS

OP-01YI Metabolic programming of exhausted CD8+ T cells in chronic viral hepatitis

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Background and Aims: Exhausted T cells (T_{EX}) with limited function accumulate in chronic infections such as hepatitis B and -C virus infection. T_{EX} are characterized by an increased inhibitory receptor expression and substantial alterations in their transcription profile. Regulation of energy metabolism has been suggested as a mechanism driving the dysfunction of exhausted T cells, however, the metabolic programming of HBV- and HCV-specific T cells in chronic infection and its links to T cell function remain unclear.

Method: To address these important questions, we set out to profile key metabolic pathways involved in energy metabolism in patients with cHBV and cHCV using metabolism-directed flow cytometry and transcriptome profiling combined with in vitro experiments aimed at studying individual metabolic pathways.

Results: We found that in chronic infection, HCV-specific T cells display enhanced glucose uptake but diminished mitochondrial polarization in comparison to HBV-specific T cells, suggesting a more severe type of exhaustion. Partial improvement of this phenotype was observed in patients receiving DAA therapy, especially in T cells lacking CD127 expression which are associated with terminal exhaustion. Transcriptome analysis of metabolic pathways revealed an upregulation of *ACSS1/ACSS2* mRNA encoding for acetyl-CoA synthetase in HCV-specific T cells, suggesting higher ability to metabolize acetate as a potential anaplerotic TCA substrate. In agreement with this notion, addition of acetate to exhausted $CD8^+$ T cells from cHCV patients was able to counteract functional T cell exhaustion.

Conclusion: In sum, these results indicate that exhausted HBV- and HCV-specific $CD8^+$ T cells exhibit differential metabolic programming. Detailed understanding of metabolic regulation may allow metabolism-directed interventions to improve T cell function.

OP-02 A transient early HBV DNA increase during PEG-IFNa therapy of hepatitis D is associated with subsequent HDV RNA response and HBsAg loss

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Background and Aims: HBV DNA levels are low or even undetectable in the majority HDV infected patients. Treatment with peginterferon alfa-2a (PegIFNa) is currently the only treatment option for hepatitis D leading to HDV RNA suppression in 25-40% of patients. The impact of PegIFNa on HBV DNA in HDV infected patients has not been studied in detail.

Method: We analyzed data of a prospective, multicenter randomized treatment trial ("HIDIT-2", Wedemeyer, Yurdaydin et al., Lancet ID 2019). One-hundred-twenty HDV RNA positive patients were randomized to receive either 180 µg PEG-IFNa-2a weekly plus tenofovir disoproxil fumarate (300mg once daily; PEG-IFNa/TDF, n=59) or placebo (PEG-IFNa/PBO; n=61) for 96 weeks. HBV DNA was determined with the Ampliprep/Cobas TaqMan assay (limit of quantification 20 IU/ml). Complete virological data including Hepatitis B Core-Related Antigen (HBcrAg) were available for 99 patients.

Results: Patients were categorized into four groups according to HBV DNA levels at baseline: 0-19 IU/ml (n= 9, 8.3 %), 20-199 IU/ml (n=58, 53 %), 200-1999 IU/ml (n=18, 17%) and > 2000 IU/ml (n=24, 22%). At treatment week 96, HBV DNA levels decreased by one, two or three categories in 29 (29%), 19 (19%) and 2 (2%) patients respectively while HBV DNA remained in the same category in 43 patients (43%) and even increased by one category in seven patients. At treatment week 96, HBV DNA was still quantifiable in 71.4 % of patients receiving PEG-IFNa/PBO but also in 75.5% of PEG-IFNa/TDF treated patients. Serum ALT correlated significantly with HBV DNA levels at all-time points during and after treatment (r between 0.23 and 0.39). Surprisingly, a transient HBV DNA increase between treatment weeks 12 and 48 was observed in 24 patients (22%; 1.3-4.95 log10 IU/ml) irrespective of the treatment arm (12 in TDF-treated and 12 placebo-treated individuals). This HBV DNA increase during therapy was positively associated with HBsAg loss (p=0.049, odds ratio 5.1) and HDV RNA suppression (p=0.007, odds ratio 4.1) at week 96. Furthermore, patients with transient HBV DNA increase had significantly higher ALT levels at week 12, 36 and 48 and significantly lower HBcrAg levels at week 48, 96 and 120 after start of treatment.

Conclusion: HBV DNA remains quantifiable in more than three quarter of hepatitis D patients after 96 weeks of PEG-IFNa plus tenofovir combination therapy despite low pre-treatment viral loads. A significant early increase of HBV DNA during PEG-IFNa-2a therapy can be observed in more than 20% of patients, irrespective of the addition of tenofovir. This transient HBV DNA rise may indicate PEG-IFNa-induced cell death and is associated with end-of-treatment HDV RNA suppression and HBsAg loss.

OP-03YI A CRISPR/Cas9 screen to identify host factors required for the hepatitis E virus life cycle

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Background and Aims: Hepatitis E virus (HEV) is a positive-strand RNA virus whose genome harbors 3 open reading frames (ORF). ORF1 encodes the viral replicase, ORF2 the capsid and ORF3 a small protein involved in virion secretion. However, our current knowledge of the molecular mechanisms allowing productive HEV infection remains scarce, especially with respect to the host factors required for the viral life cycle. Our study aims at identifying host factors necessary for viral replication.

Method: A genome-wide CRISPR/Cas9 screen was performed in permissive human cell lines harboring newly developed subgenomic HEV replicons allowing for positive and negative selection, followed by next-generation sequencing of resistant cell populations and bioinformatic analyses.

Results: "Suicide" HEV replicons expressing a neomycin resistance gene for positive selection and a herpes simplex virus thymidine kinase for negative selection (HEV83-2_TKNeo) have been prepared and characterized in depth. Two screens consisting in the parallel electroporation of ganciclovir (GCV)-sensitive (HEV83-2_TKNeo) and -insensitive (HEV83-2_Neo) replicons were performed. The two cell populations selected by GCV treatment were analyzed by next-generation sequencing and a list of candidate host factors was established. Validation of the 20 top candidates using siRNA-mediated knockdown and further analyses is ongoing. A screen for host factors of hepatitis C virus (HCV) replication, for which a large body of literature exists, was performed in parallel for validation of our experimental strategy.

Conclusion: The genome-wide CRISPR/Cas9 screen allowed to identify a number of candidate host factors of HEV replication which are currently being validated. These studies should yield new insights into the viral life cycle and virus-host interactions required for productive HEV infection.

OP-04 Hepatitis b virus x protein is required for viral gene expression, indirectly controls cccdna amplification and is dispensable for dna synthesis

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Background and Aims: The role of hepatitis B virus (HBV) X protein (HBx) in the HBV life cycle is largely enigmatic. Although strong evidence support the requirement of HBx for transcription from the nuclear covalently closed circular DNA (cccDNA), HBx's role in cytoplasmic DNA synthesis and replication is still controversial (Science. 2001 Dec 14;294(5550):2376-8). As viral pre-genomic RNA (pgRNA) is the template for HBV DNA synthesis, a block at the level of gene expression results in a reduction in DNA synthesis and replication, and therefore the relative contribution of HBx for each of these major steps in HBV life cycle is hard to assess.

Method: To reliably uncouple DNA synthesis and replication *per se* from viral gene expression, we transfected hepatoma cells with in vitro transcribed HBV pgRNA, thereby bypassing first round transcription to initiate the viral life cycle from the DNA replication step.

Results: Here we show that transfection of pgRNA harboring an early termination codon in the HBx open reading frame (X^{ko}) results in an intact synthesis of HBV DNA whereas HBsAg production is markedly reduced compared to the wildtype control. Complementation with wildtype HBx in trans has no effect on DNA copy number but completely restores HBsAg production. In contrast, complementation with the HBx R96E mutant that cannot bind DDB1 fails to restore HBsAg production. Surprisingly, nuclear cccDNA was moderately increased in X^{ko} pgRNA transfected cells, possibly due to a shift favouring nuclear cycling of newly synthesized DNA due to paucity of HBsAg.

Conclusion: HBx is essential for HBV gene expression but is dispensable for viral DNA synthesis. In addition, HBx may indirectly control intracellular cccDNA amplification by promoting HBsAg expression from the newly synthesized cccDNA.

OP-05YI Type I interferon exposure augments follicular T helper cell phenotype and functionality

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Background and Aims: A robust follicular T helper (Tfh) cell response is critical for viral control, since these specialized CD4 T cells support B cells to generate a strong antibody response. During chronic infection the antiviral T cell response is partly regulated by type I interferons (IFNs). They suppress Th1 and promote Tfh cell differentiation during viral persistence in mice but are known to inhibit human Tfh cell differentiation *in vitro*. Interestingly, Tfh cells accumulate in the IFN-rich milieu of the Hepatitis C Virus- (HCV-) infected liver during chronic infection. It is still incompletely understood how far IFN-signaling influences Tfh cell responses. To investigate the influence of IFN exposure on human Tfh cell maintenance and functionality, we developed a clone-based *in vitro* model.

Method: Tfh (CXCR5+, PD-1+, CXCR3-) and Th1 (CXCR5-, CXCR3+, CCR6-) clones with unknown antigen-specificity derived from HD or HCV-infected patients were used as reference. To investigate long-term effects of *in vivo* IFN exposure, HCV-specific CD4 T cell clones of chronically infected direct acting antiviral (DAA)-cured patients were compared to Influenza- (Flu-) specific CD4 T cell clones derived from a non-persistent infection of HD. STAT1 phosphorylation, phenotype and cytokine production of CD4 T cell clones or HD PBMCs with or without previous IFN exposure were analyzed by flow cytometry. B cell helper capacity was assessed by coculture of naïve B cells with the T cell clones.

Results: STAT1 phosphorylation was observed after type I, but not type II or III IFN exposure. Maintained CXCR5+, PD-1+ Tfh cells showed a higher level of activation (CD38+, ICOS+) after type I IFN exposure of PBMCs. Transcriptional activity (TCF-1 and FoxP3), cytokine production (IFNg, IL-21) and B cell helper capacity of HD Tfh clones were enhanced by type I IFN influence. Moreover, HCV and Flu clones showed striking phenotypical and functional differences. HCV clones maintained high CXCR3 and PD-1 expression and showed a higher expression of CD38 and BTLA. Their cytokine expression profile and B helper capacity resembled Tfh clone characteristics, whereas Flu clones presented Th1 clone features pointing out a Tfh promoting effect of long-term IFN exposure.

Conclusion: Our results suggest that type I IFNs augment Tfh phenotype, activation and functionality. This supports our observations of intrahepatic Tfh accumulation and their release into circulation upon DAA therapy in chronically HCV-infected patients.

OP-06YI Prospective study of HBV cccDNA kinetics in a French multicenter cohort of liver transplant patients (ECOGREFFE) reveals very early graft infection despite NUC+HBIG prophylaxis

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Background and Aims: Combined Nucleoside Analogues (NAs) and hepatitis B immunoglobulins (HBIG) therapy accounts for the low risk of HBV recurrence after liver transplantation (LT). The duration of the double prophylaxis remains a matter of debate and the lack of non-invasive biomarkers for intrahepatic HBV replication prevents the implementation of personalized duration of treatment.

Method: Blood samples from 34 patients transplanted for HBV-related disease were collected at LT registration, the day of the LT, 3 and 12 months after LT. A tissue sample from the native liver, after liver reperfusion and at 12 months after LT were collected and snap frozen at -80°C. Intrahepatic HBV cccDNA, DNA replicative intermediates (tHBV DNA) and 3.5Kb-RNA were quantified by digital-droplet PCR. Serum HBcrAg and quantitative HBsAg were analysed using the Lumipulse platform (Fujirebio - LLOQ of 3 logU/ml and 5 mIU/ml, respectively).

Results: At the time of LT, serum HBV DNA was undetectable in 86.2% of patients. The median age was 54.3 years, 24 had hepatocellular carcinoma, 28 were HBeAg-negative and 2 were co-infected with HDV. HBV DNA was detectable in 97% of the native livers with a median value of 0.036 copies/cell for tHBV DNA and 0.0012 copies/cell for cccDNA. 3.5Kb RNA was found in 20 patients with a median of 0.0029 copies/cell. All recipients received NA and 1-year HBIG treatment after LT and had undetectable serum HBV DNA 1 year post-LT. 14/29 reperfusion biopsies were positive for HBV DNA and 3 were positive for 3.5Kb RNA. 13/20 liver biopsies available 1 year post-LT were positive for HBV DNA and 7 for 3.5Kb-RNA with a mean of 0.006 copies/cell for tHBV DNA, 0.0006 copies/cell for cccDNA and 0.0012 copies/cell for 3.5Kb RNA. Pre-LT serum HBcrAg was significantly correlated with native liver tHBV DNA ($r=0.67$, $p<0.0001$), cccDNA ($r=0.56$; $p=0.0004$) and 3.5Kb-RNA ($r=0.71$; $p<0.0001$). Pre-LT qHBsAg correlated with native liver tHBV DNA ($r=0.63$; $p=0.0003$) and 3.5Kb-RNA ($r=0.62$; $p=0.0005$) but not with cccDNA levels. At 3 months and 1 year post-LT, 7 and 5 patients, respectively, were still positive for serum HBcrAg. Albeit negative with classic techniques, 5 patients scored positive for HBsAg 1 year post-LT by highly sensitive Lumipulse test.

Conclusion: Our study shows a rapid HBV infection of the graft despite NA + HBIG prophylaxis. One year post-LT, a majority of patients had detectable intrahepatic HBV markers. No direct correlation between pre-LT serum HBcrAg and HBsAg with HBV recurrence was identified. Correlation of intrahepatic HBV markers with serum circulating HBV RNAs is under investigation.

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ePOSTER ABSTRACT PRESENTATIONS

P01-01 Filling the gaps: HCV treatment at a needle exchange program in Sweden (project Actionne)

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Background and Aims: Malmö needle exchange program (MNEP) in southern Sweden opened in 1987 and has since reached > 5000 persons who inject drugs (PWID), 600 visitors/8000 visits annually. Partly due to successful transfer to opiate agonist therapy (OAT) the majority of participants use other substances, mainly amphetamine. Despite a low prevalence and incidence of HIV and HBV, the prevalence (>60%) and incidence (31/100 pyr) of HCV have remained high. The aim of this study was to improve access to liver assessment and treatment among NEP participants. Active injectors contribute to a large extent to onward transmission and are under risk of developing significant liver damage, but have had only limited access to HCV treatment.

Method: Project ACTIONNE is a prospective open label study to evaluate treatment of chronic Hep**a**titis **C** infec**tion** in PWID attending a **N**eedle **E**xchange Program. Fifty patients (approximately 15 % of all viremic annual visitors) will receive treatment with glecaprevir/pibrentasvir for 8 weeks (F0-3) or 12 weeks (F4). The primary endpoint is SVR at 12 weeks. Secondary endpoints are re-infections over a follow-up time of 5 years, completion/adherence rates, viral kinetics and resistance patterns, effects on quality of life and risk behaviour. Drug use is monitored during treatment. All steps within the cascade of care take place at the NEP, including assessment by infectious disease and addiction care specialists.

Results: Inclusion period was from April 2018 to May 2019. Fifty NEP participants with HCV were included). So far, 47/50 patients have reached end of treatment and 45/50 the time point for SVR12, all HCV RNA negative. Median age 46 years, 78 % male, 90% F0-F3 and 10% F4, with 49 % gt 1a, 47 % 3a and 4 % gt 2b. Half of the patients report main use of iv amphetamine, 30 % heroin and 20 % use of mixed substances. Other benefits such as improved nutrition, sleep, self-esteem and decreased drug intake during and after treatment have been observed.
Conclusion: The full cascade of care for HCV, from diagnostic testing, assessment, treatment and follow-up can be offered through a well-functioning NEP, equipped with the right medical resources for HCV treatment. Implementing treatment in the NEP routine is expected to have an impact on both individual and group levels concerning HCV related morbidity and mortality.

Disclosure of interest: The Actionne study is an investigator initiated study, receiving funding from AbbVie. AbbVie does not, however, have any access to data, nor impact on its interpretation, publication or this submission.

P01-02YI Development and Characterization of Oral combination vaccine against Hepatitis B and Influenza

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Background and Aims: Vaccination has not only become vital but a lot of revolutionary changes are being observable in the field of vaccine delivery. Vaccine antigens administered by the oral route are often degraded during gastrointestinal transit. Bile salt stabilized vesicles i.e. bilosomes are found to be effective in preventing antigen degradation and enhance mucosal penetration. The aim of the present work was to prepare a combination vaccine system against hepatitis-B (HBsAg) and influenza(r-H1N1Ags).

Method: Bilosomes containing HBsAg and r-H1N1Ags were prepared by a lipid cast film method. Antigen loaded bilosomes were characterized *in-vitro* for their shape, size, percent antigen entrapment and stability. Fluorescence microscopy was carried out to confirm the uptake of bilosomes. The *in-vivo* study comprised of estimation of IgG response in serum and sIgA in various body secretions using specific ELISA.

Results: Bilosomes formed were multilamellar and were stable in gastric and intestinal fluids. Fluorescence microscopy suggested that bilosomes were taken up by gut-associated lymphoid tissues. In-vivo data demonstrates that bilosomes produced both systemic as well as mucosal antibody responses upon oral administration at higher dose levels as compared to intramuscular immunization but fail to produce any synergistic effect.

Conclusion: Thus, HBsAg potentiates the production anti-r-H1N1 antibody. Also measurable sIgA in mucosal secretions were observed. Thus, bilosomes are a promising carrier for oral combination vaccines. This approach could be adapted for human use because mucosal surfaces are initial sites of infection and it therefore seems logical to attempt to develop vaccination strategies that evoke appropriate localized responses to counteract early events of pathogenesis.

P01-03YI Antibody coated Liposomes for Transmucosal Vaccination

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Background and Aims: The critical role of vaccine delivery system in “rational vaccine design” has been widely recognized. Thus research work was envisaged involving development of IgG antibody immobilized on the surface of hepatitis B surface antigen (HBsAg) antigen–loaded liposomes. which offered increased uptake of nanoliposome through transmucosal surface of nasal route and sustaining release of HBsAg to evoke relatively high IgA titre in mucosal surface.

Method: Liposomes were prepared by a lipid cast film method & then IgG antibody was cross linked on the surface. Coated liposomes were characterized *in-vitro* for their shape, size, polydispersity index, entrapment efficiency, zeta potential and stability. Fluorescence microscopy was performed to confirm the deposition pattern in respiratory tract. The *in-vivo* part of the study was conducted to visualize targeting potential, localization pattern, and immunogenicity. In addition, immune response was compared with alum-HBsAg vaccine injected intramuscularly.

Results: Observation of fluorescence images of nasal mucosa, lungs and spleen, revealed that these antibody coated liposome, were significantly taken up by mice respiratory mucosal surface, which made them promising carriers for mucosal vaccination. Considerable immune responses were produced by the developed system that may be due to the induction of MALT as well as contribution of the peripheral airways. The serum anti-HBsAg titer, obtained from the postnasal administration of IgG-coupled liposomes, was significantly higher than plain liposomes. Moreover, IgG-coupled liposomes generated both humoral (i.e., systemic and mucosal) and cellular immune responses upon nasal administration, while the alum-adsorbed antigen displayed neither cellular (cytokine level) nor mucosal (IgA) response. The formulation also displayed enhanced transmucosal transport, improved *in-vitro* stability, and effective immunoadjuvant property.

Conclusion: The higher immunity induced by ACL-HBsAg may be attributed to its cationic nature, antibody coating and subsequent mucoadhesive property. Thus mucosal immunization with lipid vesicle through nasal administration may be effective in prophylaxis of diseases transmitted through mucosal routes as well as systemic infections. The strategy can be made more appropriate by determination of paracellular transport, nasal mucociliary clearance, mucosal toxicity assessment etc.

P01-04 Prevalence of hepatitis C virus infection among men who have sex with men in Moscow

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Background and Aims: Behavioral factors contribute to the hepatitis C virus (HCV) infection spreading among men who have sex with men (MSM); high-risk sexual behavior and recreational drug use are among the main factors.
Aim. To estimate the prevalence of HCV infection among MSM cohort in Moscow.

Method: Research was conducted in a collaboration with the MSM volunteer organization in Moscow from September 2017 to December 2018. Participants of research were anonymously tested for HCV-antibodies with oral HCV rapid antibody test. At the same time, we conducted screening them for human immunodeficiency virus (HIV infection). Totally 826 MSM were tested. The median age of the participants was 34 years old.

Results: The prevalence of anti-HCV among the total MSM cohort was 2.1% (17 tests were positive). Anti-HCV seropositivity was significantly associated with HIV-positive serostatus. Among MSM with HIV, anti-HCV was detected in 6.9%, and among MSM without HIV, it was detected in 1.4%. Only 47% of anti-HCV-positive men (8 people) agreed to continue the examination and to get antiviral therapy. Among them, 1 (12.5%) was HCV RNA-negative (without a history of PVT, HIV-negative), the remaining 7 (87.5%) had 1 virus genotype (6 (85.7%) - 1a, 1 (14.3%) - 1b), four (57%) had HIV coinfection, and one perosn (14.3%) was reinfected. There was no marked liver fibrosis among the research participants (according to the liver elastography results, the 0–2 stage was determined by METAVIR scale).

Conclusion: HCV infection is more common among HIV positive MSM than among MSM without HIV. The prevalence of HCV infection among HIV negative MSM is comparable to the prevalence among the general population. To reduce the spread of HCV infection among MSM in Russia, early detection and treatment of infection, development of educational programs and effective behavioral interventions are needed.

P01-05YI Health-related quality of life evaluation using questionnaires in patients with chronic hepatitis c virus infection before receiving direct-acting antivirals treatment

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Background and Aims: Presenting a clinical prospective study about quality of life in patients with chronic hepatitis C virus before receiving DAA treatment. Chronic hepatitis C virus has a negative impact on physical and mental status, influences not only patients' functional health, but also work abilities.

Method: A group of patients was analyzed before starting DAA treatment. The design of the study is prospective and starts from November 2018 at Hospital for Infectious and Tropical Diseases "Dr. Victor Babeș" and at "Victor Babeș" Private Medical Clinic, Bucharest. Patients received two questionnaires, patient-reported outcomes (PRO): 36-Item Short Form Survey (SF-36) and Hospital Anxiety and Depression Scale (HADS).

Results: The total number of patients was 37, men and women, with ages between 34 and 81 years. Gender distribution was not uniform, 29 (62%) women and 8 (38%) men. Most of them are living in urban area, 27(73%) and 10 (27%) in rural area. Stages of liver fibrosis were evaluated by Fibroscan and the results were: 7 patients with F1 Metavir, 10 patients with F2 Metavir, 14 patients with F3 Metavir and 6 patients with F4 Metavir. SF-36 has 36 items, 2 major components and 8 scale scores (ranging from 0 to 100, score 0 means severe affected and score100 means unaffected): Mental Component Score and Physical Component Score. There was observed a correlation between fibrosis and physical function (PF) low score with p=0.011. BMI was correlated with emotional role low score with p=0.022. Patients from rural area had a low score in PF with p=0.051. Analyzing the questionnaires, all the patients had low scores in vitality scale (VT) and in general health perception (GH). There was observed a correlation between fibrosis and physical function (PF) low score with p=0.011. BMI was correlated with emotional role low score with p=0.022. The other questionnaire, HADS is composed of 2 scales Anxiety and Depression, both ranging from 0-21, higher scores suggesting more severe distress. HADS analysis revealed 17 patients with normal scores – ranging from 3 to 7 points, 11 patients with modified scores – possible cases (ranging from 8 to 10 points) and 9 patients with abnormal scores – probable cases (ranging from 11 to 21 points). From those with modified and abnormal scores, 11 patients had more questions suggesting anxiety, 4 patients suggesting depression and 5 had equal scores in-between anxiety and depression. BMI was also correlated with HADS high score with p=0.008.

Conclusion: People with chronic hepatitis C virus have an increased incidence in mood disorders like anxiety or depression with important impact in social activities and quality of life.

P02-01 Efficacy and safety of elbasvir/grazoprevir treatment in treatment-experienced and co-morbid HCV GT1b patients

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Background and Aims: Treatment of HCV infection has become more effective and safer since more direct antiviral agents are available. In clinical trials a treatment of HCV with combination regimen of elbasvir (EBR) and grazoprevir (GZR) was associated with high SVR rate (up to 100% in treatment-naïve patients with GT1b). Fixed-dose combination tablet of EBR+GZR has recently been approved in Russian Federation (October 2018).
Aim of the study was to evaluate efficacy and safety profile of EBR+GZR treatment of HCV G1 treatment-experienced patients and patients with co-morbidities in real clinical practice.

Method: Sixty-eight patients with HCV GT1b received EBR 50 mg plus GZR 100 mg once daily for 12 weeks. All demographic and laboratory data during treatment period was recorded. The primary endpoint was sustained virologic response 12 weeks (SVR12) after the end of therapy. HIV co-infection was not an exclusion criteria.

Results: This analysis includes 68 patients from 2 clinical centers in Russia with available data for SVR12. Enrolled patients characteristics: male – 44%, median age - 50 (45-58) years, high viral load (HCV RNA>8*10^5 IU/ml) - 30 %, advanced fibrosis (F3-F4 using TE) (n=18, 26%), co-morbidities (chronic kidney disease, HIV, cardiovascular disease, T2DM, lymphoproliferative disorder) – 60%. SVR12 achieved 98,5% (67/68) patients. Relapse were registered in 1 patient with high viral load, T2DM and BMI> 30 at baseline. No serious adverse events as well as treatment discontinuation were registered.

Conclusion: HCV treatment using grazoprevir / elbasvir is well-tolerated and associated with high SVR rates in patients with advanced fibrosis, treatment-experienced and co-morbid patients. Grazoprevir / elbasvir regimen in real clinical practice has shown similar results to clinical trials.

P02-02 Hepatitis e virus infection among people living with hiv / aids virus in kano state

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Background and Aims: Hepatitis E is an emerging viral disease causing acute hepatitis worldwide which may result into a chronic hepatitis especially in immunocompromised individuals. Hepatitis E Virus is transmitted primarily via the fecal oral route and placenta. The study determined the prevalence of Hepatitis E Virus (HEV) among people living with Human Immunodeficiency Virus (HIV) in Kano State. One hundred and eighty (180) subjects were enrolled for the study and their sera were screened for Hepatitis E Virus Antigen using Enzyme Linked Immunosorbent Assay (ELISA). Twelve (6.7%) were found positive for HEV antigen comprising 7(58.3%) males and 5(41.7%) females and was common (5, 41.7%) in age group 35-44 years. Preponderance of HEV antigen was also found among subjects with primary school level of education(5, 41.7%), entrepreneurs (8, 66.7%) and those with HIV duration of 3-5 years (8, 66.7%), and were on the first line of antiretroviral treatment (ART) (10, 83.3%) or used borehole as a source of water (8, 66.7%). Statistical analysis shows a significant relationship between HEV and gender ($P<0.05$) of subjects. The study provided evidence that HEV is present among people living with HIV in Kano State. There is need to intensify enlightenment campaign among the populace about the disease and associated morbidity and mortality so as to limit its spread in the community.

P02-03YI HCV treatment with DAA in children and adolescents in Russian Federation (single center study)

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Background and Aims: The efficacy of direct antiviral drugs (DAAs) in the treatment of HCV in adults is up to 100%. However, to date, antiviral therapy in children and adolescents with DAAs in the Russian Federation is not studied yet, since the standard of care for children with HCV infection was, until recently, alpha-interferon (a-IFN) in combination with ribavirin.
Aim of the study is to evaluate efficacy and safety of DAA treatment in children and adolescents in Russian Federation.

Method:
Twenty patients with HCV (genotype 1) were treated with DAA: ombitasvir/paritaprevir/ritonavir and dasabuvir (OBV/PTV/r+DAS) (N=13) and sofosbuvir plus daclatasvir (N=7) at the Department of Hepatology of MONIKI. M. F. Vladimirsky and JSC “United medical system” after ethic committee approval. Treatment duration was 12 weeks in both groups. Fibrosis stage were evaluated using transient elastography (TE). The criteria for exclusion were the presence of HIV infection and other liver diseases.

Results: This interim analysis includes data for 20 patients available to 13.10.2019. Median age was 15 (12;17) year, 35% patients were males. The main transmission route was vertical (70%). The median duration of infection was 9 (1;17) years. Three patients were treatment-experienced (15%). Fibrosis stage according to TE were F0- 65%; F1- 25%; F2- 10%. SVR24 rate for 17 patients completed treatment course are 100% despite of treatment regimen. Three patients are currently receiving antiviral treatment (HCV RNA negative at week 4). No treatment related SAEs as well as treatment discontinuation were registered. The most common AE were: fatigue (20%) and headache (20%).

Conclusion: HCV treatment with DAAs is associated with a high SVR rate and a good safety profile, as well as a high compliance in adolescents. No serious adverse events leading to treatment discontinuation have been identified, which confirms the safety of the use of interferon-free therapy in adolescents.

P02-04YI Hepatitis B infection: is it a real public health threat in upper Egypt?

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Background and Aims: Egypt is well known for its high HCV prevalence. The Egyptian government has implemented a very effective program to eradicate HCV, which includes mass screening campaigns to diagnose the asymptomatic cases and widespread treatment program. On the other hand, there is little information available about the prevalence of HBV in Egypt.

Method: Luxor HCV Treatment Center was established in 2016 by Tahya Misr Fund to help fight HCV infection in Luxor city and the surrounding areas in Upper Egypt. The center adopted a unique mass screening program for both HBV and HCV. Participants aged 16 years and older were screened, at no cost from their side, for anti-HCV antibodies (anti-HCV) and hepatitis B surface antigen (HBsAg) using third generation enzyme immunoassays (Enzygnost® Anti-HCV and HbsAg). In this report, we will focus on HBV screening results and compare it to results of the 2015 Egyptian Health Issues Survey (EHIS), a large nationwide screening study.

Results: From June 2016 to May 2017, 67,007 persons were screened for HBsAg at Luxor center, including 31,945 males (47.7%) and 35,062 females (52.3%). The mean age was 43.6 years. 2947 persons (4.4%) were found positive for HBsAg. HBsAg prevalence was significantly higher in males versus females (6.2% vs. 2.75% OR = 2.3; $p < 0.0001$). The age structure of HBsAg prevalence has a steep increase to age 31 (7.7%) followed by a decline to age 60 and then flattens. In EHIS 2015, 26,047 persons aged 1-59 years were screened for anti-HCV, Hepatitis B core antibody (HBcAb) and HBsAg, including 12,319 males (47.3%) and 13,728 females (52.7%). The overall prevalence of HBcAb was 9.9% (11.3% in males - 8.7% in females), compared to 1% for HBsAg (1.2% in males - 0.8% in females). 274 persons from Luxor area were screen, in which HBcAb and HBsAg prevalence was 18% and 1.7% respectively. The age specific pattern of anti-HCV, HBcAb and HBsAg in EHIS and Luxor study are shown in **Figure (1)**.

Conclusion: HBV infection rate is very high in Egypt as indicated by the high prevalence of HBcAb (9.9%) reported in EHIS. Luckily, the majority of these infections are spontaneously resolved, and 1% only progress to chronic infection (HBsAg positive). Luxor study showed a higher HBsAg prevalence (4.4%), which is more significant in males and in the middle age group. HBV screening and vaccination of high risk groups should be enforced in this area of Upper Egypt.

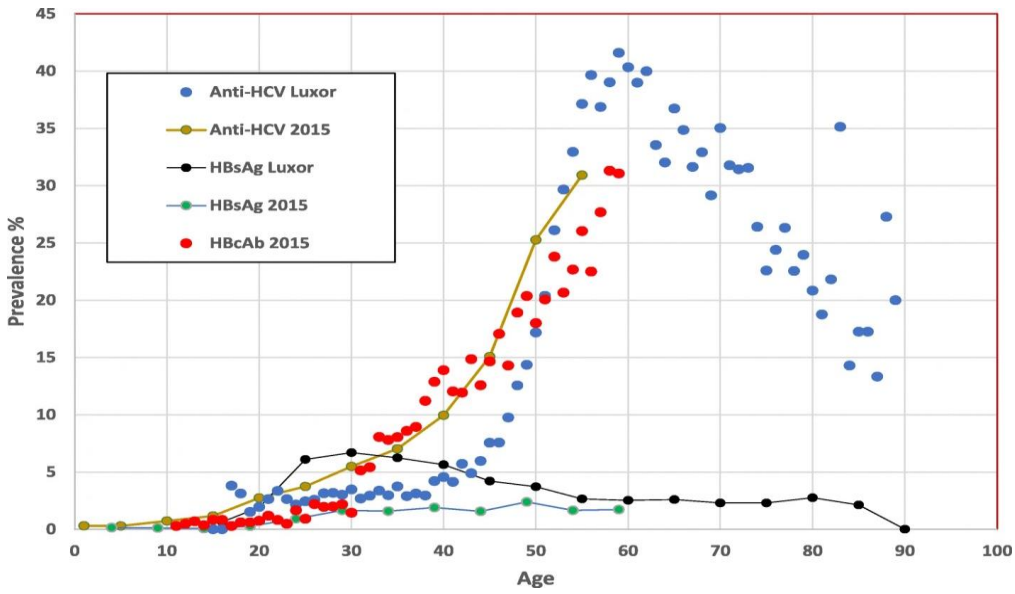
Anti-HCV Luxor
Anti-HCV 2015
HBsAg Luxor
HBsAg 2015
HBcAb 2015
Prevalence %
Age
0
5
10
15
20
25
30
35
40
45
0
10
20
30
40
50
60
70
80
90
100

Figure:

P02-05 Clinicopathologic Characteristics of Follicular Lymphoma in Hepatitis C Virus-Infected Patients

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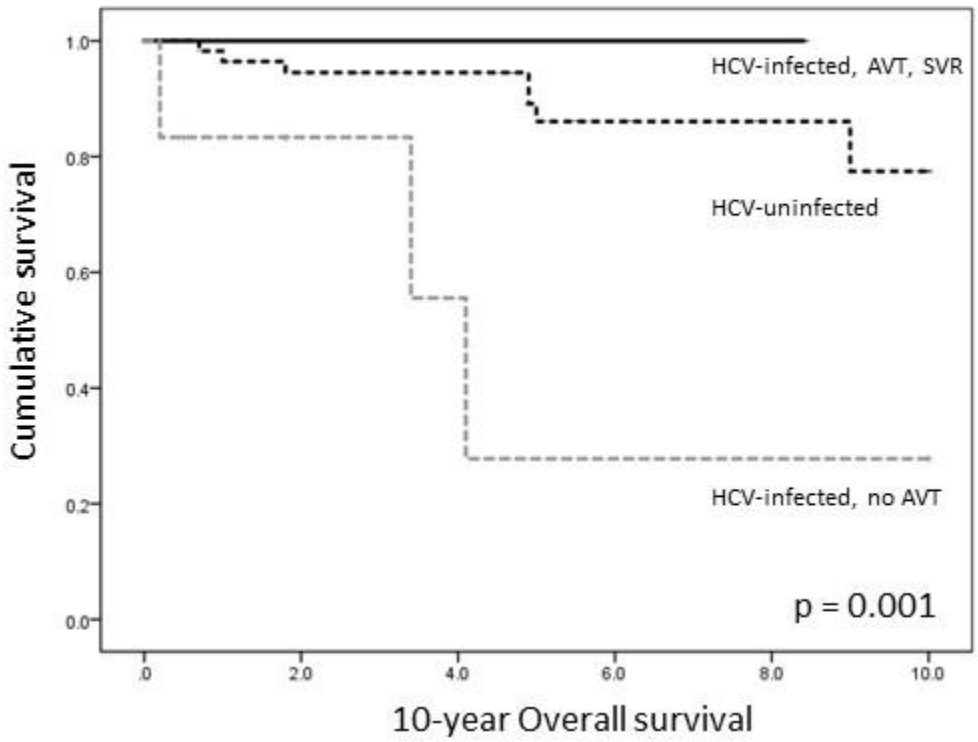
Background and Aims: Follicular lymphoma (FL) is the most common subtype of indolent non-Hodgkin lymphoma. It has been hypothesized that chronic hepatitis C virus (HCV) infection stimulates *IGH-BCL2* clone proliferation, leading to development of FL. Furthermore, regression of FL after antiviral treatment without chemotherapy has been reported in HCV-infected patients. To clarify the relationship between HCV and FL, we compared the prevalence of *IGH-BCL2* translocation and other clinicopathologic characteristics between HCV-infected and HCV-uninfected FL patients and determined the impact of HCV eradication on the oncologic outcomes of HCV-infected FL patients.

Method: The study included HCV-infected patients (cases) with FL seen at our institution during 2004-2018. Cases were matched with HCV-uninfected FL patients (controls) according to year of lymphoma diagnosis, sex, and hepatitis B serology.

Results: We studied 19 cases and 57 controls. More cases than controls had splenic involvement of FL (26% vs 5%, p=0.02), higher histologic grade (grade 3 in 56% vs 24%, p=0.01), absent or weak CD10 expression (42% vs 11%, p=0.005), and absent BCL2 expression (33% vs 4%, p=0.004). Compared to controls, cases had a lower rate of detection of *IGH-BCL2* translocation (31% vs 68%, p=0.02). Finally, cases with a sustained virologic response (virologic cure of HCV) had a better 10-year overall survival rate than did cases not treated with antivirals or controls (p=0.001).

Conclusion: HCV-infected patients with FL have unique clinicopathologic characteristics including improved overall survival with HCV eradication. The pathogenesis of FL in HCV-infected patients seems unrelated to anti-apoptotic effect of *IGH-BCL2* rearrangement.

Figure:


Cumulative survival
1.0
0.8
0.6
0.4
0.2
0.0
HCV-infected, AVT, SVR
HCV-uninfected
HCV-infected, no AVT
p = 0.001
.0
2.0
4.0
6.0
8.0
10.0
10-year Overall survival

P03-01 Therapy of hepatitis C in people who inject drugs: focus on adherence

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Background and aims: In the Czech Republic, intravenous drug use (IVDU) represents the major factor of HCV transmission, but the numbers of treated individuals among people who inject drugs (PWID) remain low. The refusal or deferral of treatment is based on the false presumption of low treatment efficacy due to bad adherence to therapy. The aim of our study was to assess HCV treatment efficacy in PWID with a focus on factors determining adherence to therapy.

Methods: All consecutive patients who started DAA anti-HCV therapy from 1st January 2017 to 6th August 2018 were included. The patients were divided into two groups: individuals with a history of IVDU (PWID, N = 101) and control group (N = 177), without IVDU in the past. The patients' data were obtained from patients' medical charts.

Results: SVR12 was achieved by 99/101 (98%) patients in the PWID group, 2 patients were lost to follow-up. In the control group, the SVR rate was identical, 98% (4 relapsers, 1 lost to follow-up). SVR24 was achieved by 89/101 (88.1%) patients in the PWID group (1 relapser, 1 reinfected, 10 lost to follow-up). In the control group, SVR 24 rate was 92.1% (163/177), the SVR 24 rate did not differed significantly between groups.
PWID patients had a significantly higher number of postponed appointments (28.7% PWID vs. 4% controls, p = 0.001), however, postponing visits led in only one PWID to a lack of medication and skipped dosing. In the control group, 2 patients postponed their appointments and ran out of medication. The difference in missed visits in the follow-up period was not statistically significant at SVR12 visit, whereas PWID patients missed the SVR24 visit more often (10.9 % vs. 1.1 %, p = 0.004). In multivariate analysis of the whole cohort, IVDU was not a factor contributing to a worse adherence to treatment. Stable housing was a significant predictor of excellent adherence, whereas being a foreigner and self-referred to therapy negatively influenced adherence. In the PWID group, older age and stable housing were factors positively contributing to adherence, a stable job was a factor decreasing adherence. In the control group, none of the analysed demographic and social factors had impact on adherence to therapy.

Conclusions: The treatment efficacy in the PWID group was excellent. Stable housing and older age contributed to a better adherence, whereas a stable job was a factor decreasing adherence to therapy owing to the concern about job loss.

P03-02YI Development of Bipolymer based novel Nanoparticles in Microsphere system as vaccine adjuvant

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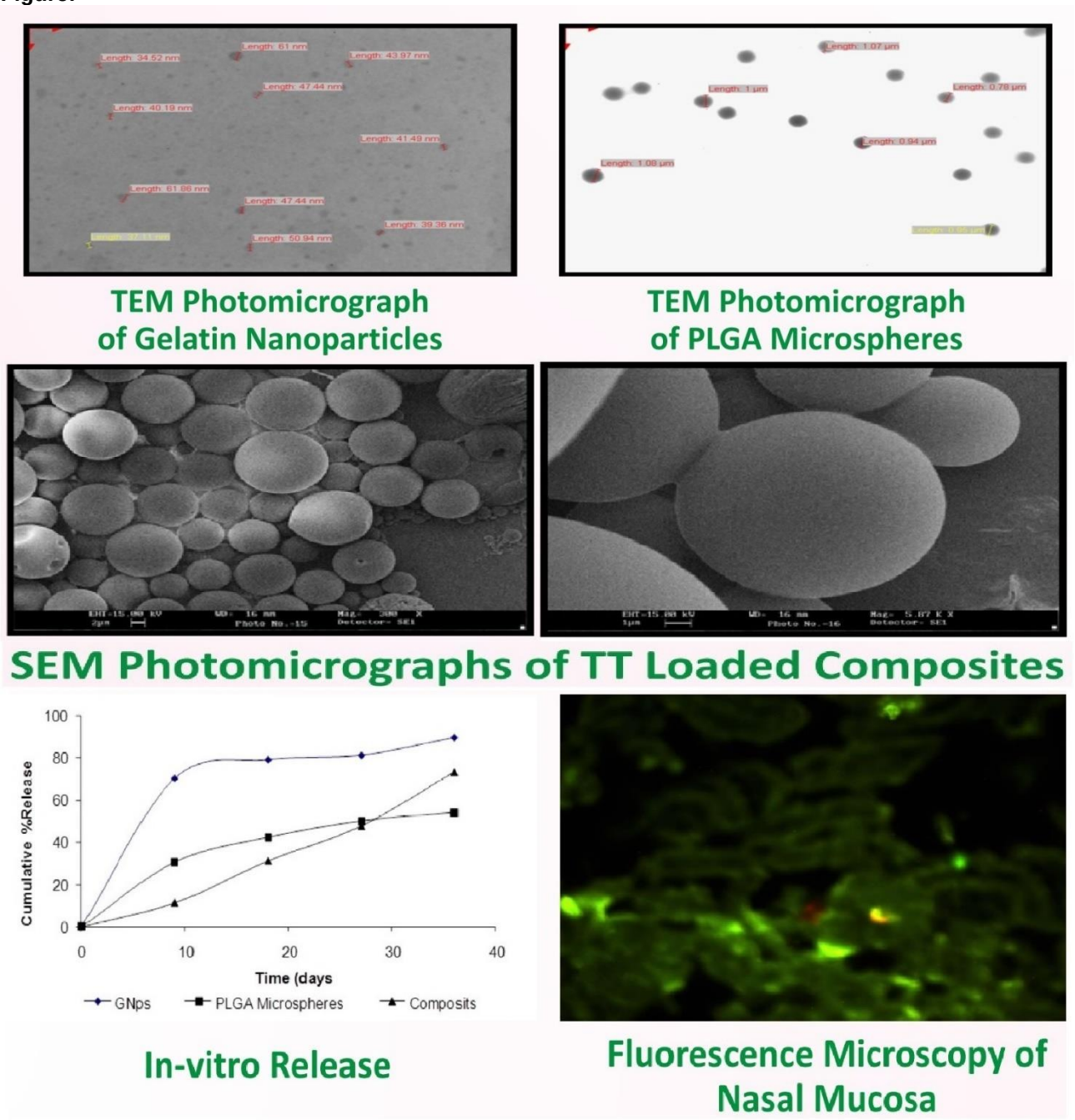
Background and Aims: Novel strategies are required for the achievement of safe and effective immunization beyond the conventional strategies. Frequent booster dosing can be avoided by the development of a mucosal/adjuvant vaccine delivery system, which can safely produce high and long lasting immune responses. Mucosal immunization is an attractive alternative to parenteral as with the appropriate delivery system it is possible to stimulate both humoral and cell-mediated responses.
The research work envisaged promotes the advantages and overcomes the disadvantages of the hydrophilic and hydrophobic polymeric systems, by a combined hydrophilic (gelatin nanoparticles, GN) with a hydrophobic polymeric system (PLGA microspheres). This combination creates a new biodegradable system for HBsAg delivery.

Method: GN & PLGA microspheres were prepared by double emulsification method and composite system was prepared by phase separation method. Antigen loaded composites were optimized and characterized *in-vitro* for their shape, size, %antigen entrapment and stability. Fluorescence microscopy was carried out to confirm the uptake of composites. The *in-vivo* part of the study comprised of estimation of IgG response in serum and sIgA in various body secretions using specific ELISA. The external morphology was studied by Scanning & Transmission Electron Microscopy.

Results: The *in-vitro* studies exhibited an initial burst release from gelatin nanoparticles, degradation of antigen from PLGA microspheres & a continuous release from composite system. This supports the hypothesis to formulate single shot vaccine with such system (to mimic booster dosing). The fluorescence studies showed the selective uptake of composites by NALT.

Conclusion: Humoral response generated by single dose of composites was comparative to marketed formulation that received the booster dose. Further, composite system generated the effective sIgA antibody which was not elicited by the marketed formulation. Thus, it could be concluded from the present study that bipolymer based composite system are capable to provide sufficient protein stability and can be a promising candidate for development of single shot vaccine, not only against Hepatitis but against all those diseases that invade the host by the mucosal surfaces.

Figure:


TEM Photomicrograph of Gelatin Nanoparticles
TEM Photomicrograph of PLGA Microspheres
SEM Photomicrographs of TT Loaded Composites
Cumulative %Release
Time (days
GNps
PLGA Microspheres
Composits
In-vitro Release
Fluorescence Microscopy of Nasal Mucosa

P03-03YI Lipid based Nanoparticulate system for effective vaccine delivery

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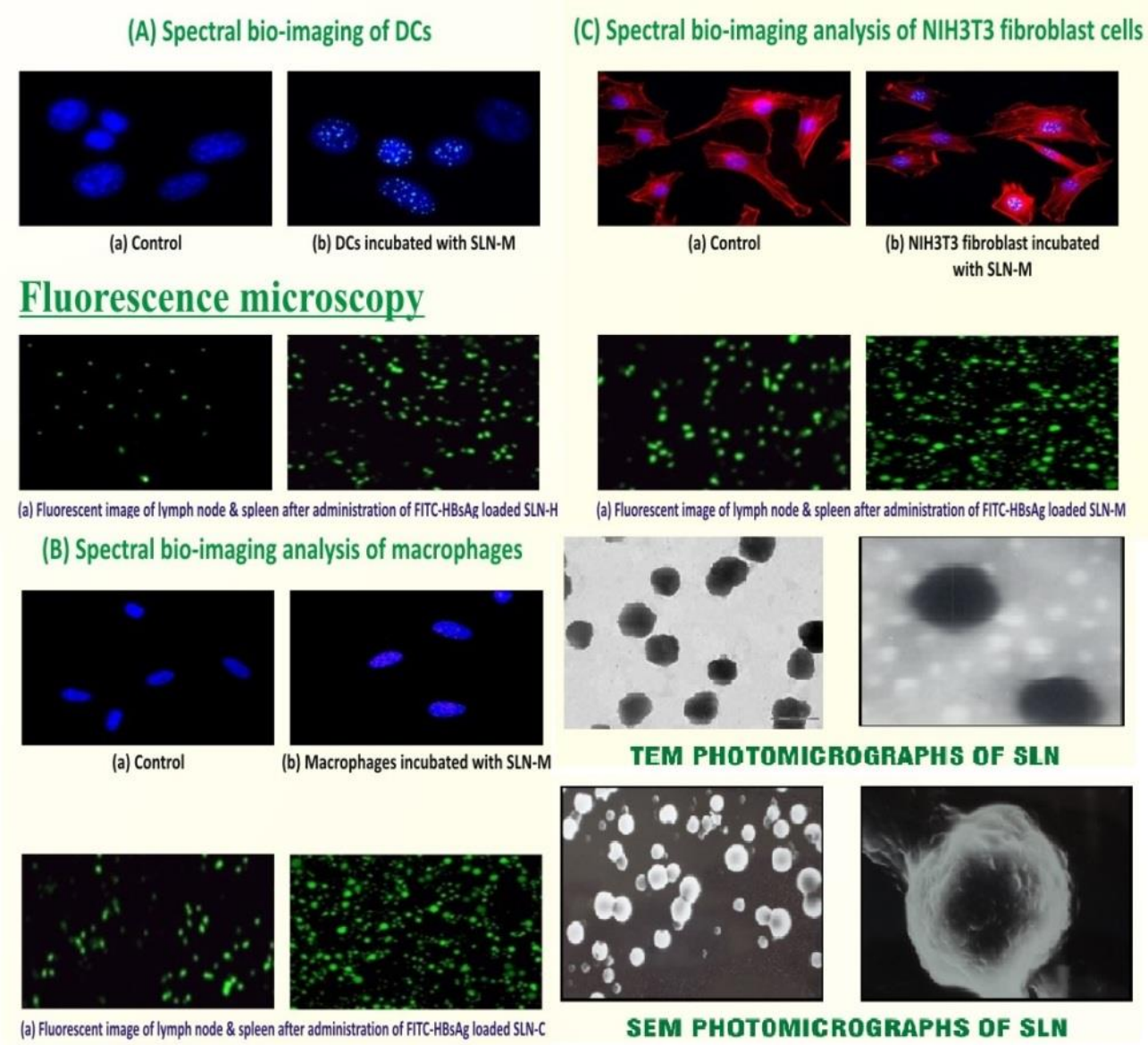
Background and Aims: The search for innovative ways of vaccination has intensified recently with declining vaccine coverage and growing public concern about new virulent disease outbreaks. Immunization is a prophylactic approach through which body is shielded from any incoming pathogenic invasion. The work envisages concern exploring potential of Solid Lipid Nanoparticles(SLN) in efficient protein delivery(HBsAg) through surface modifications, which will in turn; enhance loading efficiency, cellular uptake of SLN using subcutaneous route.

Method: The SLN were prepared by Solvent Injection Method. SLN were optimized for various parameters, by considering particle size, polydispersity index(PI) and entrapment efficiency. The characterization parameters included Transmission & Scanning Electron Microscopy, X-Ray Diffraction Analysis, *In-vitro* release, Kinetics of uptake by flow cytometer, Evaluation of cell apoptosis, T-cell proliferative response assay, TH1/TH2 cytokine profile and Internalization studies by spectral bioimaging. The *in-vivo* study comprised of fluorescence studies and estimation of IgG response in serum and sIgA in various body secretions using specific ELISA.

Results: The particulate system is better carrier system for immunization because of less diffusivity and restricted movement. SLNs themselves act as signal for the phagocytic cells. Surface modified SLNs can entrap greater amount of antigen, provide its sustained release and rapidly internalized by the antigen presenting cells. *In-vitro* T cell proliferation and induction of TH1 type of immune response clearly marks the potential of this novel carrier system. Fluorescence uptake studies showed better uptake of surface modified SLNs. Higher and more sustained antibody titer obtained with surface modified SLNs suggests their better immunological potential. Thus, subcutaneous immunization could be an efficient alternative approach for vaccination against hepatitis.

Conclusion: The formulations developed in this study can be further explored for incorporation and delivery of other proteins, peptides and should subsequently be subjected to pilot plant scale-up as well as clinical trial to establish their potential for subcutaneous immunization against hepatitis-B**.**

Figure:


(A) Spectral bio-imaging of DCs
(a) Control
(b) DCs incubated with SLN-M
Fluorescence microscopy
(a) Fluorescent image of lymph node & spleen after administration of FITC-HBsAg loaded SLN-H
(B) Spectral bio-imaging analysis of macrophages
(a) Control
(b) Macrophages incubated with SLN-M
(a) Fluorescent image of lymph node & spleen after administration of FITC-HBsAg loaded SLN-C
(C) Spectral bio-imaging analysis of NIH3T3 fibroblast cells
(a) Control
(b) NIH3T3 fibroblast incubated with SLN-M
(a) Fluorescent image of lymph node & spleen after administration of FITC-HBsAg loaded SLN-M
TEM PHOTOMICROGRAPHS OF SLN
SEM PHOTOMICROGRAPHS OF SLN

P03-04YI An undetected Hepatitis C outbreak preceded the 2011 HIV outbreak among persons who inject drugs in Athens, Greece: Insights from a mathematical modelling study

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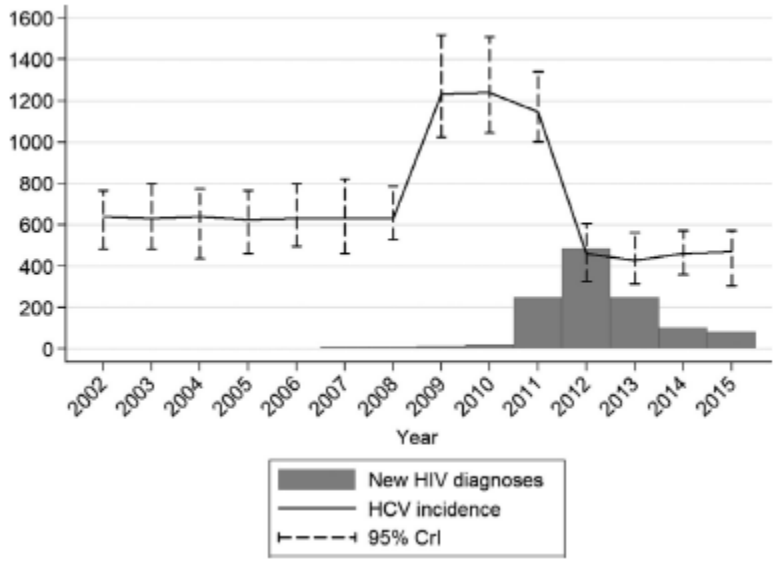
Background and Aims: People who inject drugs (PWID) comprise one of the major transmission risk groups for human immunodeficiency virus (HIV) and hepatitis C virus (HCV). In 2011, Athens experienced an unexpected large HIV outbreak among PWID, which was limited by targeted public health interventions. Meanwhile, Athens also faced an increase in HCV prevalence during 2009-2012. The aims of this study are (i) to examine whether a HCV epidemic preceded the HIV epidemic, and (ii) to estimate the indirect effects of the HIV interventions on HCV infection.

Method: A dynamic, stochastic, individual-based model was developed to simulate HCV transmission among PWID. The model was calibrated to reproduce the observed HCV prevalence among PWID in Athens, Greece.

Results: Two years prior to the HIV outbreak, an undetected HCV outbreak had occurred. The HCV incidence increased from 640 (95% Credible Intervals: 495, 842) cases in 2008 up to 1260 (1060, 1500) in 2009. The mean time from initiation of injecting drug use to HCV acquisition decreased from 29 months in 2008 to 13 months in 2009.
Without the HIV interventions, the expected HCV incidence in 2015 would be 210% higher than observed (450 vs. 980 new infections in 2015). The averted HCV incidence cases attributed to the HIV-implemented interventions were 2200 (1950, 2480), during 2012-2015.

Conclusion: Our results highlight that before the 2011 HIV outbreak in Athens, an undetected HCV outbreak occurred in 2009. Prevention measures for HIV that took place in Athens metropolitan area in 2012 reduced significantly the incidence of HCV.

Figure: Estimation of the number of HCV incident cases produced by the model and the number of new HIV diagnoses reported to the Hellenic Center for Disease Control and Prevention (HCDCP). The solid black line and shaded grey areas show the median and 95% credible intervals for the model projections.


1600
1400
1200
1000
800
600
400
200
0
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
Year
New HIV diagnoses
HCV incidence
95% CrI

P03-05 The impact of treatment emerging resistance associated substitution on outcomes of re-treatment using new generation HCV treatments: Nation-wide real world analysis

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Email: yael.gozlan@sheba.health.gov.il

Background and Aims: Resistance associated substitutions (RAS) mark the rare cases of HCV patients who failed second-generation direct-acting antivirals (DAA). Previously, using next-generation sequencing (NGS), we have reported that 86% (42/49) of treatment failures in Israel harbor RAS. Here we followed these and other patients who failed second generation DAA, attempting to assess the rate of re-treatment success.

Method: Data was collected from all 65 patients who failed (or stopped) second generation DAA treatment between 9.2015- 2.2019. NS3 and NS5A population sequencing analysis was completed for those patients not tested by NGS. Follow-up clinical data (including subsequent treatment regimen and treatment outcome) was collected. In 2 cases resistance testing was performed after re-failure.

Results: 65 patients failed (or stopped) treatment. RAS (NS5A or NS3 by population sequencing) were identified in 78.5% (51).Follow-up information was available for 52% (34). One patient died. All other 33 patients were re-treated with SOF/VEL/VOX (15, 45%), SOF/VEL (5, 15%), GLE/PIB+/- SOF (3, 9%) LED/SOF+/- DCV (4, 15%), GRZ/EBV+/- SOF (5, 15%), PAR/OMB/RTV+DAS (1, 3%) all with or without RIB, for 8-24 weeks according to guidelines.
73% (24/33) finished treatment successfully: 13 achieved SVR; 11 were HCV undetectable at end of treatment (SVR 12 data were not yet available). Of those, one failed with NS3 D168V RAS (%), 19 (79%) had NS5A RAS (30, 31 or 93 or both).
2 patients (6%) failed re-treatment: AGT 1b, oncology patient, F4 that did not respond to SOF/LED, re-treated with SOF/VEL and failed again. He had NS5A Y93H RAS in samples taken following both these two treatment failures. The second patient (GT3, F4) failed SOF/LED (24W) and relapsed with SOF/VEL/VOX+RIB (12W). NS5A Y93H RAS was identified in samples taken following both treatment failures.
The rest of the patients are still under treatment (3) or lost to follow-up (5).

Conclusion: RAS are common in second generation treatment failures. However, SVR rates of patients re-treated with third generation therapies is high. Assessment of RAS in DAA failures (using either NGS or population sequencing) could still be beneficial in reducing length, cost and simplification of re-treatment.

P04-01 The role of sphingolipids as potential biomarkers of failure to direct acting antiviral therapy in chronic HCV infection

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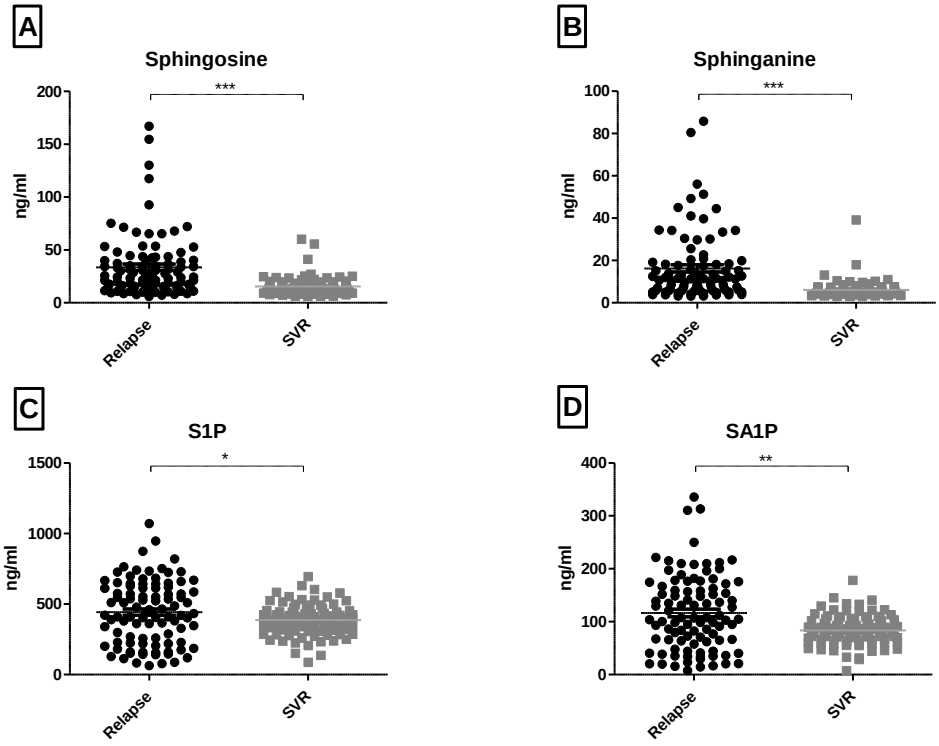
Background and Aims: Elimination strategies of chronic hepatitis C virus (HCV) infection aim to optimize the high antiviral potency of direct acting antivirals (DAA's). Our group has already shown that serum sphingolipid levels associate with fibrosis progression in patients with HCV infection and also with responsiveness to interferon treatment. Aim of the current study is to decipher the role of sphingolipids as biomarkers of response to antiviral therapy with DAA's in patients with chronic HCV infection.

Method: In the present study we used liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) in order to retrospectively quantify various sphingolipid metabolites in baseline serum samples of 98 HCV patients with DAA-failure compared to an age- and sex-matched cohort of 100 HCV-patients with sustained viral response (SVR).

Results: Sphingosine and sphinganine serum concentrations were significantly upregulated at baseline ($P< 0.001$) in patients who experienced a relapse compared to patients with SVR (Fig. 1A,B). Also their phosphate derivatives, sphingosine-1-phosphate (S1P) and sphinganine-1-phosphate (SA1P), showed higher baseline concentrations in relapse patients ($P< 0.05$ and $P< 0.05$ respectively). (Fig. 1C, D). In multivariate analysis sphinganine (OR 0.8494, CI 0.07393 – 0.9759, P = 0.021223), SA1P (OR 0.9818, CI 0.9653 – 0.9987, P = 0.034801) and the glycosylated ceramides GluCerC18 (OR 1.0683, CI 1.0297 – 1.1104, P = 0.000786) and GluCer24:1 (OR 0.9961, CI 0.994 – 0.998, P = 0.000294) constituted independent predictors of treatment response.

Conclusion: Serum sphingolipid concentrations, in particular sphingosine and sphinganine and their phosphate derivatives S1P and SA1P as well as glucosylceramides, are able to identify at baseline the minority of HCV patients with DAA-failure. Sphingolipids may resemble additional valuable biomarkers for national treatment strategies aiming to eliminate HCV infection.

Figure: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SVR

P04-02 Hepatitis C virus dysregulates polyamine and proline metabolism and perturbs urea cycle

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Background and Aims:
Hepatitis C virus (HCV) is a wide spread etiological agent of human hepatitis. In most cases HCV infection establishes chronic liver disease which can lead to liver fibrosis, cirrhosis, and hepatocellular carcinoma. Despite tremendous progress in investigation of HCV molecular virology and development of efficient direct acting antivirals, HCV pathogenesis is not fully understood. In some cases clearance of the infection does not lead to complete reversion of virus-induced liver pathologies. Therefore, investigation of changes that the virus causes in the infected cells still presents a challenging task.

Method:
Metabolic changes were studied in Huh7.5 cells infected by JFH-1 infectious clone and in a stable Huh7.5 cell line harboring a subgenomic replicon. Gene expression was quantified by RT-qPCR, western blotting, and measurement of enzymatic activity in cell lysates. Metabolite levels were estimated by HPLC and LC-MS.

Results:
HCV infection led to upregulation of transcription of the key polyamine-metabolyzing enzymes ornithine decarboxylase (ODC), spermidine/spermine-N1-acetyl transferase (SSAT), and spermine oxidase (SMOX). However, it was accompanied by a decrease in their protein levels which might reflect their enhanced turnover. HCV also affected urea cycle enzymes, specifically by a pronounced downreguation of arginase 1 (Arg1). In contrast, the virus upregulated proline dehydrogenase (PRODH), the key enzyme of proline catabolism. These changes were accompanied by a decrease in a level of proline and increase in a level of arginine. All these changes were not transient and not attributed to a specific virus isolate, as they were detected in stable Huh7.5 cell lines harboring subgenomic replicons of genotypes1b or 2a. Finally, compounds that inhibited polyamine biosynthesis or inhibited polyamine oxidases exhibited strong antiviral activity in both HCVcc and replicon systems.

Conclusion:
These data clearly show that HCV affects the urea cycle, proline and polyamine metabolism. They indicate that the virus switches source for ornithine production from arginine to proline/glutamine. In addition, targeting polyamine metabolism with low weight inhibitors represents a novel strategy for development of anti-HCV drugs.

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P04-03 HCV course, efficacy and safety of DAA treatment in patients with inherited blood disorders

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Background and Aim: HCV infection is very common in patient with inherited blood disorders (IBD) (up to 70%[1,2]) due to frequent blood transfusion and characterized by fast progression in HIV- co-infected patients[3]. On the contrary, data on HCV mono-infection course in this population is limited [4].
Aim: to evaluate disease course, efficacy and safety of direct antiviral agents (DAA) treatment in patients with HCV and IBD.

Material and methods: 46 patients with HCV infection and hemophilia A, B or Willebrand's disease were treated with either of the two DAA regimens: Sofosbuvir in combination with Daclatasvir (N=7) or ombitasvir/paritaprevir/ritonavir and dasabuvir (OBV/PTV/r+DAS) (N=39) for 12 weeks. Fibrosis was evaluated with transient elastography. HIV co-infected patients were not included. All the patients were treatment naive.

Results: Median age of patients was 42 years. Time period from first HCV detection ranged from 1 to 31 years. Only 8% of patients had F4 fibrosis. Sustained virological response was achieved in 100% (N=7) of those who received Sofosbuvir and daclatasvir and 97.5% (N=38/39) of OBV/PTV/r+DAS group. One patient developed Y93H mutation. No serious adverse event was registered. The most significant side effect was fatigue.

Conclusion: Treatment with DAA in patients with IBD is well-tolerated and effective. Liver cirrhosis is known as pro-coagulation, however our study has shown a low occurrence of advanced fibrosis in patients with IBD. Probably, the slower fibrosis progression in patients with hemophilia correlates with the level of the coagulation factor. Further study is required to determine the role of hemostasis/coagulation in liver disease progression.

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P04-04 After the achivement of sustained virological response by direct acting antivirals, does the age influence the recovery of the hepatic reserve?

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Background and Aims: By direct acting antivirals (DAA) therapy, complete eradication of HCV became possible in most HCV related liver disease. And recovery of hepatic reserve is reported. In Japan we treat elderly patients (average 70-year-old) using DAAs. So, we examined a change of the hepatic reserve according to the age.

Method: The object is 403 hepatitis C cases (male: 164, female: 239, genotype 1:299, 2:104, median age 69 years old) who achieved SVR by DAA and followed more than 3 years. Seventy-five cases (18.6%) had history of HCC. We measured blood biochemistry serially and evaluated the hepatic reserve using albumin-bilirubin (ALBI) grade. The grade of hepatic reserve is as follows, grade 1：2：3＝ ≤ -2.60：< -2.60 to ≤ -1.39： >-1.39. Dividing 3 age groups (Group A:<70 years old n=205, GroupB:70 to 79 years old n=156, Group C:≥80 years old n=42), we analyzed the influence of background factors including patient's age, blood chemistries and computed tomography findings (portosystemic shunt) on the recovery of hepatic reserve.

Results: The distribution of ALBI grade (1/2/3) at the SOT (start of therapy) and 3 years after DAA therapy were (241/157/5) and (350/49/0). Limiting advanced fibrosis cases (FIB-4 index ≥ 3.25), the ALBI grade (1/2/3%) at SOT was 61%/39%/0% in group A, 91%/9%/0% in group B and 46%/53%/1% in group C. All age group showed the good improvement of ALBI grade and achieved down grading of grade 2 or 3 to grade 1 (A: 76%, B:79%, C:81%). And 117 of 162 cases (72%) with ALBI grade 2 or 3 showed the improvement of hepatic reserve to ALBI grade 1 after DAA therapy. Then we analyzed the factor which disturbed achievement of the ALBI grade 1 after 3 years of DDA therapy. By the logistic regression analysis, HCC occurrence after DAA therapy was the most related factor (HR:3.08, P=0.0138), and the next was male gender (HR:3.45, P=0.0143), hemoglobin<10.5g/dl (HR:4.19, P=0.0157), existence of portosystemic shunt (HR:3.07, P=0.0349) and ALT≥45U/L (HR:2.67, P=0.0425). The patient's age was not a significant factor.

Conclusion: About 70% of patient with ALBI grade 2 or 3 showed improvement of hepatic reserve (ALBI grade) after 3 years of therapy. HCC occurrence and portosystemic shunt were the negative factor of the improvement of hepatic reserve and an advanced age was not the negative factor.

P05-01YI Targeting hepatitis B virus with CRISPR/Cas9

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Background and Aims: CHB infections persists mostly due to the lack of therapies that can effectively target the stable HBV covalently closed circular DNA (cccDNA) minichromosome. We used a CRISPR/Cas9 approach to target HBV genome and studied the fate of cccDNA after CRISPR/Cas9 gene editing.

Method: We set up a ribonucleoprotein (RNP) delivery system in de novo HBV infected HepG2 cells expressing the HBV receptor NTCP. RNP complex was delivered after infection was established to ensure targeting of cccDNA. HBV extracellular and intracellular parameters after Cas9 editing were analyzed. Southern blot analysis were performed to determine the presence of cccDNA and RNA-sequencing to determine if mutated cccDNA is transcriptionally active.

Results: Screening of different HBV-specific gRNAs, single or in combinations, showed that CRISPR/Cas9 can efficiently affect HBV replication. Depending on the target in the HBV genome, CRISPR/Cas9 led to degradation or mutations in total HBV DNA, both resulting in reduced viral transcripts. Besides a mild decrease in the amount of cccDNA, potentially due to degradation, we consistently observed the appearance of a smaller cccDNA species, by PCR, southern blot and RNA-seq indicating that this specie is transcriptionally active. Our results suggest that this “small cccDNA” is the product of double stranded breaks induced by Cas9 simultaneously in both target sites followed by repair of the bigger fragment. Following suppression of HBV DNA replicative intermediate production by Nucleoside analogs (NAs) administrations, the addition of RNPs decreased cccDNA levels, suggesting that cccDNA is indeed directly targeted by the CRISPR/Cas9 complex.

Conclusion: Taken together, our results suggest that targeting HBV with CRISPR/Cas9 leads to mutation, cleavage and repair of cccDNA, and an effective synergy between NAs and CRISPR/Cas9 approaches. Notably, effects induced by Cas9 were sustainable indicating permanent changes in the HBV genome. Regardless of the truncation induced by a combination of gRNAs, the “small cccDNA” was still transcriptionally active and could potentially produce HBsAg. Further and longer-term in vivo studies would be necessary to determine efficient and irreversible gene editing.

P05-02 YI Reaching people who inject drugs most in need for HCV testing, diagnosis and linkage to care through a community-based program in Athens, Greece (ARISTOTLE HCV-HIV)

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Background and Aims: People who inject drugs (PWID) constitute a key population for hepatitis C virus (HCV) elimination. PWID who are current injectors, in particular, have a high risk of becoming infected and of transmitting HCV in settings with inadequate coverage of harm reduction programs. In addition, they face barriers concerning testing, diagnosis and linkage to HCV care. A community-based program was set-up in Athens, Greece, with the aim to reach rapidly PWID most in need and to increase diagnosis and treatment for HCV infection (ARISTOTLE HCV-HIV program). Here, we present the efficacy of the program in reaching the target population and in identifying patients in need of diagnosis and treatment.

Method: ARISTOTLE HCV-HIV is a “seek-test-treat” community-based program where PWID are recruited using chain-referral sampling with monetary incentives (Respondent-driven sampling). Participation includes interviewing, blood testing (anti-HCV/HBsAg/anti-HIV, HCV genotype, biochemical evaluation), evaluation of liver fibrosis using transient elastography and counseling. All services are provided on site. Linkage to HCV care is provided through a network of collaborating hepatologists and infectious diseases specialists set up for the program.

Results: In a period of 9 months (April 2018-January 2019), 1,326 PWID accessed the program; the first 1,000 participants were recruited in a period of 4 months. Of those, 78.1% were current PWID (injecting drug use in the past 30 days). Based on the official estimate of the population size of current PWID in Athens, the coverage of the program was 95.0%. The majority of participants (78.0%) were not linked to opioid substitution treatment (OST) programs, 27.0% were homeless and 15.5% reported country of origin other than Greece. Anti-HCV prevalence was 77.4% with 15.9% of PWID being HCV/HIV coinfected. At baseline, 68.6% of anti-HCV(+) PWID reported being already diagnosed but only 11.2% and 3.0% reported previous treatment with interferon and direct-acting antiviral agents (DAAs), respectively.

Conclusion: ARISTOTLE HCV-HIV succeeded in reaching rapidly a large number of PWID. The population was particularly at risk (high HCV prevalence, current injectors, homelessness, low coverage of OST) and previous treatment rates with DAAs were very low. Similar community-based programs for

HCV infection among PWID need to be performed and complement screening and linkage to care efforts for in the setting of OST programs.

P05-03YI Homocysteine mediated oxidative stress is detrimental for pregnancy complications and outcome in Hepatitis E virus infected cases: A northeast Indian patients based study

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Background and Aims: Given the background of (i) association of hepatitis viruses and homocysteine levels in liver disease pathogenesis and response to treatment and (ii) Hepatitis E virus (HEV) replication in placenta; we aimed to evaluate the significance of changes in maternal homocysteine levels and the related mechanism(s) in the pathophysiology of HEV related pregnancy complications and negative outcome.

Method: Enrolled pregnancy cases **[**Term delivery (TD,n=194) and HEV RNA positive cases with all clinical details (n=76,AVH=63, FHF=13)] were evaluated for changes in serum and placental homocysteine levels; and was inturn correlated with VitaminB12 levels, viral load, and its association with key folate pathway parameters using molecular tools.

Results: Significantly increased serum homocysteine levels were found in: (i) both HEV AVH ($p=0.023$) and FHF cases ($p<0.001$) compared to TD (ii) in FHF cases compared to AVH cases ($p=0.015$), (iii) was associated with both preterm delivery and fetal death in HEV cases, and maternal death in FHF-HEV infected preterm delivery, and (iv) correlated with high HEV viral load. Placental homocysteine expression was upregulated in HEV cases, and was associated with negative pregnancy outcome. Homocysteine levels significantly inversely correlated with serum VitaminB12 levels in HEV cases. Presence of variant *MTHFRC667T* and *TYMS1494del6 del/del* genotype was associated with (i) increased serum homocysteine levels (ii) increased risk of FHF development and preterm delivery (iv) negative pregnancy outcome in HEV cases. Placental folate transporter FR-alpha expression was downregulated in (i) HEV cases compared to TD, (ii) FHF and AVH fetal death cases.

Conclusion: Our novel data suggests that HEV inflicted increased homocysteine levels in a background of altered VitaminB12 and folate pathway parameters is critically linked to increased risk of pregnancy related complications and negative outcome in pregnancy cases with underlying HEV infection; and may be utilized as a suitable prognostic marker and targeting for disease management.

P05-04 Potential usefulness of chicken egg yolk immunoglobulins for immunotherapy of hepatitis b

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Background and Aims: Treatment of hepatitis B virus (HBV) infection employs nucleos(t)ides analogues, but they often require long-lasting administration to avoid the risk of HBV reactivation at withdrawal. Therefore, it is important to develop novel treatments to shorten the duration of NUC therapy, and to complement the effect of available anti-viral therapies. Chicken egg yolk immunoglobulins (IgY) antibody could be an alternative due to their feasibility for large-scale commercial production and the relative noninvasive methods used for their preparation. This study aims to characterize IgYs specific as immunotherapy to HBV infection.

Method: Three groups of hens were immunized with hepatitis B vaccine with and without adjuvant CPG-ODN and a negative control group were also included. Eggs were collected for 21 weeks and IgY was extracted using polyethylene glycol precipitation and purified using affinity chromatography. IgY anti-HBs characterization was performed using polyacrylamide gel electrophoresis (SDS-PAGE), dot blot and enzyme-linked immunosorbent assay (ELISA). Total protein (TP) concentration was measured using spectrophotometer. Samples presenting higher TP concentration were evaluated as capture protein for in house ELISA to detect HBsAg.

Results: The characterization of IgY revealed bands of stained peptides with molecular weight between 75 and 50kDa and 37 and 25kDa using SDS Page. It was observed antigen-antibody reaction throughout the sample period as measured by dot blot. Mean ± standard deviation (SD) of concentration of TP obtained after purification by affinity chromatography was 0.386 mg / mL ± 0.440 mg/mL. After evaluation of different dilutions of IgY as capture protein for ELISA, it was possible to distinguish positive from negative controls that presented mean ±SD of optical density (OD) values of 1.99 ± 0.18 and 0.45 ± 0.06, respectively.

Conclusion: These results suggested that egg yolk could be a practical strategy in large-scale production of specific anti-HBV IgY for immunotherapy of HBV.

P05-05YI Molecular analysis of HCV subtype 1a dispersal patterns among inmates in Greece: HCV transmissions are not related to incarceration

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Background and Aims: The most common mode of hepatitis C virus (HCV) infection in Greece is through injection drug use. Although HCV infection is more prevalent among inmates, to date it remains largely unknown whether incarceration contributes to the spread of HCV. Our aim was to investigate the HCV transmission patters among inmates with prior intravenous injection history in Athens, Greece, using molecular epidemiology methods.

Method: The study population was consisted of 121 anti-HCV(+) inmates at Korydallos Prison Hospital "Agios Pavlos" and Detention Center of Korydallos, who participated in a "test, treat, and retain" program for viral hepatitis, HIV and tuberculosis conducted between 10/2017 and 03/2018. HCV genotyping was performed in 121 samples in partial NS5B region, by using the automated HCV Typing Tool. We analysed phylogenetically the subtype 1a (N=40) sequences along with i) the most closely related globally sampled subtype 1a sequences to our study population, using the HIV BLAST tool (N=193), and ii) subtype 1a sequences isolated from people who inject drugs (PWID) in Athens during 2012-2013, used as references. Phylogenetic trees were estimated by the maximum likelihood method as implemented in FastTree v2.1 (GTR+cat model). In order to verify our results, we performed further analysis based on the Neighbor Joining method as implemented in PAUP*4.0 (GTR+G model). Local transmission networks (LTNs) were phylogenetic clusters including at least 2 sequences from PWID, receiving bootstrap values > 75% and SH-support > 0.9.

Results: Genotyping analysis revealed that 3a (N=51; 42.1%) and 1a (N=42; 34.7%) were the most prevalent subtypes in our study population. In addition, genotype 4 and subtype 1b were detected at low prevalence equal to 13.2% (N=16) and 9.9% (N=12), respectively. Phylogenetic analysis showed that the subtype 1a inmates' sequences clustered across the tree, indicating multiple introductions. Specifically, we found 7 LTNs consisting of 20 inmates' sequences in total. The largest one included 7 sequences. Analysis also revealed that all the LTNs included sequences from both inmate and non-inmate PWID (Figure). Within the largest LTN only a single sequence from a non-inmate PWID was detected. Furthermore, two genetically identical sequences were identified within the largest LTN.

Conclusion: The low number of HCV subtype 1a sequences from inmates identified with LTNs indicates that inmates were not infected by a common source. The existence of sequences from both inmate and non-inmate PWID within the LTNs indicates that transmissions do not occur exclusively among inmates. Only for 2 inmates there is strong evidence for a common source of infection. In conclusion, the HCV dispersal patterns were similar among inmate and non-inmate PWID in Greece, and therefore HCV transmissions are likely to be related to sharing practices and not to incarceration.