filopodium and lamellipodium formation. Traditionally, actin cytoskeleton remodeling is downstream of EMT reprogramming. It is, therefore, intriguing to ask why and how KLHL23, an actin modulator, inversely regulates EMT. Activation of actin cytoskeleton remodeling by either *KLHL23* silencing or treatment with actin cytoskeleton modulators augmented cellular hypoxic responses in a cell density-dependent manner resulting in HIF and Notch signals and subsequent EMT. Environmental hypoxia did not induce EMT unless actin cytoskeleton remodeling was simultaneously activated only were cells at high density. The EMT thus induced was reversed by either adenosine 5′-triphosphate supplementation or actin polymerization inhibitors. Correlations of tumor size with EMT and inverse association of the expression of KLHL23 with HIF-/Notch-signals were further validated in patient-derived xenograft HCCs in mice.
Conclusion: Simultaneously activation of actin cytoskeleton remodeling by intrinsic (such as *KLHL23* downregulation) or microenvironment cues is crucial for cell density-dependent, hypoxia-mediated EMT, providing a mechanistic link between large tumor size and invasion/metastasis. Our findings open a new door to development of prevention and treatment strategies for tumor invasion and metastasis.

SAT-159

The HDAC inhibitor belinostat enhances the anti-tumor efficacy of immune checkpoint inhibitors in a murine hepatocellular carcinoma model

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Background and Aims: Immune checkpoint inhibitors are currently being tested in different combinations in patients with advanced hepatocellular carcinoma (HCC). Nivolumab, an anti-PD-1 agent, has been approved in the US in the second line setting after Sorafenib. Epigenetic drugs have immune-mediated antitumor effects that may improve the activity of immunotherapy agents. Our aim was to study the therapeutic efficacy of checkpoint inhibitors (anti-CTLA-4 and anti-PD-1 antibodies) in combination with the histone deacetylase inhibitor Belinostat.
Method: Therapeutic efficacy and studies on antitumor immunity were performed in a subcutaneous Hepa129 murine hepatocellular carcinoma model that similar to human tumors have an infiltrate rich in CTLA-4+ regulatory T lymphocytes and effector PD-1+ T lymphocytes. Animals treated with Belinostat, an anti-PD-1 monoclonal antibody and an anti-CTLA-4 monoclonal antibody as monotherapy or in combinations as well as control animals receiving a non-immunostimulatory antibody were followed to monitor tumor growth and survival, or sacrificed at different days after treatment for obtaining tumor, spleen and blood samples for analysis.
Results: Belinostat improved the anti-tumor activity of anti-CTLA-4 but not the activity of anti-PD-1 therapy. This effect correlated with enhanced IFN-gamma production by antitumor T-cells and a decrease in regulatory T-cells. Moreover, the combination induced early upregulation of PD-L1 on tumor myeloid cells and late expression of PD-1 on tumor-infiltrating effector T-cells, suggesting the convenience of PD-1 blockade. Indeed, Belinostat combined with the simultaneous blockade of CTLA-4 and PD-1 led to complete tumor rejection in treated animals.
Conclusion: These results provide a rationale for testing Belinostat in combination with checkpoint inhibitors in order to enhance their therapeutic activity in patients with HCC.

SAT-160

Blocking the CDK1/PDK1/B-Catenin signaling by CDK1 inhibitor RO3306 increased the efficacy of sorafenib treatment with targeting cancer stem cell in preclinical model of hepatocellular carcinoma

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Background and Aims: Cyclin-dependent kinase 1 (CDK1) is the key regulator in promoting cell division whose role can't be substituted by any other CDKs. Sorafenib the only approved target therapy for advanced HCC treatment provides limited survival benefits till today. Here we aim to determine the efficacy of CDK1 inhibitor RO3306 alone or combination with sorafenib in the preclinical tumor models of hepatocellular carcinoma.
Method: The clinicopathological parameters CDK1 expression with HCC overall survival and CDK1 related PDK1 with disease-free survival were analyzed. Three HCC patients-derived xenograft tumor models were treated with RO3306 (4 mg/kg), sorafenib (30 mg/kg) alone or combination for one month. The relevant signaling CDK1/PDK1/β-Catenin was measured by western blot. The colony formation, single cell sphere formation and tumorigenicity assay were used for liver cancer stem cells (CSCs). Silencing CDK1 with shRNA and corresponding inhibitors for mechanism and related functional studies.
Results: We reported that CDK1 was frequently augment accounted up to 50% (21/42) of hepatocellular carcinoma (HCC) tissues, which was significantly associated with poor overall survival ($p = 0.008$). Moreover, the CDK1 and PDK1 association shows worse disease-free survival ($p = 0.03$). CDK1 inhibitor RO3306 in combination with sorafenib treatment significantly decreased tumor growth in patients-derived xenograft (PDX) tumor models. Western blot results demonstrated that combined administration resulted in synergistic decreased the pluripotency protein levels of Oct4, Sox2 and Nanog as well as concurrently down-regulating CDK1, PDK1 and β-Catenin. Furthermore, downregulation CDK1/PDK1/ β-Catenin associated with suppressing the process of epithelial mesenchymal transition (EMT) revealed with the downregulation of Snail1 and Snail2 while upregulation of E-Cadherin, which may show synergistic antimetastasis potential in combination treatment. Based on the evidences, we further verified that pharmaceutical combinatorial inhibition dramatically decreased CSCs single cell sphere formation and colony formation. Synergistic down-regulated AKT and pStat3 activation with enhancing suppression on tumorigenicity in vivo. In addition, low dose of RO3306 (2 uM) and sorafenib (2.5 uM) combination could inhibit CSCs growth via decreasing the S phase and promoting cell to enter into a pseudo-G1 phase with 4N DNA. Mechanism with functional study of silencing CDK1 with shRNA and RO3306 combined sorafenib both abolished its oncogenic function via the down-regulation of CDK1, with the downstream PDK1 and β-Catenin inactivation.
Conclusion: Anti-CDK1 treatment can boost sorafenib antitumor responses in PDX tumor models, providing the rational combined treatment to increase sorafenib efficacy in clinical.

SAT-161

Obeticholic acid, a FXR agonist, inhibits the cancerogenic potential of primary human cholangiocarcinoma (CCA) cells cultures

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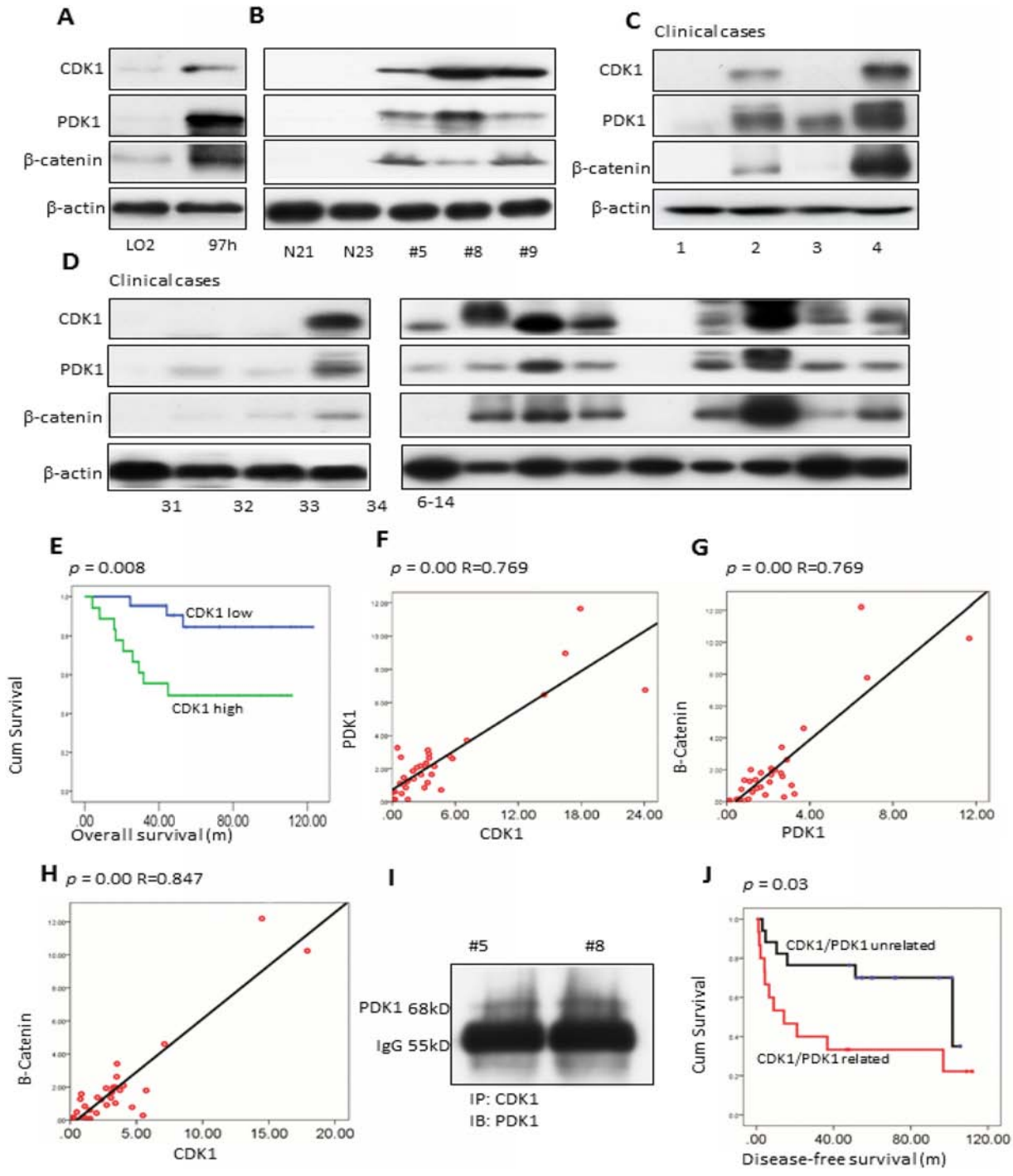

Figure: (abstract SAT-160)

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Background and Aims: Cholangiocarcinoma is an aggressive cancer, resistant to chemotherapeutics. We demonstrated that CCA is enriched of cancer stem cells associated with aggressiveness and drug resistance. FXR, involved in neoplastic transformation of stem cells and/or cholangiocytes, is down-regulated in human CCA. Our AIM was to evaluate, in primary cultures of human intrahepatic CCA (iCCA) the effects of the FXR agonist, obeticholic acid (OCA), on the cancerogenic potential of human CCA cells.

Method: Primary human cell cultures were prepared from specimens of iCCA obtained from patients submitted to surgical resection and classified into mucin- or mixed-iCCA subtypes by morphologic and immunohistochemical criteria. Increasing concentrations (0–5 μM) of OCA were added to culture media and, after 3–10 days, the effect on

proliferation (MTS assay, cell population doubling time), apoptosis (annexin V-FITC / propidium iodide), cell migration and invasion (wound healing and matrigel invasion assay) and cancerogenic potential (spheroid formation, clonogenic assay, colony formation capacity) were evaluated.
Results: FXR was downregulated (RT-qPCR) in iCCA cells vs normal human biliary tree stem cells ($p < 0.001$) and in mucin-iCCA vs mixed-iCCA ($p < 0.05$). OCA significantly ($p < 0.05$) inhibited proliferation of both mucin-iCCA and mixed-iCCA cells starting at a concentration as low as 0.05 μM (IC_{50} = 0.38 μM in mixed- and 2.1 μM in mucin-iCCA). Also CDCA (but not UDCA) inhibited cell proliferation, although to a much lower extent than OCA, consistent with the different potency in FXR activation (i.e. OCA > CDCA, no agonistic effect for UDCA). OCA significantly induced apoptosis of both iCCA subtypes and decreased the *in vitro* cancerogenic potential of iCCA cells as evaluated by impairment of colony and spheroid formation capacity and delayed wound healing and matrigel invasion. In general, these effects were more evident against mixed- than mucin-iCCA cell. When tested together with gemcitabine and cisplatin, OCA potentiated the anti-proliferative and pro-apoptotic effects of these chemotherapeutics but mainly on mixed-iCCA. OCA abolished the capacity of both mucin- and mixed-iCCA cells to form colonies when administered together with gemcitabine and cisplatin.

SAT-162
Sensitization of cholangiocarcinoma to chemotherapy by SOX17-induced down-regulation of drug export pumps ABCC3 and AGCG2

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Background and Aims: An important limitation for favorable cholangiocarcinoma (CCA) outcome is its poor response to chemotherapy. Among mechanisms of chemoresistance (MOC) involved, a reduced intracellular concentration of anticancer drugs due to active drug export through ATP-binding cassette (ABC) proteins, such as ABCG2 and ABCC3, plays an important role. SOX17, a transcription factor that inhibits Wnt/β-catenin pathway, has been reported to be downregulated in CCA. Restoration of SOX17 expression in CCA cells results in tumor suppression. Here we have investigated whether viral vector-mediated SOX17 over-expression may be also beneficial by sensitizing CCA to chemotherapy.
Method: Viral vectors containing SOX17 ORF were generated to transduce CCA cells (EGI-1 and TFK-1). Cell viability in response to incubation with commonly used anti-CCA drugs was determined by MTT test. Taqman Low Density Arrays were designed to measure mRNA abundance of ≈100 genes involved in several MOCs. Single RT-qPCR, WB and IF were used to evaluate gene/protein expression. ABC pumps activity of was determined by flow cytometry using specific inhibitors and fluorescent substrates. Firefly luciferase (Luc2) was fused to ABC promoters to carry out promoter-reporter assays. Mouse xenograft model was used for *in vivo* evaluation of chemotherapy efficacy.
Results: SOX17 overexpression selectively enhanced the cytostatic response of CCA cells to SN-38, 5-FU and mitoxantrone, but not to gemcitabine or cisplatin. The magnitude of chemosensitization was dependent on SOX17 expression levels. The analysis of changes in MOC gene expression profile revealed that SOX17 overexpression affected several of these genes, mainly those encoding ABC proteins. Thus, a significant reduction in *ABCC3/ABCG2* expression was found. Interference with ABC gene promoter activity seems to be involved in the mechanism of SOX17-induced *ABCC3/ABCG2* downregulation. Functional studies supported a reduced ability of SOX17-overexpressing CCA cells to export specific substrates of these pumps. Moreover, combined ABCG2/ABCC3 substrate specificity matched the observed selective chemosensitization. SOX17-induced better response to ABCG2/ABCC3 substrates was confirmed by *in vivo* experiments.
Conclusion: In addition to the tumor suppression effect of SOX17, its over-expression induces selective chemosensitization due to down-regulation of some ABC proteins, which reduces the ability of CCA cells to export anticancer drugs.

SAT-163
Defective NKp30-mediated function in hepatocellular carcinoma-infiltrating NK cells

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Background and Aims: Natural killer (NK) cells play a significant role in innate immune responses to cancer cells, their action being regulated by several activating and inhibitory receptors. The natural cytotoxicity receptor NKp30/NCR is critical for NK cell function. The NCR3 gene is transcribed into three major isoforms that have different biological functions. The expression levels of NKp30 isoforms can negatively impact on the prognosis and evolution of different malignancies and might be associated with advanced liver disease in HCV-infected patients. Current evidence indicates that malignant cells bypass the NK surveillance by releasing the NKp30 ligand B7H6 as soluble proteins that block NKp30 activity, suggesting that this may be an immune escape mechanism of tumor cells from NK cell-mediated killing.
We investigated the NKp30-B7H6 axis in patients with hepatocellular carcinoma (HCC).
Method: *Ex-vivo* isolated PBMC, non-tumor liver-infiltrating lymphocytes (LIL) and tumor-infiltrating lymphocytes (TIL) were examined by flow cytometry. Serum concentration of soluble B7-H6 was measured by ELISA. Expression of the three major NKp30 isoforms was analyzed by quantitative real-time PCR in PBMC, LIL and matched TIL of HCC patients and healthy controls (HC).
Results: The frequency of NKp30-expressing peripheral NK cells and the intensity of its expression were significantly lower in patients with HCC compared to HC. In contrast, NKp30-expressing NK cells were enriched in the neoplastic tissue of HCC patients compared to the non-tumorous surrounding liver. Peripheral and tumor-infiltrating NKp30-expressing NK cells showed defective degranulation capacity and cytokine release after NKp30 triggering, and had altered expression of NKp30 splice variants. Serum levels of the soluble form of B7H6 ligand were increased in HCC patients, particularly those with intermediate and advanced tumors, classified according to Barcelona Clinic for Liver Cancer (BCLC) staging classification, as compared to early stage tumors. Moreover, statistically significant positive correlations were found between sB7H6 levels and HCC nodule size and serum alpha fetoprotein values.