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ABSTRACT
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WORKSHOP

TUMOR IMMUNOLOGY

HINDERING TRIPLE NEGATIVE BREAST CANCER PROGRESSION BY TARGETING ENDOGENOUS INTERLEUKIN-30 REQUIRES IFNΓ SIGNALING

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PURPOSE: Triple-Negative (TN) Breast Cancer (BC) is one of the most aggressive malignancy. IL30 expression by tumor and infiltrating immune cells is frequent in TNBC and has been associated with worse prognosis (1). Here, we investigate the consequences of targeting host-derived IL30 on TNBC behavior and identify the underlying molecular events.

METHODS: The METABRIC dataset was used to analyze the expression of IL30 in BC and its distribution by molecular subtype. After testing their response to IL30 treatment, TNBC cells were implanted in syngeneic hosts lacking endogenous IL30 (*IL30KO*) and survival curves were plotted by Kaplan-Meier method.

RESULTS: Microarray data from 1699 BC cases, established a positive correlation of IL30 expression with TNBC. In TNBC cells endowed with IL30 receptor, IL30 boosted proliferation, migration and a broad tumor progression and immune evasion program. When implanted into *IL30KO* hosts, both IL30-responsive and -unresponsive TNBCs gave rise to poorly vascularized and slow growing tumors with low metastatic potential, which led to increased survival. Intratumoral influx of CD3+CD8+ and CD3+CD4+T lymphocytes, NKp46+cells, and their IFNγ production, were the hallmarks of tumor growth inhibition in *IL30KO* mice. Knocking-out *IFN*γ gene or blocking IFNγ pathway with neutralizing antibodies in *IL30KO* host restored tumor vascularization, abolished intratumoral T-cell recruitment and the anti-tumor efficacy due to the lack of endogenous IL30.

DISCUSSION: IFNγ is functional to the antitumor effect of targeting endogenous IL30. The ability of IL30 to affect the host environment can circumvent its ineffectiveness on cancer cells and be fundamental to tumor behavior.

CONCLUSIONS: This study consolidates our recent findings (2) and provides the proof of concept that IL30 is a valuable target to improve immunotherapy and life expectancy in TNBC patients.

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NEUTROPHILS CONTROLLING THE INTESTINAL MICROBIOTA PROVIDE PROTECTION AGAINST COLITIS AND COLITIS-ASSOCIATED COLORECTAL CANCER

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PURPOSE: Recent evidence highlighted the complexity and heterogeneity of neutrophils in cancer (1). However, their role in inflammatory bowel disease (IBD) and colitis-associated CRC (CAC) is poorly understood and controversial (2-3). Therefore, we aimed to dissect the role of neutrophils in a model of intestinal inflammation and CAC.

METHODS: We used a genetic model of neutrophil deficiency (*Csf3r-/-*) and neutrophil adoptive cells transfer (ACT) to dissect the role of neutrophils during intestinal inflammation and CAC development. *In silico* analysis on publicly available dataset of IBD patients were performed.

RESULTS: *Csf3r-/-* mice displayed increased susceptibility to colitis and CAC development compared to *Csf3r+/+* control mice. Neutrophil ACT was sufficient to reduce the severity of colitis in *Csf3r-/-* mice. 16S sequencing and metagenomic analysis showed a significant difference in the beta-diversity of faecal microbiota between *Csf3+/+* and *Csf3r-/-* mice. Broad-spectrum antibiotics administration into *Csf3r-/-* mice was sufficient to rescue the susceptibility to colitis and CAC to the level of *Csf3r+/+* controls. C*sf3r-/-* mice displayed reduced number of repairing ulcers, coupled with reduced tissue levels of IL-22 and pSTAT3. In IBD patients, *CSF3R* and *IL-22* expression were positively correlated, and *CSF3R* high patients displayed the enrichment of epithelial repair gene signatures.

DISCUSSION: Our data showed that neutrophils play an essential role in controlling the composition of the intestinal microbiota and in the activation of an IL-22-dependent intestinal epithelial repair pathway.

CONCLUSIONS: Therefore, our data suggest that neutrophils provide protection against intestinal inflammation and CAC development.

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INTRA-TUMOR COMBINATION THERAPY WITH POLY(I:C) AND RESIQUIMOD SYNERGISTICALLY TRIGGERS TUMOR-ASSOCIATED MACROPHAGES FOR EFFECTIVE ANTI-TUMORAL IMMUNITY

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PURPOSE: In cancers, tumor-associated macrophages (TAMs) play a key immunosuppressive role that limits antitumoral activity of the immune system and hinder the efficacy of most treatments. The antitumoral immune response triggered by the TLR agonists poly(I:C), imiquimod (R837) or resiquimod (R848) has been previously evaluated as monotherapies; however, their combination for the reprogramming of TAMs and the treatment of cancer has not been explored yet.

METHODS: TLR agonist treatments were evaluated *in vitro* for toxicity and immunostimulatory activity, using primary human and murine M-CSF-differentiated macrophages. For *in vivo* experiments, CMT167 lung cancer model and MN/MCA1 fibrosarcoma model metastasizing to lungs were used; tumor-infiltrating leukocytes were evaluated by flow cytometry, multispectral immunophenotyping and proteomic analysis.

RESULTS: Results demonstrate the higher efficacy of poly(I:C) combined with R848 versus single treatments or combined with R837, to polarize macrophages towards M1-like antitumor effectors *in vitro*. *In vivo*, the intratumoral synergistic combination of poly(I:C)+R848 significantly prevented tumor growth and metastasis in lung cancer and fibrosarcoma immunocompetent murine models. Regressing tumors showed increased infiltration of macrophages, CD4+ and CD8+ T cells, accompanied by a reduction of immunosuppressive CD206+ TAMs and FOXP3+/CD4+ T cells. Treated mice acquired resistance to tumor re-challenge. Proteomic experiments validate the superior activation of innate immunity by poly(I:C)+R848 combination versus other treatments; and protein-protein-interaction network analysis reveal the key activation of the STAT1 pathway.

CONCLUSIONS: These findings demonstrate the antitumor immune responses mediated by macrophage activation upon local administration of poly(I:C)+R848 combination, and support the low dose intratumoral application of this therapy to patients with solid tumors in the clinic.

IL-1R8 ACTS AS AN IMMUNE CHECKPOINT IN CD8+ T CELLS

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PURPOSE: IL-1R8 is a member of the Interleukin-1 receptor (ILR) family, acting as a negative regulator of ILR and Toll-like receptor pathways. IL-1R8 deficiency was shown to enhance NK cell effector functions restraining tumor metastasis and viral dissemination. IL-1R8 is thus considered a new immune checkpoint. Since NK and CD8+ T cells share most of the cytokine-related mechanisms responsible for their anti-tumor activity, we characterized IL-1R8 in T lymphocytes.

METHODS: Taking advantages of tumor-transplantable models and tumor samples, we characterized expression and function of IL-1R8 in T lymphocytes by FACS analysis and Single-cell RNA-sequencing. In addition, we evaluated CD8+ T cell activation *in vitro*.

RESULTS: Transcriptomic profiles indicated that IL-1R8 was upregulated during T cell maturation and it was associated with the acquisition of effector markers in physiological and pathological contexts. We demonstrated that IL-1R8 deficiency promoted CD8+ T cell-mediated protection against epithelial and mesenchymal tumor models. IL-1R8-deficient mice further benefited from immune checkpoint blockade. Moreover, depletion of CD8+ T cells abolished the anti-tumor resistance of IL-1R8-deficient mice. Finally, we observed enhanced effector functions in IL-1R8-deficient mouse T lymphocytes and in IL-1R8-silenced human CD8+ T cells generated by CRISPR-Cas9.

DISCUSSION: Here we show that IL-1R8 acts as a checkpoint for mouse and human T cells and it is regulated during the transition from naïve to mature T cells. We further demonstrate that IL-1R8 influences T cell maturation \proliferation and controls T cell effector functions, such as Interferon gamma and Granzyme B production, through an unrevealed cell-autonomous mechanism.

CONCLUSIONS: Therefore, our data indicate that IL-1R8 plays a profound impact on CD8+ T cell anti-tumor potential, thus suggesting that IL-1R8 genetic targeting represents a tool for improving the activity of lymphoid cells in cancer immunotherapy.

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IMMUNOMODULATORY EFFECT OF NEDD8-ACTIVATING ENZYME INHIBITION IN MULTIPLE MYELOMA: SENSITIZATION TO NATURAL KILLER CELL RECOGNITION AND KILLING

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PURPOSE: Neddylation is a post-translational modification that adds a ubiquitin-like protein, NEDD8, to selected substrate proteins, affecting their stability, function and subcellular localization. This regulates biological processes such as cancer progression and immune response. Neddylation is needed for the activation of the Cullin Ring Ligases (CRLs), the largest family of E3 ubiquitin ligases in eukaryotes. Multiple Myeloma (MM) is an incurable hematological cancer of terminally differentiated plasma cells (PCs) characterized by an immunosuppressive bone marrow microenvironment where, among others, NK cell functionality is impaired. MLN4924 (MLN) is an inhibitor of the NEDD8 activating enzyme (NAE). It has showed activity in clinical trials in hematological malignancies, including MM. Because NAE is the only NEDD8 activating enzyme, its inhibition results in the stabilization of CRL substrates and triggers multiple cellular responses responsible for the anti-cancer activity of MLN. Our study is focused on whether inhibition of neddylation can regulate the NK cell activating receptor NKG2D ligands (NKG2DLs) expression and sensitize MM cells to NK cell killing.

METHODS: Flow cytometry, RT-qPCR, luciferase reporter assay, western blot, confocal microscopy.

RESULTS and DISCUSSION: We found that MLN upregulates the NKG2DLs MICA and MICB on plasma membrane in different MM cell lines and patient-derived PCs. This is correlated to the enhanced degranulation observed for NK cells co-cultured with MLN-stimulated MM cells. MICA is upregulated also at mRNA level and this is the result of an increased transcription. After MLN treatment the TFs IKZF3 and IRF4, essential for MM survival and transcriptional repressors of the MICA gene, are downregulated at mRNA and protein level. They are both regulated by NF-κB, whose translocation in the nucleus is reduced by MLN. An explanation is the stabilization of IκBα, a CRL substrate. For MICB we observed an increased redistribution on the plasma membrane. Future experiments will be directed to characterize the mechanisms through which this occurs.

CONCLUSIONS: MLN sensitizes MM to NK cell recognition through the modulation of the NKG2DLs MICA and MICB.

WORKSHOP

COVID19

FIRST DOSE MRNA VACCINATION IS SUFFICIENT TO REACTIVATE IMMUNOLOGICAL MEMORY TO SARS-COV-2 IN EX COVID-19 SUBJECTS

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PURPOSE: Aim of this study is to characterize the early kinetics of the immune response to the Spike protein of SARS-CoV-2 following mRNA vaccination in healthy individuals and in ex COVID-19 patients.

METHODS: Vaccine was administered in two doses on day 0 and day 21. Serum and PBMNC were collected before and after 7, 14, 21, 28 days from the first injection. Spike-specific antibodies were evaluated by using commercially available kits. Spike-specific B and T cells were characterized by flow cytometry.

RESULTS: We enrolled 11 healthy individuals and 11 ex COVID-19 patients. Preliminary data showed that in ex COVID-19 subjects the frequencies of circulating, Spike-specific, T and B cells rapidly increased following the administration of the first dose of vaccine. Indeed, B cells peaked after 14 days from vaccination and remained stable, with no further increase after the second dose. T cells instead peaked one week after the first injection and then declined with no further increase after the second dose. Regarding humoral immunity in ex COVID-19 subjects, we observed that anti-Spike IgG, anti-RBD IgG and neutralizing antibodies peaked one week after the first dose and remained stable even after the second injection. On the contrary, healthy individuals showed a different kinetic of Spike-specific immune response following vaccination. Specific T and B cells and antibodies increased progressively after the first injection, and further raised after recall injection.

DISCUSSION: The data obtained show that one vaccine dose is sufficient to increase both cellular and humoral immune response in ex COVID-19 subjects without any additional improvement after the second dose. On the contrary, the second dose is mandatory in naïve individuals.

CONCLUSIONS: These results question whether a second vaccine shot in ex COVID-19 subjects is required, and indicate that millions of vaccine doses may be redirected to naïve persons, thus shortening the time to herd immunity.

QUANTITATIVE AND QUALITATIVE ALTERATIONS OF CIRCULATING MYELOID CELLS AND PLASMACYTOID DC IN SARS-COV-2 INFECTION

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PURPOSE: Since there is paucity of data on the features of myeloid cells involved in SARS-CoV-2 infection, we decided to evaluate them in the blood of patients with different severities of COVID-19.

METHODS: Fourty patients diagnosed with COVID-19 were categorised into three groups – patients admitted to an emergency department (ED), to an intensive care unit (ICU) and not admitted to an intensive care unit (non-ICU). Eight healthy donors were also included in the study. Peripheral blood samples from SARS-CoV-2 patients and healthy donors were analysed by standard clinical flow-cytometric assays. In particular, we collected absolute numbers and characterized DCs, monocytes and neutrophils subpopulations and their subset distribution, as well as activation status profile of these cells. Moreover, we evaluated the correlation of these changes with disease progression.

RESULTS: COVID-19 patients showed a significantly decrease in the absolute number of plasmacytoid and myeloid dendritic cells, different subsets distribution of monocytes and different activation patterns of both monocytes and neutrophils, coupled to a significant reduction of HLA-DR monocyte levels. We found that some of these alterations are typical of all COVID-19 patients, while some others vary at different stages of disease and correlate to biochemical parameters of inflammation.

DISCUSSION: Results suggest that myeloid immune cells are severely affected by SARS-CoV-2 infections.

CONCLUSIONS: Collectively, these data suggests that inflammation has a crucial role in the impaired immune response seen in patients with COVID-19. It will be important to further understand how the alterations in levels of myeloid cells and their activation impacts severity of disease.

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CHARACTERIZATION OF CIRCULATING DENDRITIC CELLS IN COVID-19 PATIENTS

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PURPOSE: Myeloid Dendritic Cells (DCs) present in the blood have been classified in cDC1 and cDC2 subsets, based on the expression of specific molecules. Recently, cDC2 has been further separated into two subpopulations namely DC2 and DC3. DCs respond early to a viral infection to activate adaptive immunity, to control viral replication and to reduce virus spread from the peripheral site. COVID-19 patients with severe symptoms show immune dysregulation and alterations of lymphoid and myeloid populations in the peripheral blood. However, the DCs response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still undefined.

METHODS: We performed flow cytometric and scRNAseq analyses of DCs obtained from blood of severe and mild COVID-19 patients, according to WHO classification. Patients were enrolled from the STORM cohort of San Gerardo Hospital in Monza. We performed in vitro assays to verify the ability of cDC2 extracted from healthy donors (HDs) to produce inflammatory cytokines after stimulation with serum from COVID-19 severe patients.

RESULTS: We verified the frequency of the DCs subsets in the blood of COVID19 patients. We found a decrease in the frequency of cDC1, DC2 and DC3 in COVID-19 patients, while we observed an increase in the presence of the inflammatory DC3, recently identified as CD14+ and CD163+. The scRNAseq analysis revealed an enhanced expression of Interferon-stimulated genes (ISGs) in COVID-19 versus HD DCs, while no upregulation of the expression of inflammatory cytokines has been observed. Overall, DC3 and DC2 responses to SARS-CoV-2 infection did not revealed substantial differences. Moreover, we found that serum from severe COVID-19 patients did not induce any cytokines production in cDC2 extracted form HD. Among the molecules involved in DC inhibition, Neuropillin-1 has been shown to decrease DC inflammatory response to LPS. We found that the inhibition of Neuropillin-1 restores that capability of DCs to produce inflammatory cytokines after stimulation with serum from severe covid19 patients.

DISCUSSION: Our results demonstrated that Sarscov2 infection induce a functional impairment of all DC subsets present in the blood. Moreover we showed that Neuropillin-1 is involved in the inhibition of DC functions in response to SARS-CoV-2 infection.

DYNAMICS OF γδ T CELLS DURING SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 INFECTION

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PURPOSE: Considering the protective role of γδ T cells in the respiratory infections1, our purpose is to analyze frequency, phenotype and effector functions of γδ T cells during Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection in hospitalized patients compared to healed subjects.

METHODS: Peripheral blood were obtained from hospital-treated patients with coronavirus disease 2019 (COVID-19) at ARNAS Civico Hospital, healed patients enrolled for hyperimmune plasma apheresis and healthy subjects as control. We characterized γδ T cells in order to evaluate exhaustion markers, phenotype, and proinflammatory cytokines production by flow cytometry following methods published in Ref. 2.

RESULTS: Compared to healthy subjects we observed a decreased percentage of total CD3+, Vd1 and Vd2 T cells in COVID-19 patients. In healed subjects, the frequency of Vd1 T cells did not reach the same values of controls. Phenotypical analysis of Vd1 subset did not exhibit any substantial difference between COVID-19 patients and healed subjects while Vd2 T cells showed a homogeneous distribution of all four subsets, with a slight increase in effector and terminally-differentiated cells in healed subjects. Percentage of PD1+ expressing Vd1 and Vd2 T cells were increased in COVID-19 patients compared to healthy subjects and healed patients while TIM3 expression did not change. γδ T cells from COVID-19 patients displayed a spontaneous production of proinflammatory cytokines while healed patients showed significantly decreased cytokine production.

DISCUSSION: SARS-CoV2 infection dysregulates γδ T cells losing their antiviral function that is partially restored when infection is solved.

CONCLUSIONS: Our preliminary results suggest that γδ T cells are involved in the immune responses towards SARS-CoV-2 infection and they could represent an effective therapeutic strategy against the virus.

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CHARACTERIZING INNATE AND ADAPTIVE IMMUNE RESPONSES TO SARS-COV2 IN PREGNANT WOMEN AND NEONATES

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PURPOSE: Pregnant women are considered a special population group because of the unique 'immunological' condition due to pregnancy. However, maternal morbidity, related to SARS-CoV-2 infection in pregnancy, is similar to that of women of reproductive age and the vertical transmission of the virus is rare. Our aim was to characterize maternal and neonatal adaptive and innate immune response to SARS-CoV-2.

METHODS: We designed an observational study including pregnant women with confirmed SARS-CoV-2 infection at delivery and their newborns. Maternal and neonatal peripheral blood samples were collected at Time 0 (48h after delivery) and at Time 1 (2 month after delivery) for the study of immune cell populations by standard flowcytometry and for the detection of anti-SARS-CoV-2 specific antibodies in the serum. Clinical and demographic data were also collected.

RESULTS: Twenty maternal-infant dyads were enrolled and 5 were lost at follow-up. We reported one case of vertical transmission. The ratio between monocytes and NK cells (MNKR) is a sensitive indicator of the individual reaction to the virus. The vertically infected newborn had lymphopenia and a high MNKR with a high relative percentage of intermediate monocytes. No cases of postnatal infection were observed among the newborns. Symptomatic mothers had higher MNKR compared to asymptomatic mothers. At Time 0, 9 mothers had positive serum IgG and IgA for SARS-CoV-2 but just in one case maternal IgG crossed the placenta and were detected in neonatal serum. At Time 1, all mothers developed SARS-CoV-2 specific IgG, while no newborns developed antibodies except the infected one.

DISCUSSION: The absence of specific IgG transfer from the mother to the infant could be explained by the low maternal antibody titer as the infection was too recent. At two months of life no newborns became infected or had SARS-CoV-2 specific antibodies, suggesting that mother-infant contact, if protected, does not represent a significant risk factor for mother to infant infection transmission.

CONCLUSIONS: Despite their different immune systems, pregnant women have clinical and laboratory outcomes similar to those of the general population. If the infection is contracted late in pregnancy is generally not critical for the fetus/newborn but transfer of maternal specific IgG is lacking.

WORKSHOP

LEUKOCYTES

ROLE OF DANGER AND MICROBIAL SIGNALS IN THE CONTROL OF THE RECRUITMENT OF NEUTROPHIL SUBPOPULATIONS DURING INFECTIONS

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PURPOSE: Neutrophils are the first line of defence against bacteria and fungi and help fight parasites and viruses. Recently, neutrophil subpopulations with distinct functions have been reported under homeostatic and pathological conditions, although their role and their mechanisms of recruitment during inflammation have not been clarified. The aim of our project is to establish the mechanism of recruitment and to functionally characterize neutrophil subsets during fungal, Gram-negative and Gram-positive bacterial infections.

METHODS: To this purpose we used a model of skin infection with *Candida (C.) albicans*, *Pseudomonas (P.) aeruginosa* and *Staphylococcus (S.) aureus* and we performed a multi-parametric flow-cytometry analysis. We identified aged and fresh neutrophils as CXCR4+CD62Llow and CXCR4- CD62+/high respectively. Moreover, we employed mice lacking key Pattern Recognition Receptors (PRRs), PRR signalling molecules or alarmins to study the mechanisms of neutrophil subsets' recruitment.

RESULTS: Our preliminary data suggest the existence of two waves of neutrophil recruitment. The first wave mediated exclusively by danger molecules and the second wave mediated by both danger molecules and PAMPS (Pathogen Associated Molecular Patterns). Our data also suggest an increased accumulation of aged neutrophils during the second wave.

DISCUSSION: An enhanced "effector" phenotype and an increased phagocytic activity *in vitro* has been described for aged neutrophil. Accordingly, our preliminary results indicate that neutrophil aging is not merely a passive mechanism to direct their death in tissues, e.g. bone marrow and spleen. In fact, aged neutrophils are recruited at the infection site at late time points when fresh neutrophils have confined the infection. This suggests an active role of aged neutrophil in determining pathogen clearance in the skin and an active role for fresh neutrophils in protecting the tissue from an excessive damage that may be induced by more aggressive neutrophil activation.

CONCLUSIONS: Our project intends to identify mechanisms of recruitment and specific functions of neutrophil subtypes during different kind of infections. This could potentially have a clinical application, where infections represent a real threat to patient lives.

A 3D-BASED MODEL TO STUDY THE CROSSTALK BETWEEN INTESTINAL ORGANOIDS AND MAST CELLS

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BACKGROUND: Mast Cells (MCs) are long-living innate immune cells widely distributed in mucosal and connective tissues at the interface with the external environment. MCs accumulate in the intestine of patients suffering from inflammatory bowel disease and colorectal cancer where they can be activated and affect the integrity of the tissue, thus aggravating the disease. However, evidence of their direct effect is still missing.

PURPOSE: This work aims to study the bidirectional cross-talk between terminally differentiated MCs and intestinal microenvironment, both in healthy and pathological settings.

METHODS: Small intestine and colon organoids were produced from healthy mice and DSS- or AOM/DSS-treated mice and co-cultured with bone marrow-derived MCs (BMMCs) and investigated by qPCR, confocal microscopy and cytofluorimetry.

RESULTS and DISCUSSION: Resting and activated BMMCs induced different effects on healthy and pathological organoids, in terms of composition (analyzed as mRNA expression of the intestinal epithelial markers Lgr5, Lyz1, Muc2, ChgA and SI) and structural architecture (analyzed as mRNA expression and/or as protein expression of Cld4, Cdh1, ZO-1, and Ezrin). Moreover, the protease expression profile and activation status of BMMCs indicated that tumoral organoids are capable of inducing substantial MCs activation response, in the absence of other external stimuli. In this scenario, IL-33 organoid expression and TNFα release by BMMCs seem to play a central role, creating a cytokine environment in which IL-33 stimulates BMMCs to produce TNFα that in turn induces a different structural effect depending on the microenvironment.

CONCLUSION: These results indicate that MCs are important mediators of tissue homeostasis and that a different stimulatory environment can shape and direct MCs specific response towards the dampening or propagation of the inflammatory response.

IL17A DEPLETION AFFECTS THE METABOLISM OF MACROPHAGES IN THE PRESENCE OF CHEMOTHERAPY

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PURPOSE: IL17A is a member of the IL17 cytokine family released by both immune and non-immune cells such as tumor and stromal cells into the tumor microenvironment. IL17 receptors are widely expressed in different type of cells1. Our aim is to investigate how IL17A inhibition modulates macrophage (MO) differentiation and metabolism in the presence or absence of chemotherapy.

METHODS: MO were generated by bone marrow derived monocytes by both wild-type and IL17A knock out mice. They were polarized in the presence of LPS or GM-CSF+IL4 to obtain pro-inflammatory (M1-like) and alternatively activated (M2-like) MO. Phenotypic and functional markers were evaluated by quantitative PCR, immunofluorescence and flow cytometry. Metabolic pathways associated with the two dichotomic M1- and M2-like cells were also analyzed.

RESULTS: We observed some unique features of MO polarized in the absence of IL17A, and this paralleled specific changes in their metabolism and functions, such as the induction of an anti-tumor response. Interestingly, these features were maintained or enhanced when MO were treated with gemcitabine. We also demonstrated that the anti-IL17A antibody effectively reproduced features of MO derived from IL17A knock out mice.

DISCUSSION: IL17A has a controversial role in tumor. Tumor associated MO contribute to forming a typical suppressive milieu and inducing chemoresistance. Metabolic reprogramming is an elegant way to skew suppressive macrophages towards anti-tumor cells.

CONCLUSIONS: Overall, our results unveil a novel function of IL17A in modulating macrophage metabolism towards a less consuming glucose and producing lactate, which may orchestrate a more effective anti-tumoral response. This occurred even after gemcitabine treatment and, therefore, the combination of an anti-IL17A monoclonal Ab with gemcitabine could be envisaged.

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ROLE OF AUTOANTIBODIES IN SHAPING HUMAN MEMORY NATURAL KILLER CELL COMPARTMENT

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PURPOSE: NK cell adaptation to Cytomegalovirus (CMV) chronic infection is based on the expansion of a long-lived NK subset, dubbed memory NK cells, which is selectively hyperresponsive to antibody (Ab) stimulation via FcgRIIIA (CD16) ligation; hence, they are believed to impact on individual responses to pathogens, vaccination and tumor immunotherapy. In humans, memory NK cells are commonly defined by lack of FceRIg (g-) chain, although preferential expression of HLA-E receptor NKG2C, and CD57 maturation marker have been also evidenced. The requirements for memory NK pool establishment/maintenance, heterogeneity, and activation have not been fully characterized.

METHODS: CMV+ healthy donors (HD) and immune thrombocytopenia (ITP) patients were studied. Memory NK cell phenotype was assessed by multiparameter flow cytometry analysis of freshly isolated PBMC, and upon 9-day coculture with Ab-opsonized targets.

RESULTS: In a large cohort of CMV+ HD, the frequency of g- NKG2C+ and NKG2C- memory NK subsets displayed a large variability. g-NKG2C+CD57+ NK cells selectively and robustly expanded upon CD16 stimulation; additionally, they exhibit increased levels of antiapoptotic molecule Bcl2 with respect to g-NKG2C-CD57+, *ex vivo*. To support the hypothesis that chronic exposure to Ab-opsonized targets may contribute to the shaping of memory NK pool *in vivo,* we observed that g-NKG2C+CD57+ NK cells were more abundant in a cohort of CMV+ patients affected by ITP, an autoimmune disease where anti-platelet auto-Abs play a major role. Moreover, the coculture of PBMC from CMV+ HD with ITP, but not control, platelets, led to the preferential expansion of g-NKG2C+CD57+ subset, suggesting a more marked dependence of this memory NK cell subset on CD16-initiated signals.

DISCUSSION and CONCLUSIONS: Our work supports the concept that antibody production contributes to the shaping of a memory NK subset with distinct functional capabilities in CMV+ individuals.

C.C. and C.P., G.P. and R.G. equal contribution

RESTORATION OF FOLLICULAR T CELL COMPARTMENT IN PATIENTS WITH WISKOTT ALDRICH SYNDROME AFTER GENE THERAPY

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PURPOSE: Wiskott-Aldrich syndrome (WAS) is a rare X-linked primary immunodeficiency caused by mutations in the WAS gene and characterised by cellular and humoral defects and by an increased risk of autoimmunity and lymphomas1. Gene therapy (GT), an investigational treatment, may represent a potential alternative to allogeneic hematopoietic stem cell transplantation (HSCT), particularly for patients who do not have any suitable HSCT donor available. In this study, we aim at characterizing for the first time the T follicular helper (Tfh) and regulatory (Tfr) compartments, before and after GT, evaluating also CXCL13 plasma levels, a biomarker of germinal centre activity2.

METHODS: Circulating Tfh and Tfr number, phenotype, functional properties and CXCL13 plasma levels were determined in 10 pre-GT and 17 post-GT patients with flow cytometry and ELISA.

RESULTS: Frequencies of Tfh and Tfr cells in pre-GT patients were lower than controls (HCs), but were restored after GT. PD-1 and ICOS expression levels on Tfh were significantly higher before treatment compared to HCs. Interestingly, while Tfh PD-1 levels declined post GT, ICOS levels remained elevated. Finally, WAS patients had high levels of plasma CXCL13, that decreased after GT.

DISCUSSION: Although Tfh and Tfr cells were lower in frequencies, these cells had an activated phenotype in terms of PD-1 expression before GT. These alterations can represent new biomarkers for disease course as well as novel therapeutic targets to manipulate. Of note, Tfh and Tfr cell frequencies and phenotype were restored after GT as well as CXCL13 levels.

CONCLUSIONS: Our findings indicate that GT restores some of the defects observed in Tfh and Tfr cells of WAS patients and contributes to the recovery of the humoral immune response. These data support GT as a potential treatment option for WAS patients with limited options for definitive therapy.

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EPIGENETIC AND TRANSCRIPTIONAL CONTROL DURING CD8+ T CELL FATE COMMITMENT

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PURPOSE: Following activation by antigens, naïve CD8+ T lymphocytes establish specific heritable gene expression programs that define the progression to long-lasting memory or to short-lived effector subsets. Understanding lineage relationships between T cell subsets, and the molecular pathways that regulate the transitions between these distinct states, is essential for the design of vaccines and the development of new immune-therapeutic protocols. Many studies have characterized the transcription factors that control the differentiation of T cells, however the corresponding epigenetic states involved in the establishment and maintenance of memory and effector identities are still incompletely understood. Several epigenetic pathways, including DNA methylation and histone modifications, can contribute to establishing or maintaining transcriptional silencing (1).

METHODS and RESULTS: Our findings have defined a critical role of Suv39h1-dependent gene silencing in the establishment and maintenance of memory CD8+ T cell stemness, plasticity, and the transition to terminally differentiated effectors. These results suggest that during T cell fate commitment, Suv39h1/H3K9me3 silencing pathway establishes an epigenetic barrier on the stem/memory gene expression program, preventing the effector re-programing into memory cells (2). We have also recently examined CD8+ T cell heterogeneity during the different stages of differentiation, by developing an integrative approach involving the combined analysis of chromatin dynamic changes and gene expression profiles at single cell level (single cell RNA- combined with surface marker sequencing).

DISCUSSION and CONCLUSIONS: These results establish a transcriptional "map" during CD8+ T cell lineage commitment, highlighting new interclonal relationships between different CD8+ T subsets, during the different stages of CD8+ T cell differentiation. Recent results and new perspectives will be discussed in the context of long-term memory and T cell-based immunotherapies.

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E-POSTERS

1. AUTOIMMUNITY AND ALLERGIES

P1.01

ANTIBIOTIC-ASSOCIATED DYSBIOSIS AFFECTS THE ABILITY OF THE GUT MICROBIOTA TO CONTROL INTESTINAL INFLAMMATION UPON FAECAL MICROBIOTA TRANSPLANTATION IN EXPERIMENTAL COLITIS MODELS

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PURPOSE: The gut microbiota plays a central role in host physiology and in several pathological mechanisms in humans. Antibiotics compromise the composition and functions of the gut microbiota inducing long-lasting detrimental effects on the host. Recent studies suggest that the efficacy of different clinical therapies depends on the action of the gut microbiota Here, we investigated how different antibiotic treatments affects the ability of the gut microbiota to control intestinal inflammation upon faecal microbiota transplantation in an experimental colitis model and in ex-vivo experiments with human intestinal biopsies.

METHODS: Murine faecal donors were pre-treated with different antibiotics *i.e.* vancomycin, streptomycin and metronidazole before FMT administration to colitic animals. Gut microbiome, faecal metabolome and the immunophenotype of colonic lamina propria immune cells were analysed. *Ex vivo* cultures of human intestinal lamina propria cells and iNKT cell clones from IBD patients exposed to antibiotic pre-treated healthy microbiota faecal waters were phenotipically and functionally evaluated.

RESULTS: Antibiotic pre-treatment significantly influences the capability of the microbiota to control intestinal inflammation. Streptomycin and vancomycin-treated microbiota failed to control intestinal inflammation and were characterized by the blooming of pathobionts previously associated with IBD as well as with metabolites related to the presence of oxidative stress and metabolism of simple sugars. Metronidazole-treated microbiota retained its ability to control inflammation co-occurring with the enrichment of *Lactobacillus* and of innate immune responses involving iNKT cells. Furthermore, *ex vivo* cultures of human intestinal lamina propria mononuclear cells and iNKT cell clones from IBD patients with vancomycin pre-treated sterile faecal water showed a Th1/Th17 skewing in CD4+ T-cell populations; metronidazole, on the other hand, induced the polarization of iNKT cells towards the production of IL10.

CONCLUSIONS: Diverse antibiotic regimens affect the ability of the gut microbiota to control intestinal inflammation in experimental colitis by altering the microbial community structure and microbiota-derived metabolites.

P1.02

GATA6 DEFICIENCY LEADS TO EPITHELIAL BARRIER DYSFUNCTION AND ENHANCES SUSCEPTIBILITY TO GUT INFLAMMATION

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PURPOSE: The GATA-binding factor 6 (GATA6) is a zinc-finger DNA binding transcription factor involved in intestinal epithelial cell proliferation and differentiation. As inflammatory bowel disease (IBD) is marked by functional changes of the intestinal epithelial layer, we investigated the expression and functional role of GATA6 in IBD.

METHODS: GATA6 expression was evaluated in mucosal samples of IBD patients and controls by real-time PCR and immunohistochemistry (IHC). Ex vivo organ cultures of unaffected colonic explants of inactive IBD patients were stimulated with the inflammatory cytokines TNF-α, IL-6, IFN-γ and GATA6 level evaluated by IHC. *Gata6* conditional deletion in murine intestinal epithelial cells (Gata6del) was obtained by multiple injections of tamoxifen. After 4 weeks, Gata6del mice were sacrificed and intestinal damage and inflammatory cell infiltration evaluated by histological analysis and flow cytometry. Intestinal barrier integrity was assessed by FITC-dextran intestinal permeability assay and immunofluorescence of tight junctions. In parallel, some mice were injected with indomethacin to induce ileitis and sacrificed after 1 day, while others received trinitrobenzene sulfonic acid to induce colitis and sacrificed at day 3. To deplete gut microbiota, Gata6del mice were exposed to antibiotics for 2 weeks.

RESULTS: Decreased GATA6 expression was seen in the intestinal epithelium of IBD patients compared with controls, while only TNF-α reduced GATA6 level in normal intestinal epithelial cells. Gata6del mice exhibited epithelial damage and a pronounced inflammatory cell infiltration of the mucosa, thereby resulting in a greater histological score of intestinal inflammation and enhanced immune-inflammatory response. Gata6del mice showed altered zonulin-1 expression, increased intestinal permeability and dysbiosis, with consequent bacteria-driven local, but not systemic, immune response and enhanced susceptibility to gut inflammation. Antibiotic-driven depletion of gut microbiota abrogated the local inflammatory response in Gata6del mice without changing the main epithelial alterations.

CONCLUSIONS: Our data suggest that decreased expression of GATA6 contributes to intestinal barrier dysfunction and bacteria-driven immune-inflammatory response in IBD.

P1.03

HYPERMOTILITY OF CD8+ T CELLS IN ANKYLOSING SPONDYLITIS SUBJECTS: CAUSE OR EFFECT OF A CHRONIC INFLAMMATION?

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PURPOSE: Ankylosing Spondylitis (AS) is a chronic rheumatic autoimmune disease in which the strongest risk factor is the Human Leukocyte Antigen (HLA)-B27 whose pathogenetic role is still debated1. The HLA-B27 function in the antigen presentation to CD8+ T cells and the AS association of factors involved in many activities of CD8+ T cells, point out to a role of this subset in sustaining the chronic inflammation2,3. Since the migration events of lymphocytes are important in fulfilling immune functions, our purpose is to investigate the migration of CD8+ T cells4.

METHODS: We enrolled patients with AS, Rheumatoid Arthritis (RA) and healthy donors (HD) whose CD8+ T cells were analyzed by transwell migration in presence of the following chemokines: CXCL9, CXCL10, CXCL11, CXCL12, CCL20. The receptor expression and the immune profile were analyzed by specific antibodies: a-CXCR3, -CXCR4, -CCR6, -CCR7, -CD45RA, -CD28, -CD57. The telomeric length of CD8+ T cells was analyzed by rtPCR.

RESULTS: The migration analysis upon chemokine stimulation showed that CD8+ T cells of AS subjects were less able to migrate comparing to those from HD. This reduced capability was not correlated to a lower expression of specific receptors. In absence of stimuli, CD8+ T cells from AS patients demonstrated a spontaneous hypermotility not found in CD4+. By investigating their profile in terms of naïve/memory markers, the migrated cells possess prevalently effector or TEMRA phenotype with prevalence of CD28-/CD57+ cells. Preliminary data from rtPCR showed that migrated cells had shorter telomeres than non-migrated cells. RNA samples have been collected to characterize these cells at transcriptome level.

DISCUSSION: CD8+ T cells of AS patients possessed a higher basal motility with a lower migration towards specific chemokines; such phenomenon could be related to a subset of senescent/over-stimulated CD8+ T cells.

CONCLUSIONS: An alteration in the lymphocytes migration can cause a disequilibrium of homeostasis inducing conditions of chronic inflammation such as in AS. This study could be an innovative approach to investigate CD8+ T contribution to AS pathogenesis.

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P1.04

DIVING INTO INFLAMMATION: A PILOT STUDY EXPLORING THE DYNAMICS OF THE IMMUNE-MICROBIOTA AXIS IN ILEAL TISSUE LAYERS OF PATIENTS WITH CROHN'S DISEASE

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PURPOSE: Crohn's Disease (CD) pathogenesis is still unclear. Disorders in the mucosal immunoregulation and its crosstalk with the microbiota may represent an important component in tissue injury. We aimed to characterize the molecular immune response distribution within the ileal layers and to evaluate the correlated microbiota in pathological/healthy settings comparing first surgery/relapse clinical conditions.

METHODS: We enrolled 12 CD patients. A comprehensive analysis of ileal mucosa, submucosa and serosa broad-spectrum cytokines' panel was performed through a multiplex approach. In addition, ileal microbiota composition was assessed through Next Generation Sequencing.

RESULTS: We observed a distinct profile (of IL1-a, IL-1b, IL-4, IL-8, ICAM-1, E-Selectin, P-Selectin, IP-10, IL 6, and IL 18) across the CD vs healthy ileal layers; and a different distribution of IFN-g, P-Selectin, IL-27 and IL-21 in first surgery *vs* relapse patients. In addition, the phylum *Tenericutes,* the family of *Ruminococcaceae*, and the genus *Mesoplasma* and *Mycoplasma* were significantly enriched in pathological setting. Significant microbiota differences were observed between relapse vs first surgery patients regarding the class *Bacteroidia,* the genus of *Prevotella*, *Flavobacterium*, *Tepidimonas* and *Escherichia/Shigella.* Finally, the abundance of the genus *Mycoplasma* was positively correlated with IL-18.

DISCUSSION: We describe a dissimilarity of cytokines' distribution and microbiota composition within the CD and the adjacent healthy ileal tissue layers and between first operation and surgical relapse.

CONCLUSIONS: Notably, our results give for the first time a potential insight into the dynamics of the gut microbiota-immune axis in CD patients, leading to new biomarkers' detection and personalized treatment.

P1.05

TARGETED NANOPARTICLES AS A THERANOSTIC TOOL FOR THE MANAGEMENT OF COMPLEMENT-MEDIATED VASCULAR THROMBOSIS

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PURPOSE: Vascular thrombosis in anti-phospholipid syndrome (APS) is a complement-mediated process induced by antibodies that recognize beta2-GPI expressed on blood clots and vessel endothelium of patients and animal models of APS. As visualization and resolution of thrombi remain a concrete medical need in APS, a mAb targeting beta2-GPI represents a good candidate to coat fluorescent nanobubbles together with rtPA to allow selective delivery of the fibrinolytic agent to the thrombi.

METHODS: Theranostic chitosan/lipid hybrid nanobubbles (400nm) with perfluoropentane and coumarin 6 in the core and covered with anti-beta2-GPI and rtPA were prepared.
These nanostructures were analyzed in vitro for their ability to recognize beta2-GPI by immunofluorescence analysis and for rtPA activity following the dissolution of blood clot. Thrombus formation in mesenteric vessels was evaluated by intravital microscopy; the same setting was used to visualize blood clot formation and dissolution.

RESULTS and DISCUSSION: Different nanobubble formulations were tested to avoid direct complement activation, to reduce interaction with cells, erythrocytes and tissues and to maintain surface protein activities. These nanostructures selectively targeted tissues expressing beta2-GPI in vitro and thrombi formed in rats. Clots were visualized 30 seconds after infusion of targeted fluorescent nanobubbles, while uncoated nanobubbles failed to localize to clots, demonstrating the importance of the targeting agent. Infusion of commercial rtPA into rats led to rapid dissolution of thrombi that reformed after about 2 minutes due to renal clearance of rtPA. On the contrary, injection of beta2-GPI-targeted nanoparticles coated with rtPA were equally effective even with an amount of fibrinolytic agent 10 times lower than that required for soluble rtPA to induce the thrombolytic effect. Moreover, the targeted nanobubbles bound to the endothelial surface expressing beta2-GPI prevented antibody-dependent complement activation and thrombus formation for more than 2 hours.

CONCLUSIONS: Nanoparticles loaded with a fluorescent probe and covered with rtPA and non-complement fixing MBB2 targeting beta2-GPI allow selective visualization and dissolution of thrombi formed in a model of complement-mediated vascular thrombosis.

P1.06

ELUCIDATING THE EXTRACELLULAR VESICLE-ASSOCIATED DETERMINANTS MODULATING FOXP3 EXPRESSION AND SUPPRESSIVE FUNCTION OF T REGULATORY CELLS IN MULTIPLE SCLEROSIS

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PURPOSE: Multiple Sclerosis (MS) is a chronic inflammatory disease with an autoimmune etiology mainly carried out by CD4+ conventional pro-inflammatory T (Tconv) cells together with a dysfunction of CD4+CD25hiFoxp3+ T regulatory counterpart (Treg)1. Tconv cells from MS patients release a large amount of vesicles (EVs) showing differential expression of microRNA (miRNAs) compared to healthy conditions. Some of these miRNAs could inhibit genes involved in the epigenetic regulation of Foxp3, the transcription factor necessary for Treg generation and function. The aim of our study was to evaluate whether EVs released by in vitro TCR-stimulated Tconv cells isolated from MS patients have an augmented ability to inhibit function of Treg cells by affecting Foxp3 expression.

METHODS: We analysed 4 Relapsing-Remitting-MS (RR-MS) subjects at diagnosis and 4 healthy donors (HD). We performed a characterization of the main Treg cell-related markers (CCR7, CTLA4 and PD-1) expressed on iTreg cells generated in the presence of Tconv-derived EVs from either RR-MS or HD. Finally, the main regulators of Foxp3 expression (mTOR and STAT3/5 pathways) were evaluated both in RR-MS and HD by western blot.

RESULTS
Tconv-derived EVs from RRMS subjects reduce the induction of Foxp3all and Foxp3E2 at 24h and 36h in both HD and RRMS iTreg cells. Also CCR7 and PD-1 expression is downregulated by Tconv-derived EVs from RR-MS subjects at 24h both in HD- and RR-MS- iTreg cells. At molecular level, Tconv-derived EVs from RR-MS subjects promote the induction of STAT5 and S6 during the generation of iTreg cells from RR-MS subjects. Conversely, STAT3 levels are reduced by Tconv-derived EVs from RR-MS, both in RR-MS and HD.

DISCUSSION: Our results show that Tconv-derived EVs from RRMS could affect the induction of the main pathways involved in Foxp3 induction thus modifying the generation and function of iTreg cells.

CONCLUSIONS: These data suggest the specific establishment of an autocrine pro-inflammatory loop in RRMS, via miRNA-dependent epigenetic regulation of Foxp3.

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P1.07

PHENOTYPICAL AND FUNCTIONAL CHARACTERIZATION OF FOXP3EXON2+ TREG CELL SUBSET IN THE TUMOR MICROENVIRONMENT OF BREAST CANCER SUBJECTS

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PURPOSE: Regulatory T CD4+CD25+Foxp3+ (Treg) cells are a cellular subset involved in the establishment of peripheral tolerance through the inhibition of effector T (Teff) cells and suppression of the immune-mediated tissue destruction toward self-reactive clones1. Treg cells can have an important impact on the tumor growth since their frequency in the tumor microenvironment (TME) could affect cancer progression and clinical outcomes2. TME could impact on Treg cell functional features through the modulation of their intracellular metabolism, such as glycolysis3. We performed the phenotypical and functional characterization of Treg cells expressing the Foxp3Exon2 (Foxp3E2+) splicing variant, in tumor-infiltrating lymphocytes (TILs) and in peripheral blood of naïve-to-treatment (ER+PR+) breast cancer subjects (BC).

METHODS: We enrolled 30 BC subjects at diagnosis and 30 age- and sex-matched controls. We performed multiparametric cytofluorimetric analysis on Peripheral Blood Mononuclear Cells (PBMCs) and TILs. *In vitro* proliferation of conventional T (Tconv) cells labeled with the division-tracking dye CFSE was evaluated. Finally, we assessed Foxp3 splicing variant expression in *in vitro* induced-Treg (iTreg) generated from Tconv cells after 24 and 36 hours of stimulation with anti-CD3/CD28, by Western Blot.

RESULTS: An increased frequency of Foxp3E2+ Treg cells and suppressive capacity was observed in PBMCs of BC subjects. Moreover, Foxp3E2+ Treg cells from BC subjects have higher expression of Treg cell-suppressive markers (such as CTLA-4, PD1). During iTreg cell induction, we found that tumor supernatants enhances the peripheral conversion of Tconv into Foxp3E2+ iTreg cells through an increased glycolytic engagement.

DISCUSSION: Increased Treg suppressive capacity correlates with different disease status and clinical score. Glycolysis supports human Treg generation and function by regulating the expression of Foxp3E2, accounting for an enhanced immunosuppressive function.

CONCLUSIONS: Our results support the intricate interplay among Foxp3E2+ Treg cells, metabolism and TME and pave the way to explore innovative therapeutic interventions for the immunotherapy of BC.

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P1.09

PLATELET PHAGOCYTOSIS CONTROLS THE ACCUMULATION OF MICROPARTICLES IN THE PLASMA OF PATIENTS WITH SYSTEMIC SCLEROSIS

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PURPOSE: It is unclear why activated platelets and platelet-derived microparticles (μPs) accumulate in the blood of patients with systemic sclerosis (SSc).

METHODS: Platelet-neutrophil interaction, phagocytosis, P-selectin, PSGL-1, HMGB1 expression and distribution on platelet and μPs, the concentration of byproducts of NETs were assessed by flow cytometry and immunochemistry in 81 donors. 25 patients had SSc, 26 had stable coronary artery atherosclerosis (CAD). 30 sex- and age-matched healthy volunteers served as controls. Soluble E-selectin, lung parenchyma and microvasculature were assessed in NSG mice after μPs injection in the tail vein.

RESULTS: P-selectin+ platelets and platelet-derived HMGB1+μPs accumulate in the blood of SSc patients . The expression of the receptor for P-selectin, PSGL-1 was decreased, raising the possibility that phagocytes fail to recognize/phagocytose activated platelets, which in turn might keep releasing μPs. In support, SSc neutrophils did not contain platelets, consistently present within CAD neutrophils. HMGB1+μPs elicit the generation of NETs, which were detected in the plasma of SSc patients only. P-selectin/PSGL-1 interaction resulted in platelet phagocytosis *in vitro* and influenced the μPs ability to elicit NETs, endothelial damage and migration of leukocytes through pulmonary microvasculature in mice.

DISCUSSION: Previous studies have shown that removal of platelets requires the recognition of platelet P-selectin. The best characterized receptor for P-selectin is PSGL-1, a highly conserved moiety that mediates the adhesion of platelets to neutrophils and is required for their phagocytosis. The PSGL-1 defect, that reproduces in mice a SSc-like disease, could explain the defective platelet clearance and μP accumulation in SSc, prompting vascular inflammation and fibrosis.

CONCLUSIONS: Strategies aimed at restoring platelet phagocytosis could offer valuable tools for molecular interventions in SSc, an unmet medical need.

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P1.11

EXPRESSION OF CD26 (DIPEPTIDIL-PEPTIDASI IV) IN MUSCLE AND BLOOD CELLS OF PATIENTS WITH IDIOPATHIC INFLAMMATORY MYOPATHIES

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PURPOSE: Idiopathic inflammatory myopathies (IIM) comprise systemic autoimmune diseases preferentially targeting the skeletal muscle. T cells play a pathogenic role but the rarity and heterogeneity of IIM make it difficult to identify mechanisms. The dipeptidil-peptidasi CD26 has a role in T cell activation and antigen presentation. Aim of the project was to evaluate CD26 expression in biopsies and circulating blood cells of IIM patients and healthy controls according to the clinical, histological and serological characteristics of the disease

METHODS: Expression of CD26 was evaluated by flow cytometry in monocytes, B, CD8+ and CD4+ T cells and by immunofluorescence in muscle biopsies. We analyzed 22 patients and 17 age- and sex-matched healthy controls.

RESULTS: CD26 was mainly expressed by CD4+ and CD8+ cells. The expression of CD26 in circulating CD8+ T cells negatively correlated with the concentration of plasma aldolase and C reactive protein. Furthermore, the frequency of CD26+ cells among blood CD8+ T cells negatively correlated with disease activity. T regulatory cells (Tregs) expressed high levels of CD26. In the muscle, CD26 was undetectable in healthy subjects but consistently expressed in IIM patients, with its expression being consistently higher in patients with DM. Muscle CD26 is expressed by lymphocytes and endothelial cells and within the matrix in particular in tissues undergoing substantial necrosis, negatively correlating with muscle strength.

DISCUSSION: The increase in CD26 expression on blood leukocytes in patients might reflect the involvement of the protein in effector T lymphocyte activation. Moreover, patient Treg CD26 could be associated with a decreased inhibitory capacity by adenosine deficiency. CD26 is expressed in skeletal muscle of patients with severe disease.

CONCLUSIONS: CD26 represent a target of molecular intervention for treatment-refractory myositis.

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P1.12

MONITORING OF BIOLOGICAL DRUGS IN THE TREATMENT OF AUTOIMMUNE RHEUMATIC DISEASES: ADVANTAGES AND LIMITATIONS

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PURPOSE: Following the development of Rheumatoid Arthritis monitoring the different mechanism of action of two biological drugs: Etanercept, fusion protein and Adalimumab, monoclonal antibody. They act on the tumour necrosis factor leading to a down-regulation of pathogenic action.

METHODS: A total of 33 patients affected by Rheumatoid Arthritis were tested. The present study is based on three methods of fundamental importance in the diagnosis of autoimmune rheumatic diseases: INDIRECT IMMUNOFLUORESCENCE, ELISA, IMMUNOBLOTTING.

RESULTS: It's interesting underline the different response to the treatment in the patients treated. Infact 10 patients treated with Etanercept and 8 patients treated with Adalimumab showed efficacy in therapeutic treatment.

DISCUSSION: Biological drugs represent a new step in the treatment of autoimmune diseases. Their use has even proved decisive in some diseases such as Rheumatoid Arthritis, delaying the pathological process and consequently improving the painful symptoms. The use of biological drugs opens the way to "new frontiers of therapy" in other diseases such as cancer. Immunotherapy represents a step forward the treatment of diseases that until now have been considered inauspicious.

CONCLUSIONS: The Rheumatoid Factor is a marker in monitoring therapy with pro-inflammatory cytokine antagonists. Autoimmunity represents a step forward the treatment of diseases that until now have been considered inauspicious.The "non-responsiveness" to biological drugs is at the basis of important phenomena such as: immunogenicity, immunological habits of individual patients, spreading epitope, overlap syndrome.

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P1.13

NATURAL KILLER CELLS AS POSSIBLE MEDIATORS OF GRAVES' DISEASE CURE

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PURPOSE: Graves' Disease (GD) is a common autoimmune hyperthyroidism, whose definitive cure is still unmet. Natural Killer (NK) cells, in particular CD56bright subset, might play a regulatory role, controlling T cell responses. A randomized, controlled trial (EudraCT 2017-00505011) was set to determine whether vitamin D (VitD) and Selenium (Se) co-supplementation improves methimazole (MMI) efficacy in GD treatment. NK cells were studied as possible mediators.

METHODS: Patients with newly diagnosed GD underwent consecutive screening for the conditions of VitD and Se. Phenotypic and functional studies on NK cells were performed. Distinct fluorophore-conjugated mAbs were used for Flow Cytometry: anti-CD3, CD56, CD16, NKGD2, CD49d, CD69, CD161, CD107a. Eligible patients were randomized to MMI treatment alone or combined with Se+cholecalciferol supplementation.

RESULTS: At baseline, 41 eligible GD patients (45.8±10.3 years, 36 women and 5 men) exhibited higher number of circulating total NK and NKbright cells, compared to 75 age and sex matched healthy controls (HC). CD69+NK cells were higher, while CD161+NKs were lower in GD patients compared to HC. Total NK, NKbright and NKdim cells had lower degranulation ability in GD. Six months experimental treatment resulted in greater reduction of FT4 levels (p 0.003), compared to MMI. Total NK and NK subsets significantly decreased in both groups, as compared to baseline levels. A greater reduction emerged in the experimental group (p 0.03). In both groups, degranulation ability improved from baseline. CD69+NKs decreased from baseline, but a significant decrease was observed uniquely in experimental group.

DISCUSSION and CONCLUSIONS: Although preliminary, our results suggested NK dysfunction in untreated GD. Treatment with VitD and Se associated to MMI seemed efficient in favouring GD remission, by modulating NK cells subsets.

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P1.14

JANUS-FACED LIPOSOMES AS THERAPEUTIC TOOLS TO DRIVE T SUPPRESSOR PHENOTYPE IN MULTIPLESCLEROSIS

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PURPOSE: Phosphatidylserine liposomes are proved to promote innate antimycobacterial activity and to limit the inflammatory response. In this study we used a new class of liposomes, empty (PSPS) or conjugated with the myelin basic protein (PSPS-MBP), as immunomodulatory agents that can promote the induction of Tregs in Multiple Sclerosis (MS), while simultaneously reducing the production of pro-inflammatory cytokines.

METHODS: We enrolled 40 Relapsing-Remitting MS (RR-MS) patients during the active phase of the disease. We performed Flow-cytometry (Treg and Th1/17 panels), ELISA and qRT-PCR on CD4+ T cells cultures (expression levels of cytokines).

RESULTS: Flow cytometric analysis of CD4+ T cells from MS patients cultured with different conditions revealed a modulation of the Th1/Th17 compartments. The stimulation with PSPS lead to a trend of slight reduction of effector T cells, with a major effect on the Th17 subpopulation. Flow cytometric analysis also showed a modulation of the T reg compartment. In fact, the stimulation with PSPS lead to a trend of increase of CD25high-CD127low-FoxP3pos cells. PSPS seemed to act on Treg subpopulations, especially upregulating Inducible T reg (iTreg) compartment, specifically CD39+ Suppressor Tregs, modulating IL10 levels and down-regulating the Th1/Th17 response.

DISCUSSION: Multiple sclerosis is an autoimmune disease that can lead to severe disability in affected patients and whose treatment options are still quite limited. The use of liposomes could be an effective alternative to traditional treatments. In fact, our study demonstrates the ability of the liposomes PSPS and PSPS-MBP to immunomodulate the response of T cells, promoting the activation of iTreg and the down-regulation of Th1/Th17 populations and of the pro-inflammatory cytokines produced by them.

CONCLUSIONS: Thus, taken together, these data suggest that liposomes could represent a useful tool for new personalized therapeutic strategies in order to promote Treg or to reduce Th1 response and to decrease or eliminate the side effects due to the traditional drugs currently used for treatment of MS patients.

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2. B-LYMPHOCYTES

P2.01

TARGETED CHITOSAN NANOBUBBLES: NEW APPROACH FOR ANTI-MICRORNA-BASED THERAPY IN BURKITT LYMPHOMA MODELS

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PURPOSE: Standard therapeutic regimens for Burkitt's Lymphoma (BL) are based on polychemotherapy. Despite survival rates higher than 70-90%, patients often relapse or are refractory to treatments1. The management of these patients represents a great challenge. This aim was address combining RNAi and nanomedicine: chitosan nanobubbles (NBs) were loaded with antagomiR17 and conjugated to antiCD20 antibodies. This approach represents a novelty in the field; in fact, until now, just drugs-loaded nanosystems were proposed [2-4].

METHODS: NBs were characterized *in vitro* and *in vivo*. *In vitro* studies were made incubating NBs with CD20-expressing B cells and evaluating their binding, internalization and effect (decrease of antagomiR17 level); *in vivo* studies were made in a mouse model of CD20-expressing B cell malignancy and included biodistribution studies and the evaluation of the therapeutic effect.

RESULTS: NBs have a diameter of 400nm, a positive charge and a long-term stability. NBs bind and are internalized by CD20+ BL cells thus decreasing miR17 levels *in vitro* and *in vivo*. NBs were localized mostly in liver, kidneys and the tumor mass. The presence of the targeting mechanism (antiCD20 antibodies) did not increase NBs biodistribution in the tumor mass. However, targeted NBs arrest tumor mass progression increasing the survival of animals while untargeted NBs did not.

DISCUSSION: Different targeting strategies (folic acid5, pluronic F1273) has been used to address BL cells but anti-CD20 antibodies remain of key importance for the active vehiculation of nanosystems. anti-CD20 antibodies were fundamental for the targeting and the internalization of NBs *in vitro* and *in vivo* allowing the therapeutic effect of antagomiR17.

CONCLUSIONS: The combination of RNAi and anti-CD20-conjugated nanomedicine can be effective in controlling lymphomas providing a rationale for adopting this approach for the treatment of BL. NBs can be loaded with multiple drugs to allow a combinatorial therapy, potentially increasing anti-tumoral effect.

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P2.02

THREE-DIMENSIONAL IN-VITRO MODEL FOR THE STUDY OF DIFFUSE LARGE B CELL LYMPHOMA ANTINEOPLASTIC TREATMENTS

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PURPOSE: Diffuse large B cell lymphoma (DLBCL) represents the most common type of non-Hodgkin lymphoma. Although the curability rate is high, around 30% of patients will relapse or exhibit refractory disease1representing approximately 30–40% of all cases in different geographic regions. Patients most often present with a rapidly growing tumour mass in single or multiple, nodal or extranodal sites. The most common type of DLBCL, designated as not otherwise specified, represents 80–85% of all cases and is the focus of this review. There are also rare types of lymphoma composed of large B-cells, in aggregate about 15–20% of all neoplasms that are sufficiently distinctive to recognise separately. DLBCL not otherwise specified (referred to henceforth as DLBCL,2. About 15% of DLBCL patients have bone marrow (BM) involvement. There is evidence that BM mesenchymal stromal cells (MSC) protect DLBCL cell lines and primary DLBCL cells through a combination of soluble factors and cell-to-cell contact. We sought to develop a three-dimensional (3D) *in-vitro* model to study MSC/DLBCL interaction with the aim to establish a tool for evaluating patient-specific therapies.

METHODS: Human decellularized femoral bone fragments were used as a scaffold and recellularized with MSC. 3D spatial configuration was analyzed with two photon microscopy. Then, DLBCL cells were allowed to flow into the model by a microfluidic system and spatial interaction was studied. Viability of DLBCL cells was also evaluated by *in-vitro* co-cultured with human MSC.

RESULTS:
We optimized a two-step recellularization protocol providing direct MSC seeding on the scaffold surface and MSC flowing through it by an in-house made device. We digitally recreated the 3D structure of the model identifying that MSC autonomously adhered and grew on scaffold. MSC form a 3D web creating niches in which DLBCL cells stably adhere (Figure 1,2) suggesting their stable physical interaction. The co-culture studies demonstrated that DLBCL cells apoptosis is reduced in presence of MSC.

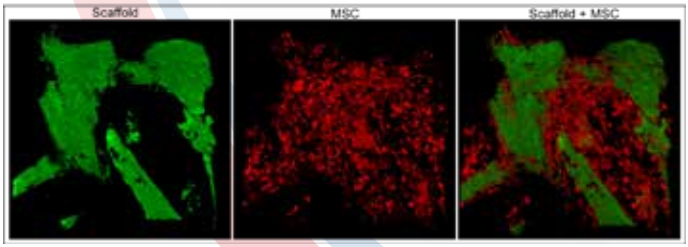

Figure 1. Two photon microscopy 3D visualization.

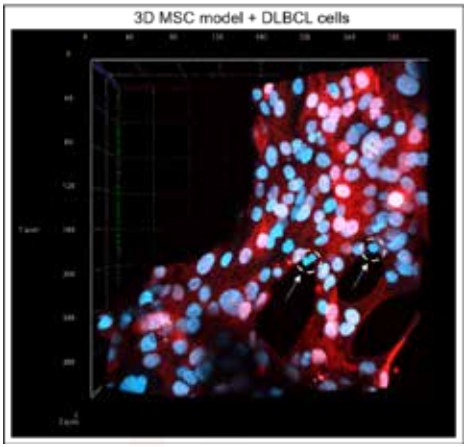

Figure 2. Arrows indicating DLBCL cells.

DISCUSSION: *Ex-vivo* derived human MSC adapted to our 3D model can be exploited to study their potential ability to modulate DLBCL growth and sensitivity to lymphoma therapies. Our preliminary results confirm that MSC prevent DLBCL cells apoptosis.

CONCLUSIONS: Our 3D model of MSC/DLBCL interaction is a promising tool to develop a patient-specific approach for anticipating the response to treatment of patients with refractory DLBCL

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P2.03

CRISPR/CAS9 AND MRNA-BASED TECHNOLOGY FOR GENE EDITING AND EXPRESSION IN HUMAN B CELLS

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PURPOSE: Robust and efficient protocols for gene editing and expression are highly needed in order to facilitate study of gene function in human B cells. B cells are notorious for being hard-to-manipulate making it challenging to modulate their endogenous gene expression or to induce expression of exogenous genes. Conventional methods based on plasmid/siRNA transfection or viral transduction are not efficient in B cells. Hence, the development of optimized methods adapted to B cells gives an opportunity of developing screening platforms to assess function of multiple genes in assays *in vitro*.

METHODS: In this work, using tumour B cells from patients with leukemia, we set up conditions for CRISPR/Cas9-based gene knockout and mRNA-driven gene overexpression in B cells freshly isolated from peripheral blood. We also developed a platform for high-throughput *in vitro* production of mRNA encoding multiple genes allowing to scale up eventual screening assays.

RESULTS: We show, on one hand, that by electroporating B cells with ribonucleoprotein complexes containing recombinant Cas9 nuclease and synthetic guides one can achieve >70-80% of homozygous knockout of a given gene in freshly isolated B cells. On the other hand, electroporation with *in vitro* transcribed mRNA allows to finely modulate gene expression levels in B cells. Gene overexpression persists for up to 4 days and longer.

DISCUSSION: Advantages of the developed system include the ability to manipulate freshly isolated B cells without impacting their viability and without prior expansion, which is particularly relevant for studies that aim to assess function of genes regulating B cell proliferation and differentiation.

CONCLUSIONS: CRISPR/Cas9- and mRNA-based platforms for gene editing and expression are readily applicable to minimally manipulated primary human B cells.

P2.04

PRECLINICAL DEVELOPMENT OF A MODULAR NANO-PLATFORM FOR THE TREATMENT OF B-CELLS MALIGNANCIES

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PURPOSE: B-cell malignancies have high-risk characteristics and significant toxicity associated with chemotherapy, requiring alternative approaches for the treatment of relapsed/refractory patients. Polymeric nanoparticles (NPs) labeled with a targeting mechanism and loaded with therapeutic agents can represent a modular platform for a safe and selective delivery of chemotherapeutic drugs, antagomiR, and enzymes.

METHODS: The nano-system is based on PLGA-PVA polymers, coated with Human Serum Albumin to simulate the soft part of protein corona and protect NPs from macrophage opsonization. NPs surface was decorated with an anti-CD19 ScFv-Fc as targeting agent. NPs were incubated with plasma and calcium, or with serum to investigate the residual coagulation and complement (C) activity, respectively. Viability tests *in vitro* were performed employing erythrocytes, Nalm-6 and Bjab cells, Acute Lymphoblastic Leukemia and Burkitt Lymphoma cell line, respectively. These cells were also employed to set up *in vivo* models of B-cell malignancies in zebrafish larvae in which NPs were injected in the bloodstream.

RESULTS: This NPs-platform usually demonstrated a round shape of ~300nm, negative charge, and concentration of 1012 NPs/mL. These nano-systems showed a safe toxicological profile, avoiding a direct lysis of red blood cells, clotting formation, C activation and cell cytotoxicity. NPs were able to bind and to be internalized in both Nalm-6 and Bjab cells. In zebrafish models, NPs were safe and easily diffused in tissues trough the bloodstream. Surface modifications reduce macrophages elimination and favor the targeting of B-cells.

DISCUSSION: The increase of efficacy and safety of chemotherapeutic drugs is closely related to the prevention of undesired interaction through a specific delivery for whom NPs represent a relevant and innovative approach. The proposed platform ensures modest interaction with the immune system and allows the specific delivery of the therapeutic payload to the site of interest, avoiding side effects.

CONCLUSIONS: Anti-CD19 stealth NPs represents a safe and effective tool for the specific delivery of probes and drugs for the diagnosis and the treatment of B-cell malignancies, but also a promising modular platform for other pathologies.

P2.06

THE DIALOGUE BETWEEN B CELLS AND MAST CELLS SUSTAIN IGA RESPONSE IN THE INFLAMED INTESTINE

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PURPOSE: In many intestinal pathologies the presence of mast cells (MCs) correlates with the accumulation and activation of B cells, however, the reciprocal influence of MCs and B cells remains poorly investigated.

METHODS: The present study took advantage of bioinformatics analysis as well as in vitro cultures and animal models of MCs deficiency or intestinal inflammation.

RESULTS: Although the role of MCs in promoting B cell-related functions is widely accepted, the impact of the absence of MCs on B cell distribution, phenotype, and function is poorly understood. Bioinformatics analysis revealed that plasma cells (but not naïve B cells) accumulation positively correlates with activated MCs in human intestinal inflamed tissues, suggesting a specific interaction occurring between these two cell types in the ulcerative colitis setting. By using MC-deficient mice, we uncovered MCs impact onmaintenance of B cell homeostasis in the intestine. In fact, under physiological conditions, MC-deficient mice present higher titers of serum IgA, while during inflammatory conditions while failed to increase both the of IgA+ titers and CD138+ cells numbers in the small intestine and colon as well as the level of IgA in the serum.

DISCUSSION: MCs act as a cellular modulator of IgA synthesis under homeostatic conditions while enhancing IgA production following the onset of colitis. Whether the effect on IgA production is the result of the direct B/MC interaction or rather an indirect effect of MCs involving a third cellular partner (e.g., Treg, Th17) remains to be investigated.

CONCLUSIONS: We demonstrated, in vivo, that MCs regulate B-cell homeostasis in the intestine and the production of IgA both in physiological conditions and during intestinal inflammation.

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P2.07

IMMUNE COMPLEXES SUSTAIN CPG-INDUCED PROLIFERATION OF RHEUMATOID FACTOR-SPECIFIC B CELLS IN HEPATITIS C VIRUS-CURED MIXED CRYOGLOBULINEMIA BY REVERSING TLR TOLERANCE

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PURPOSE: Hepatitis C virus (HCV) causes mixed cryoglobulinemia (MC) by driving clonal expansion of innate-like B cells expressing B cell receptors (BCR), often encoded by the VH1-69 gene, with dual rheumatoid factor and anti-HCV specificity1. Clearance of HCV is commonly associated with response of MC, but B cell clones remain in most patients some of whom have persistence or relapse of vasculitis2. We investigated whether immune complexes (ICs) may act as autoantigen sustaining survival of pathogenic B cells.

METHODS: We selected 6 MC patients, 5 cured of HCV infection, which had circulating monoclonal B cells that could be traced by an antibody to the VH1-69-encoded protein. We investigated the capacity of anti-Ig (F(ab')2 anti-human Ig) or of ICs (heath-aggregated human IgG) to induce phosphorylated AKT (pAKT) and CpG-driven proliferation in patients' VH1-69pos clonal and VH1-69neg normal B cells.

RESULTS: Anti-Ig induced pAKT in both VH1-69pos and VH1-69neg B cells, whereas ICs induced pAKT exclusively in VH1-69pos cells. VH1-69pos B cells constitutively expressed TLR9 mRNA but failed to proliferate in response to the TLR9 ligand CpG. Stimulation with ICs alone did not induce proliferation but, similarly to anti-Ig, restored the capacity of VH1-69pos B cells to proliferate in response to CpG. By contrast, ICs decreased CpG-induced proliferation of VH1-69neg B cells.

DISCUSSION: We found that stimulation with immune complexes (ICs) induced Akt phosphorylation and restored TLR9-driven proliferation in clonal RF B cells of MC patients, closely recalling the rescue of B cells from TLR tolerance by BCR signaling.

CONCLUSIONS: ICs normally produced in low amount can rescue pathogenic B cells of HCV-cured MC patients from TLR tolerance3; thus, TLR9 stimulation by microbial or endogenous nucleic acids might explain their HCV-independent survival and persistence or relapse of disease.

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3. CELL SIGNALING

P3.01

CLASS IA PHOSPHOINOSITIDE 3-KINASES REGULATE SUBCELLULAR AND FUNCTIONAL DYNAMICS OF INDOLEAMINE 2,3-DIOXYGENASE 1

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PURPOSE: Indoleamine 2,3-dioxygenase 1 (IDO1) is a cytosolic enzyme that controls immune responses via tryptophan metabolism, mainly through its enzymic activity. When phosphorylated, however, IDO1 acts as a signaling molecule in plasmacytoid dendritic cells (pDCs), thus activating genomic effects, ultimately leading to long-lasting immunosuppression. Whether the two activities - namely, the catalytic and signaling functions - are spatially segregated has been unclear.

METHODS: Confocal microscopy, western blot analysis of sucrose isopycnic gradient, mutagenesis to generate a construct coding for mutated IDO1. Pull-down, coimmunoprecipitation. Real time PCR, HPLC. Skyn test assay.

RESULTS: We found that, under conditions favoring signaling rather than catabolic events, IDO1 shifts from the cytosol to early endosomes (EE). The event requires interaction with class IA phosphoinositide 3-kinases (PI3Ks), which become activated, resulting in full expression of the immunoregulatory phenotype in vivo in pDCs as resulting from IDO1-dependent signaling events.

DISCUSSION: IDO1's spatial dynamics meet the needs for short-acting as well as durable mechanisms of immune suppression, both under acute and chronic inflammatory conditions. These data expand the theoretical basis for an IDO1-centered therapy in inflammation and autoimmunity.

CONCLUSIONS: Under conditions favoring signaling rather than catalytic activity, IDO1 shifts from the cytosol to EE. Class IA PI3K have a dominant role in the immunoregulatory signaling pathway of IDO1 in pDCs.

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4. COVID19

P4.01

SEROPREVALENCE OF SARS-COV2 IN IBD PATIENTS TREATED WITH BIOLOGICAL THERAPY

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PURPOSE: A similar course of COVID-19 in patients with inflammatory bowel diseases (IBD) and in the general population has been reported (1,2). However, disease prevalence in IBD patients is presently unknown. In this prospective observational study we aimed at determining SARS-CoV2 infection prevalence in IBD patients treated with biological therapy during the first pandemic wave.

METHODS: 354 sera from IBD patients under biological therapy recruited from three different locations in Italy and Germany were evaluated for antibody presence by RBD ELISA (3,4). Control groups were i) age-matched healthy subjects tested in the same time period in Milan, Italy; ii) healthy subjects collected in the pre-COVID era; iii) IBD patients under biological therapy collected in the pre-COVID era.

RESULTS: 8 out of 354 patients tested positive for the anti-RBD-SARS-CoV2 IgG antibody (prevalence 2.3%). IgG positive patients' percentage recruited from Milan was significantly higher than those recruited from other locations (prevalence 5.4% vs. 0.4% $p < 0.005$). IgG positive patients reported a significantly higher incidence of fever, anosmia and ageusia, and were more likely to have entered in close contact with COVID-19 positive subjects before the study enrolment. Biological therapy did not prevent the mounting of efficient humoral responses in infected IBD patients.

DISCUSSION: SARS-CoV2 IgG seroprevalence in IBD closely reflects values measured in background populations, whose prevalence was of 7% in Milan in the same period. This result is in line with an Italian nationwide study indicating SARS-CoV2 seroprevalence of 7.5% and 0.3% in Lombardy and in Sardinia, respectively, and of 0.9% among blood donors in Germany. Thus, our data confute the hypothesis that IBD patients under biological therapy may be protected from SARS-CoV2 infection.

CONCLUSIONS: Seroprevalence of SARS-CoV2 in IBD patients treated with biological therapy reflects values measured in the local general population. Specific symptoms and contact history with SARS-CoV2-infected individuals strongly increase the likelihood of SARS-CoV2 seropositivity.

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P4.02

CYTOKINE AND IMMUNE CELL PROFILING IN PERIPHERAL BLOOD FROM PATIENTS WITH COVID-19 TREATED WITH TOCILIZUMAB

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PURPOSE: To determine the effects of the anti-IL-6 receptor antibody tocilizumab (TCZ) in patients with COVID-19 and identify predictors of clinical outcome.

METHODS: 35 patients with COVID-19 treated with TCZ at the Azienda Unità Sanitaria Locale-IRCCS (Reggio Emilia, Italy) from February to April 2020 were included (median age: 64, range: 35-83 years). 29 patients received intravenous TCZ at 8 mg/kg, twice, 12 hour apart. 6 patients received subcutaneous TCZ at 162 mg twice simultaneously, one in each tight.1 Response to therapy was defined as an improvement of at least one point from the status at the beginning of TCZ using a six-category ordinal scale.1 Lymphocyte subsets were analyzed with the AQUIOS Tetra flow cytometer (Beckman Coulter). G-CSF, I-TAC, IFNα, IFNβ, IFNγ, IL-10, IL-17A, IL-18, IL-6, IL-7, IL-8, IL-9, IP-10, MCP-1, MIF, MIG, TNFα, VEGF were quantified in plasma by a multiplex-custom panel (eBioscience). Data were acquired before and after 3 days of TCZ therapy and analyzed by Wilcoxon matched pair and Mann-Whitney tests.

RESULTS: 16 patients showed clinical improvements after TCZ therapy while 19 patients did not. 3 day treatment with TCZ induced a decrease in neutrophil concentrations and an increase in platelet concentrations and monocyte percentages, particularly in patients with clinical improvements. Moreover, it induced a median increase of 13 folds in IL-6 and 7 folds in TNFα levels. At baseline, patients with better outcome had higher platelets/µl, higher percentages of monocytes, lower percentages of neutrophils and lower levels of IL-6, IL-10, MCP-1, MIF, IP-10. Lymphocytes subsets (CD3+CD4+, CD3+CD8+, CD3negCD19+, CD3negCD56/CD16+, CD3+CD56/CD16+) and the other cytokines did not change after therapy and were similar between responders and non-responders.

DISCUSSION: Herein data confirm the effects of TCZ on platelets, neutrophils and IL-6 reported by other authors while effects on lymphocytes could occur later.2

CONCLUSIONS: Administration of TCZ in COVID-19 patients had a restricted impact on circulating cytokines and immune cells in the short term. Quantification of blood cells and IL-6, IL-10, MCP-1, MIF, IP-10, TNFα could help in the management of COVID-19 patients.

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P4.03

IMMUNOPHENOTYPE ANALYSIS IN PATIENTS WITH SARS-COV-2 INFECTION ADMITTED TO THE IRCCS SACRO CUORE - DON CALABRIA HOSPITAL

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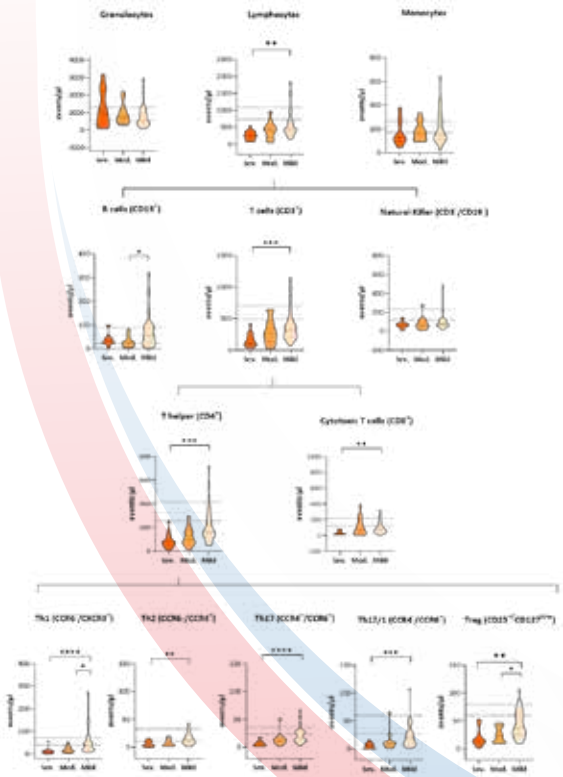
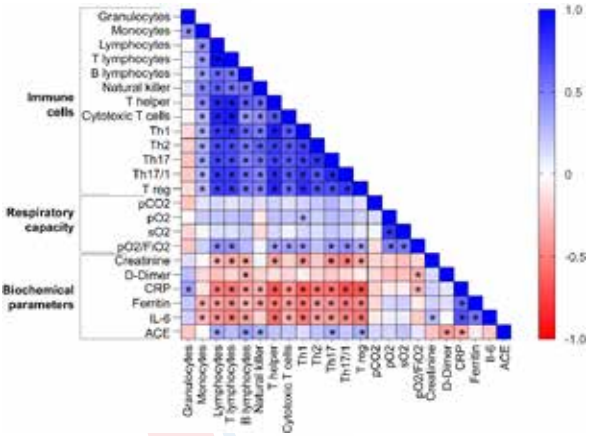
PURPOSE: This project wants to verify the hypothesis that the host immune response plays a pivotal role in the progression and outcome of the SARS-CoV-2 infection as already observed during the previous SARS-CoV and MERS-CoV pandemic [1,4]. To achieve this goal, we characterized the leukocyte immunophenotype of whole blood samples obtained from COVID-19 patients admitted to our hospital between March and May 2020. The variation in the frequency of the different lymphocyte subpopulations in association with the disease severity was evaluated and the correlation between immunophenotype and clinical data was performed.



METHODS: The immuno-phenotyping was performed by flow cytometry on peripheral blood obtained from COVID-19 patients [3]. The studied population encompassed 60 patients, 35 of which were men and 25 women, with an average age of 70 years. This population was divided into three subgroups - severe, moderate or mild - based on clinical presentation and respiratory capacity.

RESULTS: The immunophenotype analysis confirmed the association between the reduction of all lymphocyte subpopulation and the severity of the clinical presentation in COVID-19 patients. Patients suffering from a severe form harbored significantly reduced circulating virus-specific (Th1) and regulatory T lymphocyte populations (Fig1). Furthermore, the immunphenotype significantly correlated with clinical and biochemical parameters associated with the severity of SARS-CoV-2 disease. In particular, all T cell subpopulations, positively correlated with the pO2 / FiO2 ratio; on the contrary, all subpopulations negatively correlated with the inflammation markers (Fig 2).



DISCUSSION: This study demonstrates the association between the immunophenotype and COVID-19 disease severity. Our observations suggest that the analysis of the patient's immunological structure at the time of diagnosis should be taken into account to allow a better clinical stratification and management of patients. More in depth investigations including circulating cytokines and the association with patients' outcome will be performed to evaluate the clinical impact of the immonophenotype in COVID-19.

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P4.04

ACTIVATION, TRAFFICKING AND TURNOVER OF PERIPHERAL NK CELLS IN COVID-19 PATIENTSACTIVATION, TRAFFICKING AND TURNOVER OF PERIPHERAL NK CELLS IN COVID-19 PATIENTS

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PURPOSE: Evidence of immune involvement point to the participation of T, B, and NK cells in the lack of control of virus replication leading to COVID-19. NK cells contribute to early phases of virus control and to the regulation of adaptive responses but the mechanism of NK cell dysregulation tissue margination or turnover is poorly understood

METHODS: We investigated differences in peripheral blood circulating NK cell and CD34+ cell precursors in 28 consecutive COVID-19 patients compared to 18 healthy donors (HD) by multicolor flow cytometry.

RESULTS: Despite to comparable expression of activating NK cell receptor between HD and COVID-19 patients, a significant increase of the inhibitory NKG2A molecule density was observed in COVID-19 patients (17.3±9vs.3.6±1.4), without changes in the frequency of NKG2A+ NK cells. NK cells subset analysis showed that COVID-19 patients had increased proportions of tissue-trafficking CD49d+CD103+, CD69+CD103+, and CD69+CD49d+CD103+ NK cells and was also associated with the disease course. Significantly limited ability to cytotoxicity upon specific triggering was observed. The frequency of inflammatory precursors Lin-CD34+DNAM-1brightCXCR4+ was considerably increased in COVID-19 patients compared to HD (27.5±24. vs.3.01±3), as well as compared to HIV-1 patients (27.5±24 vs 9.8±11).

DISCUSSION: The observed intense NK cell activation with decreased functional activity with a related surge in inflammatory CD34+ precursors from the bone marrow (BM) suggest an unprecedented trafficking of NK cells from peripheral tissues, increased recruitment of emergency precursors from the BM and a relationship with the course of the disease. This in turn suggests possible areas of treatment and prevention.

CONCLUSIONS: The results indicate that an intense derangement of NK cell trafficking, activation, function, and turnover occurs early on, and is associated with the subsequent disease trajectory in hospitalized patients.

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P4.05

HIGH-THROUGHPUT SINGLE-CELL ANALYSIS REVEALED TRANSCRIPTIONAL RESPONSE OF DENDRITIC CELLS SUBSETS DURING SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 INFECTION

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PURPOSE: The interaction between the host immune system and the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still unclear. Dendritic cells (DCs) play a pivotal role during infections since they are specialized in antigen presentation and T cell priming. DCs are a heterogeneous population originally classified in plasmacytoid DCs, conventional DC1 (cDC1) and conventional DC2 (cDC2). Recently, single-cell RNA sequencing (scRNA-seq) studies revealed the complexity of the cDC2 subset, which was separated into two subpopulations, DC2 and DC3 [1] phagocytosis, and antigen presentation and consist of multiple specialized subtypes. However, their identities and interrelationships are not fully understood. Using unbiased single-cell RNA sequencing (RNA-seq. No specific information is available concerning the impact of SARS-CoV-2 infection on the functionality of DC subtypes and, given the pivotal role of DCs in the orchestration of the adaptive immune response, a systematic characterization of the transcriptional response of DC subsets during coronavirus disease 19 (COVID-19) may provide novel insights to understand the immune system's reaction to infection.

METHODS: We performed scRNA-seq analysis on available and newly generated datasets of peripheral blood mononuclear cells (PBMCs) from COVID-19 patients and healthy donors (HD). Using unsupervised clustering we identified DC subsets in COVID-19 patients and HD. We performed differential expression analysis to identify differentially expressed genes (DEGs) and we applied a systems biology approach to unravel signaling pathways that are altered by the identified DEGs.

RESULTS: This computational approach allowed us to isolate cDC1, DC2 and DC3 clusters. For each subset, we identified genes that are significantly up- or down-regulated during COVID-19 infection and the associated biological pathways.

DISCUSSION: Gene expression profiling revealed peculiar aspects in the responses of DC subsets of COVID-19 patients compared with HD, reflecting the activation status in response to the infection.

CONCLUSIONS: Using high-throughput single-cell analysis and advanced bioinformatics, we conducted a deep characterization of the transcriptional response of DC subsets during COVID-19 pathogenesis, providing new insights into the interaction between the immune system and the SARS-CoV-2 infection.

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P4.06

TOCILIZUMAB IN COVID-19: AN INTEGRATED APPROACH TO EVALUATE TREATMENT RESPONSE

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PURPOSE: Some patients with Covid-19 develop a hyperinflammatory syndrome resembling the cytokine storm after CAR-T cell therapy. In March 2020 tocilizumab - anti-IL-6 receptor monoclonal antibody - was proposed as immunomodulatory treatment in severe Covid-19. Indeed, IL-6 levels are correlated with SARS-CoV-2 viral load, disease severity, and prognosis. However, studies on the efficacy of tocilizumab in patients with Covid-19 are controversial, particularly considering differently enrolled populations.

METHODS: We compared in terms of clinical, laboratory, and radiologic findings 20 severe Covid-19 patients that received tocilizumab in addition to standard-of-care therapy (SOC) with 13 severe Covid-19 patients receiving only SOC. In 5 patients treated with tocilizumab we characterized via flow cytometry immune cell subsets of peripheral blood samples and in 13 patients we analyzed via 1H-NMR spectroscopy the metabolomic and lipidomic profiles of plasma-EDTA samples before and after tocilizumab.

RESULTS: Clinical respiratory status, inflammatory markers and vascular radiologic score significantly improved after tocilizumab administration. On the contrary, these parameters were stable or worsened in patients receiving only SOC. Patients treated with tocilizumab displayed an increased expression of both perforin and granzyme A in NK cells, as well as partial reversion of the metabolic alterations due to SARS-CoV-2 infection.

DISCUSSION: Despite study limitations, improvement of respiratory status (i.e. alveolar-arterial oxygen gradient) and vascular radiologic score could account for improved pulmonary vascular perfusion, as IL-6 mediates endothelial dysfunction and promotes a prothrombotic state. Treatment with tocilizumab restored the cytotoxic potential of NK cells and reverted metabolic alterations associated with SARS-CoV-2 infection. Consequently, blocking the IL-6 axis could be responsible for rapid pulmonary vascular improvement and recovery of protective antiviral potential in severe Covid-19 patients.

CONCLUSIONS: Immunopathology plays a crucial role in Covid-19, thus evaluation of response to immunomodulatory therapies should be based on an extensive approach that integrates clinical, laboratory, pathologic, and radiologic features.

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P4.07

CELL-MEDIATED AND HUMORAL ADAPTIVE IMMUNE RESPONSES TO SARS-COV-2 ARE LOWER IN ASYMPTOMATIC THAN SYMPTOMATIC COVID-19 PATIENTS, BOTH DURING INFECTION AND AFTER RECOVERING

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PURPOSE: The characterization of cell-mediated and humoral adaptive immune responses to SARS-CoV-2 in the acute and early convalescent, as well as in recovered individuals, is fundamental to understand COVID-19 progression and the development of immunological memory to the virus [1, 2].

METHODS: Multiparametric flow cytometric characterization of antigen specific T cells response and Ig specific serum levels were evaluated in 22 SARS-CoV-2 infected patients (8 asymptomatic and 14 hospitalized) during the infection phase, in 30 recovered patients after 5 months from SARS-CoV-2 infection and in 15 uninfected healthy controls.

RESULTS: We detected T cells reactive to SARS-CoV-2 proteins M, S and N, as well as serum virus-specific IgM, IgA, IgG, in nearly all SARS-CoV-2 infected individuals, but not in healthy donors. More importantly, symptomatic patients displayed a significantly higher magnitude of both cell-mediated and humoral adaptive immune response to the virus, as compared to asymptomatic. Then we found a heterogeneous magnitude of immunological memory at five months post infection since 20% of the subjects displayed a weak cellular and humoral memory to SARS-CoV-2. In particular individuals with an history of symptomatic COVID-19 was associated to higher levels of SARS-CoV-2 reactive CD4+ T cells and specific antibody levels compared to asymptomatic individuals.

DISCUSSION: The different levels of both cell-mediated and humoral immune responses to SARS-CoV-2 in symptomatic versus asymptomatic patients, suggest that a possible dysregulation of adaptive immunity in COVID-19 that could be related to different immunopathology. On the other hand, the divergence in antigen specific immune response observed in recovered patients might reveal subjects with higher risk of reinfection.

CONCLUSIONS: These results suggest that monitoring SARS-CoV-2 specific immune response in recovered patients could be important to develop effective timing in vaccination strategies.

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P4.08

EFFECTS OF TWO PHARMACOLOGICAL APPROACHES ON CYTOKINE STORM IN SEVERE COVID-19 PATIENTS

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PURPOSE: New Coronavirus disease opened many questions about the dynamics of immune responses to SARS-CoV-2 infection. The cytokine storm is a major feature of the severe development of COVID-19 [1;2]. As already available treatments, Tocilizumab was used to target IL-6, and Ruxolitinib as Jak1/Jak2 inhibitor. Investigating the role of dysregulated cytokines could enforce the perspective of a combined treatment targeting more than one molecule or pathway.

METHODS: 8 COVID-19 ICU patients and 13 COVID-19 patients were enrolled, respectively, for Tocilizumab and Ruxolitinib treatment. Phenotypic and functional properties of myeloid and lymphoid cell subsets were evaluated by flow cytometry and cytokine concentration levels were measured via Luminex Assay in plasma/ sera, before and after drug administration.

RESULTS: Before Ruxolitinib treatment, patients displayed reduction of circulating myeloid and plasmacytoid dendritic cells, activation markers on monocytes an increased terminal differentiation with impaired cytokine production by T cells, compared to healthy subjects. Ruxolitinib restored homeostasis of different immune cell subsets and induced a general decrease in levels of inflammatory cytokines, such as IL-1β, TNF-α and IL-8. Patients treated with Tocilizumab showed an evident change in many cytokines' levels, with a significant increase of IL-6 and reduction of CXCL10 levels.

DISCUSSION: The inhibition of the Jak signaling restores homeostasis with an anti-inflammatory effect, suggesting cytokine storm is strictly connected to immune response impairment. Higher levels of soluble IL-6 suggest efficiency of Tocilizumab binding with IL-6 receptor. A decrease in CXCL10 levels after anti-IL6 treatment offers a new direction for further investigation, since it increases with disease onset and possibly correlates with lymphopenia.

CONCLUSIONS: Cytokine levels variations after treatment with Tocilizumab and Ruxolitinib associate with improved clinical outcome. Thus, both IL-6 and CXCL10 could serve as biomarkers. Taken together, these results suggest cytokine storm is directly connected to illness degree in COVID-19.

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P4.09

CXCL-10 LEVELS AT HOSPITAL ADMISSION REPRESENT POTENTIAL PREDICTOR OF COVID-19 OUTCOME

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PURPOSE: Host inflammation determines whether SARS-CoV-2 infection causes mild or life-threatening disease. Tools are needed for early risk assessment.

METHODS: We studied, in a cohort of 111 COVID-19 patients prospectively followed at Opedale San Raffaele Hospital, fifty-three potential biomarkers of cell and tissue damage including alarmins, cytokines, adipocytokines and growth factors, humoral innate immune and neuroendocrine molecules, and regulators of iron metabolism. The concentration of biomarkers at hospital admission together with age, degree of hypoxia, neutrophil to lymphocyte ratio, lactate dehydrogenase, C-reactive protein and creatinine were analysed according to the transfer to intensive care unit (ICU) and survival. Classification and regression tree (CART) models were used to identify predictors of adverse outcome among biomarkers.

RESULTS: The classification tree analysis selected CXCL-10 at hospital admission, in combination with NLR and time from onset, as the best predictor of ICU transfer, while it was selected alone to predict death. CXCL-10 concentration abated in COVID-19 survivors after viral clearance and discharge.

DISCUSSION and CONCLUSIONS: CXCL-10 is the most robust predictive biomarker of patient outcome in COVID-19, and can be easily used to stratify patients according to the short-term risk of adverse outcome and establish appropriate clinical care intensity.

P4.10

SARS COV-2 CHILDHOOD INFECTION IMMUNOPATHOLOGY: THE COUNTERBALANCE OF TH2/TREG COMPARTMENT AND THE PROTECTIVE ROLE OF IMMUNOREGULATORY PROFILE

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PURPOSE: While the clinical impact of SARS-CoV-2 infection (COV) on adults has been massive, the majority of children develop pauci-symptomatic or even asymptomatic infection and only a minority of them die. The reasons of such differences are not yet established.

METHODS: We performed analyses of sera and cellular samples collected from 38 children, evaluating the impact of COV on cytokines in sera and on Th balance and B cell subpopulations. We correlated our results with clinical symptoms and compared them with infected adults and with non-infected children.

RESULTS: Unlike adult immunopathology we found low levels of pro-inflammatory cytokines in COV children, unrelated with disease severity. Interestingly, IL4 and IL2 levels were higher among asymptomatic children than the ones with moderate/severe clinical habits ($p<0,05$), while not detectable in non-infected children and COV adults. Moreover, a subgroup of COV children with mild symptoms showed relatively increased levels of IL10 in sera, undetectable in other patients. As expected, children presented wider Th and B cell subsets than adults: low numbers of IgD- B cells, and among them, of memory CD27+ B cells, significantly correlated with absent/mild symptoms, and, similarly, high amounts of inducible T regs seem to play a protective role. They were irrelevant in COV adult and healthy children. Finally, the follow up of COV children after three months, when they were all negative and asymptomatic, showed irrelevant differences of cytokines in sera, except for an unexpected increase of TNFα levels, and a significant reduction of IgD- B cells, while inducible T regs remained stable.

DISCUSSION: COV immunopathology in childhood is characterized by a complex immune response, that significantly changes according to age and disease severity. Specifically, IL6 and TNFα do not represent good prognostic biomarkers, suggesting the involvement of other mechanisms. The Th2-mediated immune response in early infected children immunoregulation could have a potential protective role.

CONCLUSIONS: Taken together these data evidence a different habit and outcome of the immunopathology of COV in the childhood, possibly hypothesizing an explanation of the low epidemiologic and clinical risk in young subjects.

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P4.11

DIFFERENT INNATE AND ADAPTIVE IMMUNE RESPONSES TO SARS-COV-2 INFECTION OF ASYMPTOMATIC, MILD, AND SEVERE CASES

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PURPOSE: SARS-CoV-2 is a novel coronavirus, not encountered before by humans. A wide spectrum of clinical expression of SARS-CoV-2 infection occurs, ranging from asymptomatic to mild to severe disease. In order to identify the immunological features of the different clinical forms of infection, we analyzed the immune response in 64 adults with diverse clinical presentations.

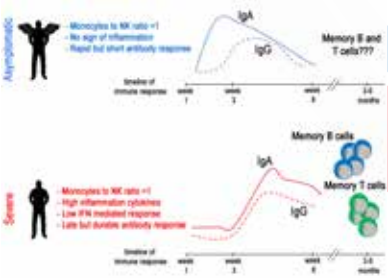
METHODS: We made a longitudinal study by standard flowcytometry comparing immune populations and we measured levels and kinetics of anti-SARS-CoV-2 antibodies in the serum.

RESULTS: High frequency of NK cells and early and transient increase of specific IgA, IgM and, to a lower extent, IgG are associated with asymptomatic SARS-CoV-2 infection. By contrast, monocyte expansion and high and persistent levels of IgA and IgG, produced relatively late in the course of the infection, characterize severe disease. Modest increase of monocytes and different kinetics of antibodies are detected in mild COVID-19. The ratio between monocytes and NK cells (MNKR) is a sensitive indicator of the individual reaction to the virus. The MNKR is below 1 in contacts and asymptomatic individuals and increases when monocytes expand, and NK cells are reduced in mild and severe patients.

DISCUSSION: The first response to a novel virus is characterized by the cooperation between NK cells and natural antibodies (NA) that contain the infection. Meanwhile adaptive immune responses develop and generate highly-specific memory cells that will clear the virus and prevent re-infection. SARS-CoV-2 has evolved in bats, which control the infection through their innate immune system, enriched for NK receptors and NA. Thus, NK and ready-to-use antibodies control viral infection in bats without the need of adaptive immune responses. Asymptomatic humans may behave like bats, controlling the infection thanks to NK cells and antibodies. The adaptive immune response is strongest in patients with severe disease causing the uncontrolled inflammatory reaction and tissue damage.

CONCLUSIONS

We propose that the MNKR and the levels of specific antibodies in the serum may be early markers of disease evolution. Low level of antibodies in the first 2 weeks and increase of the MNKR may indicate patients at risk for increased severity of disease.



P4.12

INDUCTION AND MAINTENANCE OF B-CELL MEMORY AFTER SARS-COV-2 VACCINATION IN A POPULATION OF HOSPITAL WORKERS

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PURPOSE: Coronavirus disease (COVID)-19 is a global health emergency. The project has the objective of evaluating the entity and duration of the antibody and cellular B cell response in a population of health care workers (HCWs) in the 1-year post vaccination with the COVID-19 mRNA BNT162b2 vaccine. Our aim is to assess the duration of immunity, generation and persistence of memory B cells (MBC) and effects on individuals with previous asymptomatic or mild SARS-CoV-2 infection.

METHODS: We have included in the study a total of 300 individuals, 200 without history of previous infection and 100 who have experienced SARS-CoV-2 infection. Blood samples from HCWs were collected before and 7 and 21 days after the first dose and 7 days after the second administration. During the next year, blood samples will be collected every 3 months. We will measure the frequency of Trimeric Spike (TS)-specific MBC by flow-cytometry using biotin-labelled TS and by ELISPOT to detect B cells secreting antibodies of IgM or IgG isotype specific for TS. In the serum, we will measure antibody against the N antigen, indicating a past or new infection, and vaccine-induced RBD and TS-specific antibodies.

RESULTS: Preliminary results show that BNT162b2 vaccine induces the rapid generation of specific IgM+ at day 7 and switched MBCs at day 21 (before the second dose). At day 28 circulating IgG plasma blasts can be detected. Serum anti-RBD and TS antibodies increased in 97.5% of individuals at day 21 and increased further at day 28, when 99.8% of subjects had responded to the vaccine.

DISCUSSION: The increase of TS-specific IgM B-cells by ELIspot at day 7 suggests that this vaccine rapidly activates the germline repertoire and favours a rapid adaptability of human MBC pool to SARS-CoV-2 challenge. MBC switch to IgG at day 28. Their persistence in time (1 year study) will be determined.

CONCLUSIONS: MBC generated by the vaccine will ensure the maintenance of protection in case of re-exposure to the virus. If MBC of IgM type persist in time they have the possibility of further shaping their B cell receptor, thus adapting it to viral variants.

5. INFECTIONS AND IMMUNITY

P5.01

DISSECTING THE ROLE OF IFNΓ IN THE ANTIVIRAL CD4+ T CELLS FATE

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PURPOSE: Although humoral and cellular immunity upon viral infection usually co-exist, sometimes one response emerges as dominant and is responsible for most of the antiviral activity. For example, vescicular stomatitis virus (VSV) infection induces early and potent neutralizing antibody (nAb) responses with strong Tfh development, whereas lymphocytic choriomeningitis virus (LCMV) infection induces very strong cellular responses supported by Th1 differentiation but weak nAb responses1. We have recently analyzed the transcriptional cell state of the Tfh- (VSV) and Th1- (LCMV) priming niches, and identified the spatiotemporal regulation of type I IFN expression as a critical regulator of antiviral CD4+ T cell polarization1. Our previous studies link the Tfh phenotype to the early type I interferon sensing, but they don't fully explain the strong Th1 differentiation observed during LCMV infection. In this study we aimed to elucidate the role of known Th1-polarizing cytokines in CD4+ T cell differentiation upon LCMV infection. Based on some preliminary results, we focused our attention on IFNg, whose role in influencing T helper cell polarization is still controversial.

METHODS: To address the role of IFNg in LCMV infection, we took advantage of IFNg neutralizing antibodies or we infected IFNg-deficient mice with LCMV. CD4 T cell and B cell activation were analyzed via flow cytometry. B cell responses were analyzed by antibodies production. Finally, we are employing confocal microscopy and intravital imaging to the spatiotemporal dynamics of CD4+ T cells and B cells within LNs.

RESULTS: We found that IFNg plays a key role in early CD4+ T cell differentiation upon LCMV infection, inducing Th1 cell polarization and suppressing Tfh cell development. Thus, blocking IFNg results in a shift in the equilibrium towards Tfh and humoral responses. Future studies will determine the cellular source for this Th1-polarizing cytokine and the mechanism by which IFNg exerts its function.

CONCLUSIONS and DISCUSSION: These experiments could unveil the mechanisms underlying the reduced Tfh differentiation and humoral response in the context of viral infections like LCMV and might instruct vaccine design strategies.

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P5.02

ROLE OF PLATELETS IN TUBERCULOSIS PATHOGENESIS

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PURPOSE: We have analysed the frequencies, phenotype and functional properties of platelets (PLT)in peripheral blood of patients with tuberculosis (TB)and their presence at the site of TB disease.

METHODS: Complete blood cell counts were measured in samples from active or cured TB patients, subjects with latent TB infection (LTBI) and healthy donors (HD). Luminex assay and FACS analysis were used to measure the concentrations of PLT mediators and the phenotype of PLT, respectively. Immunohistochemistry (IHC)was carried out in human tissue samples of TB. ELISA and luminometric assays were used for *in vitro* studies on the interaction between PLT and lymphocytes from TB patients or THP1-derived macrophages. Bioinformatic analysis of PLT releasate and statistical analysis of data were performed by using R Bioconductor package topGO and Graphpad prism5.0 software respectively.

RESULTS: Active TB patients have a higher PLT/lymphocyte *ratio* and an increased serum concentration of mediators related to PLT differentiation or activation, when compared to the other groups. PLT are localized around the granuloma lesions close to T lymphocytes and macrophages. Activated PLT inhibit Bacillus Calmette Guerin-induced T lymphocyte proliferation and IFN-gproduction and decrease multiplication of intracellular *Mycobacterium tuberculosis* (MTB) in infected macrophages. Proteomic analysis and neutralization studies identify TGF-b and PF4 as the factors responsible for the inhibition of T-cell response and enhancement of the bactericidal activity of macrophages, respectively.

DISCUSSION: ActivatedPLT differently modulate T lymphocyte and macrophage responses to MTBand these distinct effects are attributed to the release of soluble factors.

CONCLUSIONS: Altogether these results highlight the importance of PLT in TB pathogenesis.

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P5.03

EX VIVO STIMULATION OF MYCOBACTERIUM TUBERCULOSIS SPECIFIC TCD8+ HLAE RESTRICTED CELLS BY RECOMBINANT DETOXIFIED BORDETELLA PERTUSSIS TOXOID

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PURPOSE: Tuberculosis (TB) is one of the leading cause of death worldwide. Despite years of intensive research, Bacille Calmette-Guerin (BCG) remains the only licensed vaccine and has variable efficacy. In this study we have evaluated the immunogenic potential of a recombinant *Bordetella* (*B*) *pertussis* toxoid containing 5 *Mycobacterium tuberculosis* (MTB) epitopes recognized by HLA-E restricted CD8+ lymphocytes

METHODS: HLA-E antigen-specific CD8+ T cell lines were generated by *in vitro* stimulation of PBMCs obtained from TB infected patients upon two weeks of culture and used for evaluation of cytotoxic activity and intracellular cytokines expression. Samples were acquired on a FACS Canto II flow cytometer and data were analysed using FlowJo software

RESULTS: HLA-E–restricted CD8 T-cell lines generated upon *in vitro* stimulation with the *B. pertussis* toxoid display potent cytotoxic activities toward HLA-E-MTB peptide pulsed-target cells, superior to lines generated by culture with peptides. Furthermore, these HLA-E-MTB CD8+ T cell lines are able to produce IFN-**γ**, showing a Th1 profile

DISCUSSION: HLA-E is a conserved class Ib molecule characterized by a limited polymorphism in population. The lack of allelic variation in the peptide-binding groove may represents an advantage to design peptide-based vaccines. The recombinant protein made by insertion of 5 mycobacterial peptides into a *B. pertussis toxoid is able to induce more potent cytotoxic activity and* IFN-**γ** production than peptides alone, indicating that vaccines boosting HLA-E-restricted CD8+ T cells could represent an additional tool to achieve protective immune responses against MTB

CONCLUSIONS: Recombinant B. *pertussis* toxoid containing HLA-E-restricted MTB epitopes should be considered as a valuable approach to promote or boost activation of CD8 T cells in vaccine formulation or immunotherapy for TB

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P5.04

HOST-PATHOGEN INTERFACE IN STAPHYLOCOCCUS AUREUS-DEPENDENT OSTEOMYELITIS: EMERGING ROLES OF THE LONG PENTRAXIN PTX3

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PURPOSE: Osteomyelitis (OM) is a debilitating infection of the bone primarily caused by the opportunistic pathogen *Staphylococcus aureus* (SA)1. SA exploits several strategies to evade the immune response and subvert bone homeostasis, yet the underlying mechanisms are largely unclear2. We aimed to fill this gap by investigating the SA/bone microenvironment (BME) interface with a focus on the soluble pattern recognition molecule long pentraxin 3 (PTX3). Well-known for its functions in innate resistance to opportunistic pathogens and inflammation, PTX3 is emerging as a new player in bone homeostasis3.

METHODS: A murine model of SA intrabone (ib) infection was developed that recapitulates surgery/trauma-OM in humans. To define and characterize the role of PTX3 in the etiopathogenesis of SA-OM, wild-type (WT) and PTX3 knock-out (PTX3 KO) mice were used in the study.

RESULTS: Upon SA ib injection, >95% of mice developed bone infection in the treated limb only. Of note, the bacterial load was greater in the bone of WT *vs* PTX3 KO mice at 6 and 14 days from infection (f.i.). Accordingly, inflammation was more severe in WT than PTX3 KO animals, in terms of expansion of the innate immune cells in the spleen, and increase of inflammatory cytokines in the serum. PTX3 levels were augmented in SA-infected mice both in the serum (during the infection) and in the bone (at necropsy). Marked remodeling of the infected bone, with loss of trabecular bone and periosteal bone apposition, was observed in the SA-treated animals (both WT and PTX3 KO) at 14 days f.i.

DISCUSSION: Our results indicate a novel role of PTX3 in OM pathogenesis, pointing to an involvement of this pentraxin in the early bone adaptation strategies of SA, such as tissue adhesion, biofilm deposition, and abscess formation. In particular, we outline that in the absence of PTX3 the BME is a less permissive environment for SA infection.

CONCLUSIONS: Genetic deficiency of PTX3 protects from infection in a murine model of locally-induced SA-OM. Our data suggest a crucial role of PTX3 in the early phases of the disease. Ongoing work will provide additional mechanistic insights and possibly pave the way to treatment strategies.

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P5.05

THE SEROTONIN PATHWAY OF TRYPTOPHAN METABOLISM REGULATES INFLAMMATION IN THE LUNG

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PURPOSE: *Aspergillus fumigatus* is one of the highly abundant airborne fungal species and is responsible for significant morbidity and mortality in the population by eliciting a wide spectrum of diseases that depend not only on the degree of exposure, but also on the characteristics of the immune response [1]. A central role at the *Aspergillus*/host interface is thought to be played by tryptophan metabolites, including kynurenines and microbial-derived aryl hydrocarbon receptor-targeting indoles [2]. The role of a third metabolic pathway, known as the serotonin pathway, is largely unexplored.

METHODS: In the present study, we infected wild-type mice and mice deficient of tryptophan hydroxylase 1 (Tph1), the enzyme responsible for the peripheral synthesis of serotonin, with *A. fumigatus* conidia and assessed the severity of the disease and the underlying immune response.

RESULTS: Our results indicate that Tph1-deficient mice are more susceptible to infection with increased fungal burden and lung immunopathology, as revealed by the presence of perivascular and peribronchial inflammation. The use of a specific inhibitor of Tph1 or the exogenous administration of serotonin to wild-type or Tph1-deficient mice, respectively, corroborated these findings.

DISCUSSION: The results presented in this study extends the role of tryptophan metabolites in the regulation of the host immune response to *A. fumigatus infection* by unraveling the protective activity of Tph1, the enzyme responsible for the synthesis of serotonin. Whether serotonin itself, known to be toxic against *Aspergillus*, or other mediators along the different tryptophan pathways are responsible for the protective effects is presently under investigation.

CONCLUSIONS: At variance with what described in the gut, the serotonin pathway contributes to antimicrobial resistance in the lung by providing immune and disease tolerance.

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P5.06

THE MODULATION OF TOLL LIKE RECEPTORS/CD44 AXIS REGULATES CLINICAL FEATURES OF EXPERIMENTAL AND HUMAN MULTIPLE SCLEROSIS

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PURPOSE: Our hypothesis is based on the ability of the external environment to influence our immune system, and in particular T cells, in relation to our genetic background. We investigated a new mechanism through which pathogens and commensals can modify trafficking properties of activated T lymphocytes.

METHODS: The stimulation of the Toll-like Receptors (TLRs) expressed on them impact on the alternative splicing of the adhesion molecule CD44 inducing the T cell migration into target organs. We previously demonstrated that SJL/J and C57Bl/6 mice express different TLR2 polymorphisms, whose activation with different molecules impact differently on the ability of T cell to migrate [1]. We observed that T cell trafficking was CD44-dependent, in particular TLRs stimulations were able to up-regulate the mRNAs encoding for some specific CD44 isoforms (CD44vv), which were differentially expressed depending upon the genetic background of the mouse (i.e. CD44v8-v10 and CD44v9-v10 in SJL/J and CD44v8-v10 in C57Bl/6).

RESULTS: Analysis of different brain areas from SJL/J mice affected by Experimental Autoimmune Encephalomyelitis (EAE) during onset of the disease also revealed the upregulation of CD44v8-v10 and CD44v9-v10 in T cells infiltrating the forebrain, in association with the distribution of the active lesions. Translationally, the analysis of cerebrospinal fluid (CSF) and circulating CD4+ T, similarly to murine model, revealed an association with the upregulation of CD44 isoform(s) and the presence of gadolinium-enhancing lesions and after stimulation with TLRs ligands.

DISCUSSION: Together, these data suggest that TLRs collectively represent a pathway through which pathogens or commensals, of viral and bacterial origin, modify trafficking of antigen-activated T cells by modulating the relative ratios of CD44 isoforms to induce or inhibit T cells enter in specific areas of the CNS.

CONCLUSIONS: In prospective, these molecules could represent new potential target for drug therapies and TLRs/CD44 axis may have clinical implication, providing new tools for disease activity evaluation, prognosis and treatment efficacy.

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P5.07

XENOBIOTIC RECEPTORS CROSS-TALK IN LUNG INFECTION AND INFLAMMATION

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PURPOSE: Xenobiotic receptors (XR) are chemical-sensing transcription factors that play essential roles in the transcriptional regulation of drug-metabolizing enzymes. Beyond this function, they also regulate genes involved in cell proliferation, energy metabolism and immunity. Aryl hydrocarbon Receptor (AhR) and Pregnane X Receptor (PXR) are the most common XR that actively take part in the control of immune response and despite different expression in cells and tissues, a reciprocal regulation between these two receptors has been recently suggested1;2. However, the exact mechanism is still poorly understood.

METHODS: We have investigated the role and the cross-talk between PXR and AhR in the lung by resorting to *in vivo* models of infection with *Aspergillus fumigatus* and *Pseudomonas aeruginosa* in Pxr- and Ahr-deficient mice.

RESULTS: Despite low expression level of Pxr in the lung, Pxr-deficient mice were resistant to both *A. fumigatus* and *P. aeruginosa* infections. Histopathological analysis showed no or very low level of inflammation in the lung, confirmed by the low expression level of NLRP3 gene, pro-inflammatory cytokines and reduced neutrophil recruitment. Ahr levels were elevated in Pxr-deficient mice, but surprisingly Ahr activation dampened resistance to infection in these mice.

DISCUSSION: The results showed that both PXR and AhR contribute to the antimicrobial immune response in the lung either independently or via a fine regulation of innate immune receptors, such as the inflammasome NLRP3.

CONCLUSIONS: Our study suggests that the role of PXR and AHR may go beyond the xenobiotics metabolism to include a fine regulation of the inflammatory response via a mutual cross-talk.

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6. INNATE IMMUNITY

P6.01

INNATE IMMUNE REGULATION OF THE INFLAMMATORY PROCESS

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PURPOSE: The dialogue between innate and adaptive branches of the immune system is a central paradigm of modern immunology and *is v*ital for protection against infections as well as for the pathogenesis of autoimmune, allergic and inflammatory diseases. According to the current model, innate immune sentinels dispersed throughout peripheral tissues sense, via their pattern recognition receptors (PRRs), the presence of microbial clues or endogenous moieties released during an infection, are activated and migrate to the draining lymph node (dLN). This process enables a transfer of "information" from peripheral tissue to the dLN, where the antigen-dependent adaptive immune response against the pathogen is initiated. These events are required for the development and polarization of the adaptive immune response, underscoring a critical need to gain an in-depth mechanistic understanding of this process. Here, we will discuss how the innate immune system instructs the development of the inflammatory process thus shaping the final outcome of the immune response.

P6.03

MOLECULAR AND PHENOTYPICAL ANALYSIS OF IMMUNOSUPPRESSIVE NEUTROPHILS FROM G-CSF-TREATED DONORS AND CANCER PATIENTS

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PURPOSE: Immunosuppressive CD66b+ low-density neutrophils (LDNs), also known as PMN-myeloid derived suppressor cells (PMN-MDSCs), have been consistently reported within the mononuclear cell fraction of cancer patients1. While the immunosuppressive properties of PMN-MDSCs are quite established, our knowledge on their phenotypical and transcriptional profile is limited. In this context, we recently demonstrated that immunosuppressive neutrophils are present in great abundancy [in both the LDN and the normal-density neutrophil (NDN) fractions] in G-CSF-treated donors (GDs)2. Goal of this study is to define the transcriptional and molecular features of immunosuppressive neutrophils/PMN-MDSCs from GDs and cancer patients in order to define common specific signature/markers to distinguish these cells from *bona fide* neutrophils.

METHODS: Circulating LDNs/NDNs from GDs and cancer patients, as well as control NDNs from HDs, have been purified by cell sorting. Transcriptomic profile was evaluated by Smart-Seq2. Differentially expressed genes were identified using the Likelihood Ratio Test implemented in DESeq2; genes with adjusted p-value<0.05 were considered modulated. Potential interesting antigenic markers were validated at mRNA (by qRT-PCR) and protein (by flow cytometry) levels.

RESULTS: Preliminary results suggest that immunosuppressive neutrophils from GDs and PMN-MDSCs from cancer patients display a common distinct genetic signature. Gene ontology reveals that metabolic and immune pathways are strongly altered in these cell populations. In ongoing experiments, we are validating the expression of selected genes of interest.

DISCUSSION: In this study, by analysing the transcriptome of immunosuppressive neutrophils from GDs and PMN-MDSCs from cancer patients we defined specific common signatures/markers distinguishing immunosuppressive neutrophil populations from *bona fide* neutrophils.

CONCLUSIONS: Antigenic markers/signatures identified in this study may help identifying/isolating immunosuppressive neutrophils/PMN-MDCSs from the blood and/or tumour tissue of cancer patients.

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P6.04

FUNCTIONAL HCMV-CONTROLLING NKG2C+ NK CELLS ARE INCLUDED IN THE PROGENY OF NEWLY CHARACTERIZED INFLAMMATORY COMMON LYMPHOCYTE PRECURSORS

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PURPOSE: There is still limited knowledge on the origin and development of specialized NKG2C+ NK subsets. In view of this we characterized the NK cell progeny of CD34+DNAM-1brightCXCR4+ and of Lin-CD34-CD16+CD56-Perfneg isolated from patients with HIV-1, HCV, CMV chronic viral infections with attention to possible specific effector populations.

METHODS: Highly purified precursors were obtained by flow cytometric sorting were cultured in standard NK cell differentiation medium. Phenotypic and functional analyses on progenies were performed by multiparametric cytofluorimetric assays. Transcriptional signatures of NK cell progenies were studied by microarray analysis. Inhibition of CMV replication was studied by PCR.

RESULTS: Lin-CD34+DNAM-1brightCXCR4+ precursors from chronically infected patients rapidly differentiate into cytotoxic, IFNγ-secreting CD94/NKG2C+KIR+CD57+ NK cell progenies. Lin-CD34-CD56-CD16+Perf-CD94-CXCR4+ precursors are also endowed with generation potential to memory-like NKG2C+NK cells. Maturing NK cell progenies mediated strong HCMV-inhibiting activity. Microarray analysis confirmed a transcriptional signature compatible with NK cell progenies and with maturing adaptive NK cells.

DISCUSSION: The identification of novel developmental trajectories of circulating precursors provides an additional angle on maintenance of NK cell memory and may represent a tool for monitoring inflammation and lymphoid (re)generation. Skewing of the NK cell repertoire may represent a crucial resource for the control of replicating viruses.

CONCLUSIONS: During viral infections precursors of adaptive NK cells are released and circulate in the peripheral blood

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P6.05

LONGITUDINAL STUDY OF C1q AND ANTI-C1q AUTO-ANTIBODIES IN HOMOLOGOUS AND HETEROLOGOUS PREGNANCIES

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PURPOSE: C1q, the recognition molecule of the classical pathway of the complement system, plays fundamental roles in pregnancy. Lack of C1q is characterized by poor trophoblast invasion and pregnancy failure. This molecule can be the target of an antibody response: anti-C1q antibodies are present in several infectious and autoimmune diseases (1,2,3). The presence of these auto-antibodies has been detected also in 2-8% of the general population (4). Recent evidence indicates that women who undergo medically assisted procreation (MAP) techniques have an increased risk of developing pre-eclampsia (PE), particularly oocyte donation (OD) pregnancies (5). The aim of this study was to characterize the expression of C1q in OD pregnancies and compare circulating levels of anti-C1q Ab in healthy spontaneous, homologous, heterologous and PE gestations.

METHODS: Placenta and serum of 3 groups of women, followed through three trimesters, were collected: OD: oocyte donation recipients; OM: HOMOLOGOUS MAP women; Sp: spontaneous physiological pregnancy; PE: patients diagnosed with PE C1q gene expression was evaluated in placental tissues and C1q protein and anti' C1q antibodies were analysed by ELISA in sera of three trimesters of pregnancy.

RESULTS: Placental C1q transcripts showed lower level in PE and MPA patient groups compared to Sp pregnancies. Anti-C1q antibodies (Abs) were detected in sera of all patient groups. Higher levels of these Abs were present in the 1st trimester of healthy women compared to term pregnant sera.

DISCUSSION: MAP pregnancies, even if they do not manifest PE symptoms, showed alterations in C1q levels (comparable to PE patients) which could be an indication of immunological dysfunction at foeto-maternal interface.

CONCLUSIONS: C1q mRNA levels are lower in MAP placetal tissues. Anti-C1q Ab levels increased during the 1st trimester of spontaneous physiological pregnancy, but not in MAP or PE gestations. These data indicates a possible role of anti-C1q Abs in pregnancy and in the pathogenesis of PE. The role played by these Abs at foeto-maternal interface requires further investigation.

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P6.06

EFFECTS OF WHOLE-BODY CRYOTHERAPY ON THE INNATE IMMUNE RESPONSE IN FOOTBALL PLAYERS

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PURPOSE: Whole-body cryotherapy (WBC) is the short exposure (up to 2-3 minutes) to dry air at cryogenic temperatures (up to -190°C) and has recently been applied for muscle recovery after injury to counteract the inflammatory response [1,2]. Our study aimed to identify the effects of WBC on immunological, hormonal, and metabolic responses of non-professional football players (NPFPs).

METHODS: We enrolled nine male NPFPs (age: 20±2 years) on the same team, who played and trained regularly (each day), before, during, and after the treatment. Immediately before and after 5 once-a-day sessions of WBC, we collected blood samples for the evaluation of a full set of fifty analytes, including hematologic parameters, serum chemistry, and hormones profile. We performed the phenotyping of monocytes (Mo) and we quantified plasmatic markers usually increased during inflammation [CCL2, IL-18, free mitochondrial (mt)DNA] or with anti-inflammatory effects (IL2RA, IL1RN).

RESULTS: After the WBC treatment (WBC-t) we found reduced levels of ferritin, mean corpuscular hemoglobin, mean platelet volume, testosterone and estradiol, which however always remain within the normal ranges. The percentage of total Mo increased after the treatment and among them, classical Mo decreased while intermediates and non-classical ones increased. The expression of CXCR4 decreased in each Mo subset. IL18 and IL1RN decreased in plasma after WBC-t, while IL1RA showed a tendency to decrease. Circulating mtDNA levels were not altered by treatment.

DISCUSSION: The difference observed in monocytes subsets after WBC-t could be due to their redistribution into the surrounding tissue. Moreover, the decrease of CXCR4 in Mo subsets could be consistent with their differentiation process [3,4]. Thus, WBC through yet unknown mechanisms could promote their differentiation.

CONCLUSIONS: WBC seems to modulate the innate components of the immune system in such athletes, suggesting not only a beneficial anti-inflammatory effect but also a role in tissue repair [5].

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P6.07

ROLE OF CCRL2 IN THE REGULATION OF CXCR4- AND CXCR2-DEPENDENT NEUTROPHIL HOMING

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PURPOSE: The atypical C-C chemokine receptor-like 2 (CCRL2) can regulate in vitro and in vivo the activity of C-X-C chemokine receptor type 2 (CXCR2), through the formation of heterodimers on neutrophils1. The aim of this study was to verify whether CCRL2 is able to form heterodimers with C-X-C chemokine receptor type 4 (CXCR4), a receptor that plays a key role in the retention of neutrophils in the bone marrow (BM)2. The following aim was to investigate the functional role of CCRL2/CXCR4 heterodimerization in the regulation of senescent neutrophils homing in clearance organs.

METHODS: Förster Resonance Energy Transfer (FRET) analysis was used to study the interaction of CCRL2 with CXCR4. Neutrophils from BM of wild type (WT) and CCRL2 knock-out (KO) mice were used to perform in vitro characterization and in vivo adoptive transfer experiments. The results have been assessed by flowcytometry.

RESULTS: FRET analysis showed the ability of CCRL2 to heterodimerize also with CXCR4, both in human and murine neutrophils. Neutrophils isolated from BM of WT mice, treated with pro-inflammatory stimuli for 30 hours, show a co-expression of CCRL2 and CXCR4. The use of CCRL2 KO neutrophils allowed to identify a role for CCRL2 in the regulation of CXCL12 response in vitro and in the homing of senescent neutrophils to the BM and liver.

DISCUSSION: The data obtained suggest that CCRL2 may modulate the biological functions of chemokine receptors through the formation of heterodimers.

CONCLUSIONS: We have demonstrated that CCRL2 can heterodimerize not only with CXCR2 but also with CXCR4. These heterodimers can regulate senescent neutrophils homing in clearance organs.

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P6.08

HUMAN NEUTROPHILS ACTIVATED BY TLR8 AGONISTS, WITH OR WITHOUT IFNγ, SYNTHESIZE AND RELEASE EBI3, BUT NOT IL-12, IL-27, IL-35, OR IL-39

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PURPOSE: The present study investigates whether TLR8-activated human neutrophils express and release cytokines of the IL-12 family.

METHODS: Neutrophils were isolated from buffy coats at high levels of purity (>99.7 %) and then incubated with TLR8 agonists and other stimuli. Evaluation of cytokine gene expression by RNA-seq and RT-qPCR, as well as cytokine production by ELISA, flow cytometry, immunoblotting and immunohistochemistry (IHC), was then performed.

RESULTS: Neutrophils stimulated with TLR8 and other agonists (LPS, TNFα) were found to express transcripts for *IL-12B*, *IL-23A* and *EBI3* but not *IL-12A*, *IL-27A* or *IL-35A*. In accordance, TLR8-stimulated neutrophils were found to produce and release EBI3, IL-12B, IL-23A and, consequently, IL-23. However, no IL-12, IL-27, IL-35 nor the newly discovered IL-39 were detected in supernatants from TLR8-stimulated neutrophils. Induction of EBI3 and IL-23 was found to require endogenous TNFα, as both EBI3 and IL-23 mRNA and protein levels were blocked by TNFα-neutralizing antibodies in TLR8-activated neutrophils. Interestingly, by IHC we evidenced an *in vivo* EBI3 expression by neutrophils infiltrating tissues from diverticulitis, cholecystitis and *B. Henselae* infection. Finally, we found that neutrophils co-incubated with R848/LPS and IFNγ do not express or produce either IL-12 or IL-35, while they accumulate *IL-27A* transcripts, without however releasing IL-27.

DISCUSSION: Our study demonstrates that highly pure human neutrophils express and produce IL-23, further supporting the key roles played by these cells in the IL-17/IL-23 network and Th17 responses. Moreover, activated neutrophils produce remarkable amounts of EBI3, but none of the cytokines that include it, namely, IL-27, IL-35 and IL-39, indicating that EBI3 produced by neutrophils could act as either monomer or homodimer. Finally, contrary to previous findings, we show that IFN' is unable to activate IL-12A transcription in neutrophils, consequently impeding the production of IL-12/IL-35.

CONCLUSIONS: The present study demonstrates that TLR8-activated human neutrophils produce functional IL-23 and remarkable amounts of EBI3, but not IL-12, IL-35, IL-27 or IL-39.

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P6.09

SINGLE NUCLEOTIDE POLYMORFISMS IN IL1RN GENE AFFECT THE RESPONSE TO ANAKINRA IN SYSTEMIC JUVENILE IDIOPATHIC ARTHRITIS AND ALTERS IL1RN AND IL1B GENE EXPRESSION

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PURPOSE: Systemic juvenile idiopathic arthritis (sJIA) is an inflammatory disease. Interleukin-1 (IL-1) plays a pivotal role in the pathogenesis of the disease. Accordingly, treatment with the recombinant IL-1 receptor antagonist is efficacy in a significant portion of patients. The identification of non-responder patients is of primary importance to avoid the progression towards chronic arthritis. Recently, a cluster of single nucleotide polymorphisms (SNPs) in the *IL1RN* non-translated region has been suggested as a possible predictor of non-response to anakinra. The aim of this study was to evaluate the impact of these SNPs on the expression levels of *IL1RN* and of other genes of the locus, as well as their impact on the response to anakinra in sJIA patients.

METHODS: Response to anakinra was considered as clinically inactive disease (CID) at 6 months from the therapy start, without glucocorticoids treatment. Demographic, clinical and laboratory characteristics of 56 patients were analyzed. Six SNPs in the *IL1RN* gene were genotyped by qPCR or Sanger sequencing. Haplotype mapping was performed with Haploview software. Identification of SNPs potential target genes has been performed by searching in public Capture Hi-C databases. mRNA expression of several genes of the IL1B locus was assessed by qPCR in whole blood from patients and healthy donors.

RESULTS: 73.2% of patients met the criteria for CID. The six *IL1RN* SNPs were inherited as a common haplotype in our cohort of patients and the homozygosity for at least one high expression SNP correlates with higher *IL1RN* and *IL1B* mRNA levels but not with the expression levels of *IL1A*, *CKAP2L* and *PSD4*. The presence of high expression genotypes was associated with a 6-fold higher risk of non-response to treatment.

CONCLUSIONS: Our results confirm the importance of IL-1 inhibition in sJIA. Furthermore, genetic *IL1RN* variants predict non-response to IL-1 blockade therapy and identify potential new genes involved in the pathogenesis of the disease.

P6.10

PSORIASIS-RELATED MIR203 ACTIVATES NK CELLS BY A COMPLEX PDC/MONOCYTE/NK CELL CROSSTALK

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PURPOSE: We have recently described that exosomal GU-rich microRNAs (GU-miRNAs) activate plasmacytoid dendritic cells (pDCs) by triggering TLR71 and that pDCs may enhance Natural Killer (NK) cytotoxicity and tissue damage in Lupus Erythematososus2. Because both pDCs and NK cells also infiltrate psoriatic lesions3,4, we asked whether deregulated miRNA secretion may foster psoriatic tissue damage by activating pDCs and the crosstalk with NK cells.

METHODS: Following extraction from healthy and psoriatic skin, the expression of GU-miRNAs was investigated by RT-PCR. Synthetic miRNAs were used to stimulate purified NK cells, pDCs, peripheral blood mononuclear cells (PBMCs) or co-cultures of these cells. Cell activation was assessed in terms of cytokine secretion and target cell killing. Inhibitor experiments were performed to demonstrate TLR activation by miRNAs.

RESULTS: Several GU-miRNAs were upregulated in psoriatic skin lesions, with miR203 being the most expressed. pDCs but not purified NK cells were fully activated by miR203. By contrast, when miR203 was used to stimulate total PBMCs, NK cells were able to produce IFN-g and to kill target cells. Co-culture experiments of purified cell populations revealed that both pDCs and monocytes were required for NK cell activation by miR203. This action was depent on a TLR7/8-mediated release of IFN-a, IL-12 and IL-18 which were responsible for licensing NK cell response to miR203.

DISCUSSION: pDC/monocyte-licensing of NK cell response to TLR7/8-ligands adds a further level of complexity to innate immune cell crosstalk.

CONCLUSIONS: Deregulated exosomal miRNAs potentially activate a tissue-damaging innate immune crosstalk in psoriasis and may represent a novel mechanism involved in pathogenesis.

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P6.11

DIFFERENTIAL EXPRESSION AND REGULATION OF MS4A MOLECULES IN MYELOID CELLS IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

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The *MS4A* gene family encodes 18 tetraspanin-like proteins, most of which with unknown function. MS4A1, MS4A2 and MS4A3 have been shown to play an important role in immunity and, in the case of MS4A1, to represent an invaluable therapeutic target.

PURPOSE: The present investigation aimed to define a fingerprint of the expression of MS4A family members in human hematopoietic cells.

METHODS: We performed bioinformatic analysis of RNA sequencing datasets of human leukocytes and healthy tissues, RT-PCR analysis of *MS4A* genes in human circulating leukocytes, during *in vitro* macrophage (Mφ) differentiation and activation. Immunohistochemistry (IHC) staining of MS4A proteins in rheumatoid arthritis (RA) synovial tissue, human colon and lung was also performed. *MS4A3* expression was investigated in COVID-19 circulating neutrophils and in Acute Myeloid Leukemia (AML).

RESULTS: While *MS4A6A* is highly expressed by CD14+ circulating monocytes, *MS4A4A* is expressed by Mφ, *MS4A7* is not modulated during Mφ differentiation, and *MS4A3* is a marker of common myeloid progenitors in the bone marrow. *MS4A4A*, *MS4A6A* and *MS4A7* transcript levels are regulated by glucocorticoids *in vitro*, being generally upregulated by anti-inflammatory stimuli. IHC analysis revealed a higher percentage of MS4A7+ cells in the synovial tissue of RA patients treated with steroids, supporting the *in vivo* regulation of this protein by glucocorticoids. *MS4A3* is upregulated in immature "pro-neutrophils" of SARS-CoV-2 infected individuals, and in Acute Promyelocytic Leukemia (APL) *MS4A3* expression is also increased when comparing with other subtypes of AML.

DISCUSSION: Despite the redundancy of MS4A family members' expression in myeloid cells, different regulation was observed at different stages of maturation. The high expression of *MS4A3* in neutrophils of COVID-19 and APL patients likely reflects the maturation level of these cells. As part of the transcriptional signature of "pro-neutrophils", increased in severe SARS-CoV-2 infection, MS4A3 could have a role in monitoring COVID-19 disease severity.

CONCLUSIONS: While the functions of most MS4A proteins remains unknown, their differential expression and regulation strongly supports their importance in leukocyte differentiation or function, and potential as therapeutic targets or modulators.

7. MAST CELLS AND GRANULOCYTES

P7.01

VASCULAR ENDOTHELIAL GROWTH FACTORS AND ANGIOPOIETINS AS NEW PLAYERS IN MASTOCYTOSIS

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BACKGROUND: Mastocytosis is a disorder characterized by the abnormal proliferation and/or accumulation of mast cells in (MC) different organs. More than 90% of patients with systemic mastocytosis have a gain-of-function mutation in codon 816 of the KIT receptor on MC. The symptoms of mastocytosis patients are related to the MC-derived mediators that exert local and distant effects [1]. MC produce angiogenic and lymphangiogenic factors, including vascular endothelial growth factors (VEGFs) and angiopoietins (ANGPTs) [2].

PURPOSE: The aim of this work was to evaluate the serum concentrations of VEGF-A, VEGF-C, VEGF-D, ANGPT1 and ANGPT2 in patients with different variants of mastocytosis and the expression of angiogenic and lymphangiogenic factors in human MC lines with or without D816V mutation.

METHODS: Serum concentrations of VEGFs and ANGPTs were determined in 64 mastocytosis patients and 64 healthy controls. Intracellular concentrations and spontaneous release of these mediators were evaluated in the MC lines ROSAKIT WT and ROSA KIT D816V.

RESULTS: VEGF-A, ANGPT1, ANGPT2 and VEGF-C concentrations were higher in mastocytosis patients compared to controls. The VEGF-A, ANGPT2 and VEGF-C concentrations were correlated with the symptom severity by contrast ANGPT1 concentrations were increased in all patients compared to controls. ANGPT2 levels were correlated with severity of clinical variants and with tryptase levels. VEGF-A, ANGPT1 and VEGF-C did not differ between indolent and advanced mastocytosis. ROSAKIT WT and ROSAKIT D816V contained and spontaneously released VEGFs and ANGPTs.

DISCUSSION: Serum concentrations of VEGFs and ANGPTs are altered in mastocytosis patients

CONCLUSIONS: The use of angiogenic/lymphangiogenic inhibitors could be considered for the treatment of selected patients with severe mastocytosis and high levels of circulating angiogenic factors.

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P7.02

NEUTROPHILS AND NEUTROPHIL EXTRACELLULAR TRAPS IN SEVERE BRONCHIAL ASTHMA

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PURPOSE: Asthma is a chronic respiratory disease, characterized by airway obstruction, bronchial hyperresponsiveness and low-grade inflammation1. Neutrophils (PMNs) are the first line of defence, they can bind and kill pathogens, with their antimicrobial arsenal, including granular enzymes and proteins, reactive oxygen species (ROS) and neutrophil extracellular traps (NETs)2. The role of PMNs and NETs in severe asthma is still largely unknown.

METHODS: 10 patients with severe asthma and 13 healthy controls (HCs) were prospectively recruited. PMNs were isolated from peripheral blood and evaluated for ROS production and activation status upon bacterial stimulation with LPS (lipopolysaccharide) and fMLP (*N*-Formylmethionyl-leucyl-phenylalanine). Plasma levels of myeloperoxidase (MPO), CXCL8 and matrix metalloproteinase-9 (MMP9) were measured by ELISA. Plasma concentrations of Histone CitH3 were used to evaluate NETs levels3.

RESULTS: Peripheral blood PMNs from asthma patients displayed reduced ROS production and activation status compared to HCs upon bacterial stimulation. Asthma patients displayed higher circulating levels of MPO, CXCL8, MMP9 and Histone CitH3 compared to HCs.

DISCUSSION: Neutrophilic inflammation could be involved in certain phenotypes of severe asthma. Indeed, PMNs were increased in the sputum of patients with severe asthma4, displayed increased autophagy and NET release compared to HCs5.

CONCLUSIONS: Our results show that PMNs of asthma patients display reduced ROS production and activation status upon bacterial stimuli. These patients display higher circulating levels of MPO, CXCL8, MMP9 and Histone CitH3. Collectively, our results suggest that neutrophil-derived mediators could be involved in severe asthma. A large cohort of asthma patients with different phenotypes (T2-low *vs* T2-high) will allow identifying potential neutrophil-related markers predictive of disease severity and/or therapeutic response.

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P7.03

CHARACTERIZATION OF THE TRANSCRIPTIONAL PROFILE OF CIRCULATING GRANULOCYTES IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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PURPOSE: Chronic Obstructive Pulmonary Disease (COPD) is recognized as a systemic disorder, characterized by high levels of pro-inflammatory molecules and alterations in circulating leukocytes, despite the primary site of the disease is the lung. Neutrophilia and alteration of granulocytes functions is widely accepted systemic hallmark of COPD. Nevertheless, few and often contradictory studies have been performed to characterize functional and molecular features of circulating neutrophils in COPD patients. With this background, the aim of our study is to characterize transcriptomic alterations of circulating COPD granulocytes and their association with clinical features.

METHODS: Circulating granulocytes were purified from whole blood of 22 male COPD and 20 age- and sex-matched control donors. Transcriptomic analysis was performed using the 3'mRNA sequencing, differential expression analysis was performed using DESeq2.

RESULTS: 760 protein coding genes are differentially expressed in COPD granulocytes as compared to controls (p-adj<0.05). Principal component analysis shows that combination of PC1 and PC2 is sufficient to discriminate between granulocytes from COPD and controls. Moreover, while PC1 significantly correlates with COPD status (r=0.56, p<0.001), PC2 discriminate two different groups of COPD donors and negatively correlates with pulmonary hyperinflation parameters (RV/TLC, r=0.48, p<0.05). Gene ontology analysis reveals that COPD granulocytes are mainly characterized by alteration of metabolic and biosynthetic processes and altered regulation of gene expression both at transcriptional and post-transcriptional levels. Finally, the enriched GO terms were subjected to variation and correlation analysis.

CONCLUSION: Interestingly, this analysis allowed us to identify the existence of specific GO terms associated with clinical features of the COPD subjects enrolled in this study.

8. MONOCYTES AND MACROPHAGES

P8.02

MACROPHAGE-POLARIZING STIMULI DIFFERENTIALLY MODULATE THE INFLAMMATORY PROFILE INDUCED BY THE SECRETED PHOSPHOLIPASE A2 GROUP IA IN HUMAN LUNG MACROPHAGES

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PURPOSE: Macrophages are central players in several pathological conditions related to chronic inflammation, such as cancer1. In this study, we investigated the effects of snake venom Group IA secreted phospholipase A2 (svGIA)2,3 on the release of inflammatory and angiogenic mediators4 from human lung macrophages (HLMs).

METHODS: HLMs were preincubated with lipopolysaccharide (LPS) or svGIA before stimulation with macrophage-polarizing stimuli (IL-4, IL-10, IFN-γ or the adenosine analogue NECA). TNF-α, IL-6, IL-10, CXCL8, CCL1, VEGF-A, Angiopoietin (ANGPT) 1 and ANGPT2 levels were measured in HLMs supernatants by using ELISA. HLMs were preincubated with lipopolysaccharide (LPS) or svGIA before stimulation with macrophage-polarizing stimuli (IL-4, IL-10, IFN-γ or the adenosine analogue NECA). TNF-α, IL-6, IL-10, CXCL8, CCL1, VEGF-A, Angiopoietin (ANGPT) 1 and ANGPT2 levels were measured in HLMs supernatants by using ELISA.

RESULTS: M2-polarizing cytokines (IL-4 and IL-10) inhibited TNF-α, IL-6, CXCL8 and CCL1 release induced by both LPS and svGIA. IL-4 inhibited also the release of IL-10. IFN-γ reduced IL-10 and increased CCL1 release by both the LPS and svGIA-stimulated HLMs. In addition, IFN-γ promoted TNF-α and IL-6 release from svGIA-stimulated HLMs to a greater extent than LPS. NECA inhibited TNF-α but promoted IL-10 release from LPS-stimulated HLMs according to the well-known effect of adenosine in down-regulating M1 activation. By contrast NECA reduced TNF-α, IL-10 and CCL1 release from svGIA-activated HLM. IL-10 and NECA increased both LPS- and svGIA-induced VEGF-A release. By contrast, IL-10 reduced ANGPT1 production from activated HLMs. IFN-γ and IL-4 reduced VEGF-A and ANGPT1 release from both LPS- and svGIA-activated HLMs. Moreover, IL-10 inhibited LPS-induced ANGPT2 production.

DISCUSSION: Collectively, our results demonstrate for the first time that svPLA2 can induce the release of several immunomodulatory cytokines, chemokines and angiogenic factors from HLMs.

CONCLUSIONS: Our results extend the role of sPLA2 as relevant players in the scenario of macrophage-related inflammation.

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P8.03

DECIPHERING THE FATE OF SLAN + -MONOCYTES IN HUMAN TONSILS BY GENE EXPRESSION PROFILING

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PURPOSE: slan+-monocytes represent a subset of the "non-classical" (NC) CD14dim/-CD16++ monocytes characterized by the expression of the so-called "slan" antigen. Slan+-cells can be identified also in peripheral tissues under a variety of inflammatory or neoplastic conditions. However, their origin from slan+-monocytes as well as their terminal differentiation state toward macrophages or dendritic cells (DCs) were only partially resolved.

METHODS: Cells from tonsil (slan+-cells, conventional CD1c+DCs (cDC2) and CD11b+CD14+-macrophages) and blood (cDC2, classical (CL), intermediate (INT), NC, and slan+-monocytes) were isolated by cell sorting and subjected to RNA-seq using Smart-seq2 protocol.

RESULTS: Transcriptomic data support the notion that tonsil slan+-cells mainly represent macrophage-like cells with distinct features from those of tonsil CD11b+CD14+-macrophages or cDC2. Moreover, gene expression profiles suggest that tonsil slan+-cells derive from peripheral slan+-monocytes, as also supported by results obtained from in vitro treatment of slan+-monocytes with tonsil-derived conditioned medium. Interestingly, gene onthology analysis of DEGs specific for tonsil slan+-cells revealed functions related to efferocytosis and extracellular matrix organization. Of note, we also validated new slan+-macrophage markers suitable for IHC of archivial human tissue, thus allowing retrospective studies on various diseases including cancer.

DISCUSSION: Recent studies have reported that, in tonsils, slan marks DC-like cells, as defined by morphological, phenotypical, and functional criteria. However, subsequent investigations in lymphomas have uncovered a significant heterogeneity of tumor-infiltrating slan+-cells, including a macrophage-like state. Our results indicate that the slan antigen represents a macrophage marker, possibly related to alternatively activated (M2) subtype.

CONCLUSIONS: All in all, our results not only support the use of the slan antigen to investigate the fate of NC/slan+-monocytes in tissues, but are relevant as they expand our knowledge on slan+-cells in inflammatory diseases and, by analogy, in cancer.

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P8.04

ROLE OF NAMPT/VISFATIN AND ADIPOKINES CLUSTER IN INFLAMMATION-ASSOCIATED OSTEOARTHRITIS

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PURPOSE: Ageing is intimately linked to "homeostatic frailty", a condition associated with Osteoarthritis (OA). The NAMPT (Nicotinamide phosphoribosyltransferase)/NAD+/SIRT1 axis has been recently identified as a modulator of immunometabolism of myeloid cells under stress conditions1. Impairment of this axis is observed in elderly, leading to metabolic and inflammatory disorders2. The first aim of this work is to investigate the role of adipokines (visfatin/NAMPT, chemerin, leptin, adiponectin) as new potential biomarkers of disease severity in OA patients and in a mouse model of OA. The second aim is to define the role of NAMPT in myeloid compartment using myeloid-targeted NAMPT deficient mice.

METHODS: 47 patients with knee OA and 70 healthy controls (HC) were tested for the evaluation of circulating levels of adipokines by ELISA. Experimental model of OA was induced in C57BL/6 mice by the injection of 10 U of collagenase from *C. Histolyticum* into knee joints. Synovial washout was collected after 24h for the analysis of myeloid populations by flow cytometry and serum for the evaluation of adipokines levels.

RESULTS: The analysis of circulating adipokines showed higher levels of chemerin (227.1±11.3 *vs* 172.2±5.5 ng/ml, P<0.0001) and leptin (20.01±2.5 *vs* 11.1±1.3 ng/mL, P= 0.001) in OA patients when compared to HC. Mouse chemerin resulted increased in aged mice (12 months) compared with younger ones (12 weeks) (374±19 *vs* 317.5±11.5 ng/mL, P= 0.01, n=17 *vs* 9). Characterization of synovial fluid cells showed an increased recruitment of myeloid cells in collagenase- compared to PBS-injected knees (Fold of increase: 4.29, Collagenase/PBS, P=0.01).

CONCLUSIONS: Adipokines analysis suggested that chemerin and leptin may represent potential biomarkers for OA disease. Our *in vivo* study demonstrated in wild type OA knees an increased myeloid cell recruitment. Ongoing experiments on myeloid-specific NAMPT deficient mice will address the role of NAMPT in OA development.

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9. NEUROIMMUNOLOGY

P9.01

AMYOTROPHIC LATERAL SCLEROSIS: THE ENGAGMENT OF NATURAL KILLER CELLS

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PURPOSE: Amyotrophic lateral sclerosis (ALS), associated to muscle weakness and eventual paralysis, is a motor neuron disease characterized by the degenerative alterations in upper and lower motor neurons. Previous studies (1,2) evidenced the alterations of peripheral immune cells in ALS patients even though their involvement in the pathogenesis needs to be elucidated. Here, we have investigated on NK cells in ALS patients, analysing their frequency in peripheral blood, their phenotype and their correlation with stage of disease and with the rate of disease progression (ΔFS).

METHODS: Patients with diagnosis of ALS, other moto neurone diseases (MND), among which primary lateral sclerosis (PLS) and progressive muscular atrophy (PMA), and primary progressive multiple sclerosis (PPMS) as control were enrolled. NK cells in blood samples were evaluated by flow cytometry.

RESULTS: We observed an increase in the absolute number (cells/μL) of circulating NK cells in ALS patients compared to MND and PMMS. Within ALS patients, no difference in the absolute number of NK cells was observed in bulbar and spinal ALS. Differential expression of CD16 and CD56 on CD3- cells showed a significantly higher frequency of the CD16brightCD56dim subset of NK cells, well known as cytotoxic cells, compared to the CD16dimCD56bright subset with cytokine production profile in ALS, either bulbar than spinal. Furthermore, NK cells, both as percentage and absolute number, increased regarding of the clinical stage, assessed according to King's staging system, and Spearman's correlation analysis revealed a negative correlation between ΔFS score, evaluated at the time of blood collection, and the absolute number of NK cells.

DISCUSSION: These preliminary results prompt to hypothesize that NK cells are involved in pathogenesis and disease progression of ALS.

CONCLUSIONS: The analysis of NK cells in the celebrospinal fluid of ALS patients could better clarify their involvement in the early phase of the disease and their potential as therapeutic target.

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P9.02

INTRACELLULAR CONTENT OF SUPEROXIDE DISMUTASE-1 IN T LYMPHOCYTES ASSOCIATES WITH INCREASED REGULATORY T CELLS IN MULTIPLE SCLEROSIS INDIVIDUALS UNDERGOING IMMUNE-MODULATING TREATMENT

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PURPOSE: Reactive Oxygen Species (ROS) are involved in T cell activation. ROS-dependent networks are usually mediated by peroxides, more stable and freely diffusible inside cells. Super Oxide Dismutase (SOD)-1 mediates peroxide generation. We previously described SOD-1 involvement in T cell activation [1], its reduction in Cerebro-Spinal Fluid and leukocytes of Multiple Sclerosis (MS) subjects and increased SOD-1 levels after immune-modulation [2]. This project investigates on the possibility that SOD-1 might be involved in reshaping pro-inflammatory response in MS.

METHODS: SOD-1 content in T cells, phenotype and cytokine profile have been evaluated by immune-fluorescence and flow-cytometry.

RESULTS: We found that MS subjects can be divided in two sub-groups according to SOD-1 content in T cells. Group 1, with SOD-1 level similar to controls, and Group 2, with SOD-1 higher than controls. Group 2 showed increased Treg expressing Foxp3-exon 2, largely associated to effective suppression, as compared with Group 1. Addition of recombinant SOD-1 to activated T cells mediates increased IL-17 production. This effect was dependent on the activity of the enzyme. Indeed, rhApo-SOD-1, without enzymatic activity, was unable to affect T cell cytokine profile.

DISCUSSION: Compelling evidence indicate that SOD-1 represents a major target of mTOR enzyme [3], largely associated with Treg expansion and differentiation. Our results, strongly support such finding. Moreover, the possibility to target SOD-1 for innovative therapeutic approaches and/or for prognostic purpose represents an intriguing working hypothesis.

CONCLUSIONS: Our data suggest that high SOD-1 content in T cells might affect mTOR/SOD-1 functional relationship also interfering with T cell cytokine profile in MS. The mechanisms underlying such effects need to be investigated.

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P9.03

THE USE OF ARUNDIC ACID AS NOVEL THERAPY FOR MULTIPLE SCLEROSIS

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PURPOSE: In recent years, numerous studies have investigated the neuroprotective effects of arundic acid (AA), which is known to inhibit the astrocytic synthesis of the alarmin S100B in animal models of central nervous system (CNS) diseases (1). In the light of recent findings indicating a protective role exerted by Pentamidine, which is known to block S100B action, in Multiple Sclerosis (MS) (2), we explored a possible role of AA aiming for novel effective therapeutic solutions for MS.

METHODS: The administration of AA in mice affected by experimental autoimmune encephalomyelitis (chronic progressive form, P-EAE) was able to block or delay the onset of the acute phase of the disease, as well as to decrease the intensity of symptoms and improve biomolecular and histopathological parameters.

RESULTS: The treated P-EAE group of mice showed lower severity of cumulative disease score compared with vehicle-treated mice, and particularly in the early phase of disease onset that includes the first acute phase and the onset. The measurement of enzymatic activity of NOS and ROS in P-EAE mice showed that the treatment with AA has an antioxidant effect decreasing ROS and NOS to control levels. Quantitative PCR assay performed in total mRNA samples extracted from different brain areas of mice showed a reduction of cytokines IL1beta and of S100B in AA-treated compared to untreated P-EAE animals. The ability of AA to modulate S100B was particularly evident in posterior brain regions as demonstrated also using ELISA assay. We also performed morphological studies to dissect the impact of AA on different areas of the brain during P-EAE. The treatment with AA was efficient in the control of astrocytosis and demyelination at different levels of CNS, while it downmodulated infiltrates and microglia activation particularly in the cerebellar areas and in spinal cord.

DISCUSSION: In the light of these effects, AA might show effective applications in the prevention and/or treatment of MS.

CONCLUSIONS: The administration of AA during the acute phase of the disease, concomitant with the blood-brain barrier damage that is known to accompany this phase, facilitating the passage to the nervous tissue of the drug.

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P9.04

GENE EXPRESSION PROFILING IDENTIFIES SPECIFIC SIGNATURES IN PERIPHERAL BLOOD MONOCYTES OF MULTIPLE SCLEROSIS PATIENTS

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PURPOSE: Identify transcriptional differences between monocytes of Multiple Sclerosis (MS) patients and healthy controls (HCs); 2) identify novel biomarkers for potential use in the diagnosis and in MS treatment.

METHODS: Peripheral blood monocytes of female HCs, relapsing-remitting (RR) and primary-progressive (PP) patients have been studied by using a Transcriptomic approach. qRT-PCR was chosen to validate our findings. We first validated 5 HC; 6 RR2; 4 RR1; 1 PP and then we extended the analysis to a 2nd cohort of 7 HCs; 5 RR; 7 PP.

RESULTS: The RRs of the 1st cohort were stratified into two subgroups, recalled as RR1 and RR2. The RR1 patients' expression pattern was more similar to the PP, whereas the RR2 profile was more similar to the HCs. The most dysregulated process was that of Cholesterol Biosynthesis, both in RR1 and in PP of the 1st cohort. qRT-PCR confirmed these data in both 1st and 2nd cohort but with specific differences based on the analysed patient.

DISCUSSION: Monocytes play a crucial role in the MS pathogenesis1, so we decided to investigate their role through a transcriptomic approach. We seen that RR patients were very variable and that the Cholesterol biosynthesis process was induced in PP and RR1 monocytes. The activation of these genes could predict the disease activity and different forms of MS, so we tested the same signatures in a 2nd cohort of MS patients. Interestingly, the results obtained by qRT-PCR showed a strong upregulation of the Cholesterol genes also in this cohort.

CONCLUSIONS: We hypothesize that metabolic changes in monocytes may be crucial for MS progression. A general upregulation of the Cholesterol genes was observed, but since we observed differences based on the analysed patient, a personalized approach should be applied.

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10. NK CELLS AND ILC

P10.01

PERIPHERAL BLOOD NATURAL KILLER CELLS IN PROSTATE CANCER PATIENTS ACQUIRE THE DECIDUAL-LIKE CD56BRIGHTCD9+CD49A+ PHENOTYPE AND SUPPORT ANGIOGENESIS IN VITRO, ACTING ON ENDOTHELIAL CELLS AND POLARIZING MACROPHAGES TOWARD THE M2-LIKE/TAM PHENOTYPE

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PURPOSE: Natural killer (NK) cells, effector lymphocytes of the innate immunity, have been shown to be altered in several cancers, both at tissue and peripheral levels. We aimed to phenotype and functional characterize peripheral blood (PB) NK cells in prostate cancer (PCa) patients and to investigate the direct and indirect (crosstalk with monocytes/macrophages) contribution of NK cells to PCa progression and angiogenesis.

METHODS: NK cell subset distribution in PB of PCa patients was investigated by multicolour flow cytometry. Protein arrays were performed to characterize the secretome on FACS sorted NK cells. Conditioned media (CM) from FACS-sorted PCa PB-NKs were used to determine their ability to induce pro-inflammatory/pro-angiogenic phenotype/functions in endothelial cells, monocytes, and macrophages. CM from three different PCa (PC-3, DU-145, LNCaP) cell lines were used to assess their effects on human NK cell polarization *in vitro*, by multicolor flow cytometry.

RESULTS: We found that PCa PB-NKs acquire the CD56brightCD9+CD49a+CXCR4+ phenotype and are exhausted. Similar effects were observed on healthy donor-derived PB-NK cells, exposed to CM of three different PCa cell lines. PB-NKs from PCa patients released factors able to support angiogenesis and increased the expression of CXCL8, ICAM-1, and VCAM-1 mRNA in endothelial cells. Secretome analysis revealed the ability of PB-NKs from PCa patients to release pro-inflammatory cytokines/chemokines involved in monocyte recruitment and M2-like polarization. Finally, CMs from PB-NKs from PCa patients recruit THP-1 and peripheral blood CD14+ monocytes and polarize THP-1 and peripheral blood CD14+monocyte-derived macrophages towards M2-like/TAM macrophages.

DISCUSSION: Our results show that PB-NKs from PCa patients acquire pro-inflammatory/pro-angiogenic phenotype and functions, acting directly on endothelial cells and indirectly by using polarized monocytes/macrophages as bystander cells.

CONCLUSIONS: Our data provides a rationale for a potential use of PB-NKs to profile the NK cell polarization state in PCa patients.

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P10.02

EDUCATION OF MURINE NATURAL KILLER CELLS – CELL BIOLOGICAL AND MOLECULAR CORRELATES

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PURPOSE: Natural Killer (NK) cells express activating and inhibitory receptors recognizing ligands such as MHC class I molecules expressed on target cells. The Ly49 receptor family is responsible for recognition of MHC I molecules in mice. The NK system adapts to self by rendering cells "hyporesponsive", if they do not receive inhibitory signals from surrounding cells, which means that NK cells cannot attack normal healthy cells even if the latter are devoid of MHC class I molecules. Here, we investigated the correlation between responsiveness and intracellular granule patterns in murine NK cells.

METHODS: NK cells were isolated from splenocytes of C57BL/6 (B6) and B6 β2microglobulin -/- (β2m-/-) mice by immunomagnetic depletion. Granzyme content was measured by flow cytometry and confocal microscopy. Surface markers were stained for Ly49C, biotin Ly49I, NK 1.1, CD3. NK cell subpopulations were sorted with flow cytometry, followed by intracellular staining of Granzyme A.

RESULTS: Granules in hyporesponsive Ly49C+ NK cells subset are smaller, less intensely stained and more numerous.

DISCUSSION: NK cells become more responsive if they bind to self MHC class I molecules. This adaptation process is called education. NK cells without such inhibitory interactions would instead slowly leak out content from small granules, which makes them less responsive and unable to attack healthy normal cells, while still maintaining a low baseline function.

CONCLUSIONS: Preliminary data support the hypothesis. Further analyses investigating additional mouse strains and Ly49I interactions with non-MHC class I ligands will provide more robust evidences about the role of education on NK cells responsiveness.

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P10.03

FREQUENCIES AND FUNCTIONAL PROPERTIES OF INNATE LYMPHOID CELLS ARE ALTERED IN MELANOMA PATIENTS AND MODULATED BY IMMUNE CHECKPOINTS INHIBITORS

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PURPOSE: The use of monoclonal antibodies targeting immune checkpoints has improved the prognosis of malignant melanoma (1). However, the underlying mechanisms are still unclear. Since checkpoint receptors are expressed by helper innate lymphoid cells (ILCs) (2), we analyzed peripheral ILC subsets and secretory function in metastatic melanoma patients undergoing anti-immune checkpoints therapies.

METHODS: *Cells.* PBMCs from healthy donors and stage IV melanoma patients were isolated by gradient centrifugation and frozen in 90% FBS + 10% DMSO. Patients samples were collected before and after 2 months of therapy. To measure cytokine production by ILCs, PBMCs were stimulated for 3h with PMA (10 ng/ml) plus Ionomycin (500 nM) in the presence of Golgi Plug (BD Bioscience). *Flow cytometry.* Stained PBMCs were acquired on LSRFortessa and analysed with FlowJo. *Immunohistochemistry.* Double and triple stainings were performed to detect ILC subsets in tumor tissues. *Statistics.* Statistical significance was calculated using GraphPad Prism 5.0. Paired Student t-test or Wilcoxon signed-rank test were used for related groups, unpaired Student t-test or Mann-Whitney U test were used for unrelated groups. A p-value < 0.05 was considered significant.

RESULTS: Total ILCs were higher in melanoma patients than in healthy subjects and heavily infiltrated the tumor tissue. Levels of circulating ILC subsets were altered, with a decrease in c-Kit+ ILC2 and ILC3 and an increase in ILC1. Moreover, ILC1 and ILC3 had an impaired capability to secrete TNFα. Nivolumab treatment reduced total ILCs, increased the percentage of c-Kit- ILC2 and restored the capability of ILC2 and ILC3 to secrete IL-13 and TNFα, respectively.

DISCUSSION: ILCs, which are altered in melanoma, can be affected by Nivolumab in terms of percentages and secretory capability.

CONCLUSIONS: Strategies restoring ILCs secretory activity and/or modulating their plasticity could improve immunotherapy outcomes.

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P10.04

DECIPHERING THE ROLE OF NK CELLS/ILC1 IN LIVER METASTASIS FROM COLORECTAL CANCER

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PURPOSE: The liver is the major metastatic target organ for colorectal cancer (CRC)(1)with at least 25% developing colorectal liver metastases (CRLM, but little is known about the related innate lymphoid cell (ILC) immune contexture(2,3). The aim of the study is to analyze the involvement of ILCs in metastasis engraftment and progression by investigating their phenotypic plasticity and behavior in the metastatic niche.

METHODS: By using an established CRC *in vivo* mouse model of liver metastasis, we analyzed by multiparametric flow cytometry the distribution and phenotype of type 1 ILC subsets isolated from liver of tumor-bearing mice and the mechanisms underlying tumor infiltration.

RESULTS: We show that type 1 ILCs are involved in controlling hepatic metastasis formation and progression. We report an increase of NK1.1+ cells in the tumor context, associated with low ILC1 frequency mirrored by an increment of NK cells and by an expansion of a CD49b+ population with an ILC1-like phenotype. Tumor-infiltrating ILC1-like cells co-express markers specific for NK cells and ILC1 and produce IFNg and TNFa upon activation. By adoptive cell transfer experiments, we demonstrate that ILC1-like cells arise from circulating NK cells recruited in liver metastasis. Ultimately, we report that CXCR3 deficiency inhibits ILC1-like cells accumulation within the hepatic tumor.

DISCUSSION: The observed phenotypic shift of type 1 ILCs unravel a potential role played by these subsets during liver metastasis development. We suggest that NK cells retain their role in tumor surveillance in an early stage of tumor growth but undergo a phenotypic change in the tumor microenvironment that may represent a cancer-mediated mechanism of immune evasion.

CONCLUSIONS: Our work contributes to the understanding of molecular mechanisms underlying the effect of cancer-specific factors on ILC phenotype and function and may open new interesting therapeutic paths.

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P10.05

IMMUNOMODULATORY PROPERTIES OF EXTRACELLULAR VESICLES CARRYING NKG2D ACTIVATING LIGANDS: A DOUBLE EDGE SWORD IN CANCER IMMUNOSURVEILLANCE

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PURPOSE: Natural Killer (NK) cells play a key role in cancer immunosurveillance. NKG2D is an activating receptor that binds to stress induced ligands belonging to MIC and ULBP families. The secretion of NKG2D ligands (NKG2DLs) through protease-mediated cleavage or by extracellular vesicle (EV) is a mode to control their cell surface expression and a mechanism used by cancer cells to evade NKG2D-mediated immunosurveillance. Mounting evidence has shown a crucial role of cancer derived-EVs in the modulation of anti-tumor immune response. We focus our attention on the allelic variant MICA*008 since it is highly frequent in the Caucasian population and it is expressed at high levels on EVs. Our aim was to evaluate the role of MICA*008 associated to EVs in the modulation of NK cell-mediated functions.

METHODS: Exosomes and microvesicles were isolated by ultracentrifugation and characterized by electron microscopy, dynamic light scattering, and Western blot. EV-uptake was evaluated by immunofluorescence and FACS analysis and confocal microscopy. IFN-γ levels were measured by real-time PCR. NK cell cytotoxicity was evaluated by the 7-AAD assay.

RESULTS: We found that MICA*008 is present on the surface of both exosomes and microvesicles. Interestingly, our findings show that NKG2D is specifically involved in the uptake of vesicles expressing its cognate ligand. We provide evidence that EVs expressing MICA*008 are able on the one hand to activate NK cells but, following prolonged stimulation, they induce a sustained NKG2D downmodulation related to an impairment of NKG2D-mediated functions. Focusing on MM as a clinically and biologically relevant model of tumor-NK cell interactions, EVs expressing MICA were detected in the bone marrow of a cohort of MM patients.

DISCUSSION: Our data show that EV-associated MICA*008: i) can engage NKG2D and induce NK cell activation; ii) persistent stimulation mediated by EV-associated MICA*008 leads to NKG2D downmodulation related to an impairment of NKG2D-mediated functions; iii) NKG2D is specifically involved in the uptake of MICA*008+EVs.

CONCLUSIONS: All together these data shed light on the role of EV-associated ligands in the modulation and triggering of NKG2D activity in the tumor microenvironment.

P10.06

DISSECTING ROLES FOR TYPE 1 INNATE LYMPHOID CELLS IN COLITIS AND COLITIS-INDUCED COLORECTAL CANCER

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PURPOSE: Innate lymphoid cells (ILCs) comprise distinct lymphocytes, with Natural Killer (NK) cells being the founding member of the family. Although the role for ILCs in intestinal homeostasis and in protection against pathogens is now well established, recent evidence are linking all ILC subsets to autoimmune and inflammatory diseases, as well as to cancer. Herein, we aim to investigate the dynamic phenotypic changes as well as the role of ILCs in the pathogenesis of colorectal cancer (CRC) in mouse models.

METHODS: Cells were isolated from the large intestinal lamina propria (LiLP) of DSS-treated mice and from tumors of distinct CRC mouse models, and the ILC compartment was dissected by multiparametric flow cytometry. Data were then analyzed by high-dimensional cluster analysis, and the identified subsets were assessed for selective expression of cell-specific surface markers, transcription factors, and effector molecules.

RESULTS: We observed a remarked polarization of the ILC compartment towards type 1 ILCs both in the inflamed LiLP and within tumor-infiltrating ILCs, where we observed a strong increase of NK cells. Furthermore, NK1.1+ cell depletion in acute colitis and throughout CRC development protected mice from weight loss and polyp formation, respectively.

DISCUSSION: The observed phenotypic shifts in ILC distribution present in inflamed intestinal tissues and within the tumor infiltrate suggest an active role of type 1 ILCs during CRC development. We propose these cells as responsible for colitis pathogenesis and for cancer progression, since their depletion limits both wasting disease and polyp formation.

CONCLUSIONS: Our data shed light on common and specific phenotypic patterns for ILCs associated with normal and inflamed colon, as well as, with tumor. A deeper understanding of TI-ILCs and of the signals involved in shaping their effector functions will unveil novel mechanisms underlying CRC progression.

P10.07

GRANZYME A AND CD160 EXPRESSION DEFINES DISTINCT ILC1 SUBSETS IN THE MOUSE LIVER

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PURPOSE: Type 1 innate lymphoid cells (ILC1) are tissue-resident lymphocytes which provide early protection against bacterial and viral infections. Discrete transcriptional states of ILC1 have been identified in homeostatic and inflammatory contexts. However, the extent to which these states delineate ILC1 subsets with different functional properties is not completely understood. Thus, the aim of our study is to deconvolute ILC1 heterogeneity and to provide evidence for functional diversification of distinct liver ILC1 subsets.

METHODS: C57BL/6J, *Rag2*-/- and *Nfil3*-/- mice were employed. Lymphocytes were stained with fluorochrome-conjugated antibodies and analyzed by flow cytometry. ILC1 and NK cells degranulation was assessed by CD107a assay. For cytokine detection, cells were stimulated with PMA/Ionomycin or with IL-12 plus IL-18.

RESULTS: We found that CD160 and granzyme A (GzmA) were semi-mutually expressed in liver ILC1, identifying two discrete subsets. Their relative frequencies in *Nfil3*-/- mice were not significantly altered compared with wild type mice, indicating that both subsets had distinct requirements for *Nfil3* from NK cells. By assessing the functional properties of ILC1 subsets, we observed that GzmA+ ILC1 showed higher degranulation, while CD160+ were better IFN-g-producers. Finally, we found an increase of CD160+GzmA+ ILC1, upon stimulation, both *in vitro* and *in vivo*.

DISCUSSION: In our work we identified functionally distinct liver ILC1 populations endowed with different cytotoxic potential and ability to produce IFN-g in response to cytokines. Moreover, proinflammatory stimuli promoted generation of CD160+GzmA+ ILC1, suggesting that these two markers could help define distinct states of ILC1 activation in inflammatory context.

CONCLUSIONS: Our study provides evidence for a spectrum of phenotypically and functionally distinct ILC1 subsets in the liver. Understanding the modality for liver ILC1 to contribute to the immune response can be of relevance in the context of liver pathology.

P10.08

CHIMERIC ANTIGEN RECEPTOR ENGINEERED NK-92 CELLS AGAINST PROSTATE SPECIFIC MEMBRANE ANTIGEN: AN OFF-THE-SHELF CELLULAR THERAPEUTIC FOR TARGETED ELIMINATION OF PROSTATE CANCER CELLS

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PURPOSE: Despite the remarkable success in B cell malignancies after adoptive transfer of CD19 CAR T cells, CAR T cell therapy in solid tumors has shown less encouraging clinical results (1), above all caused by tumor escape mechanisms. In order to overcome such limitations, NK-92, a permanent and IL-2-dependent cell line with a high cytotoxicity in vitro, has been engineered in preclinical models with CAR. In this project, we exploited a CAR directed against the human antigen hPSMA that is overexpressed in prostate tumors. This project aimed at transducing NK-92 cell line to obtain a hPSMA-specific CAR NK-92 cell population, to be thereafter characterized in vitro and in vivo for antigen-specific functional activity.

METHODS: NK-92 cell line was transduced with a lentiviral vector (LV) carrying a CAR antihPSMA. The cell population obtained was then sorted and analyzed for degranulation capacity, IFNγ production and lytic activity against hPSMA+ (PC3-hPSMA, LNCaP) or hPSMA– tumor cell lines. In vivo therapeutic efficacy of CAR-transduced NK-92 was evaluated initially using Winn-Assay and than in subcutaneous and orthotopic tumor models.

RESULTS: CAR-expressing LV efficiently transduced NK-92 cells, which in turn produced cytokines, degranulated and exerted a relevant cytotoxic upon challenge with PSMA+ prostate tumor cells, irrespective of 10 Gy γ-irradiation. In all the in vivo, tumor models CAR-transduced NK-92 shown a statistically significant inhibition of tumor growth.

DISCUSSION: Chimeric antigen receptor-engineered NK-92 could offer a valid and cost-effective alternative to primary CAR NK or T cells, in particular in cases, where a suitable donor is not available or the sophisticated infrastructure needed for cell isolation, expansion and genetic modification is missing.

CONCLUSIONS: This work demonstrates that CAR-engineered NK-92 cells display a high and specific recognition of hPSMA+ PC both in vitro as is in vivo, and could represent an efficient strategy as a new therapeutic intervention against prostate carcinoma, thus paving the way to an Off-The-Shelf cellular therapeutic for targeted elimination of cancer cells and induction of protective antitumor immunity.

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11. PRIMARY IMMUNODEFICIENCIES

P11.01

A CVID-ASSOCIATED VARIANT IN THE CILIOGENESIS PROTEIN CCDC28B DISRUPTS IMMUNE SYNAPSE ASSEMBLY

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PURPOSE: Ciliogenesis proteins orchestrate vesicular trafficking pathways that regulate immune synapse (IS) assembly in non-ciliated T cells (1-4). We hypothesized that ciliogenesis-related genes might be disease candidates for common variable immunodeficiency with impaired T-cell function (T-CVID) (5).

METHODS and RESULTS: We identified a heterozygous, predicted pathogenic variant in the ciliogenesis protein CCDC28B (6) present with increased frequency in a large CVID cohort. We show that CCDC28B participates in IS assembly by regulating polarized T-cell antigen receptor (TCR) recycling. This involves the CCDC28B-dependent, FAM21-mediated recruitment of the actin regulator WASH to retromer at early endosomes to promote actin polymerization. The CVID-associated CCDC28BR25W variant failed to interact with FAM21, leading to impaired synaptic TCR recycling. CVID T cells carrying the *ccdc28b* C211T allele displayed IS defects mapping to this pathway that were corrected by overexpression of the wild-type allele.

DISCUSSION and CONCLUSIONS: These results identify a new disease gene in T-CVID and pinpoint CCDC28B as a new player in IS assembly.

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P11.02

ALTERED NKG2D/NKG2D-LIGAND PATHWAY IN ATAXIA-TELANGIECTASIA PATIENTS

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PURPOSE: Ataxia-telangiectasia (A-T) is a neurodegenerative disorder caused by biallelic mutations in the gene encoding the ATM kinase, a master regulator of the DNA damage response (DDR) pathway (1). Most A-T patients show immunodeficiency (reduced T- and B-cell numbers and serum immunoglobulins) that has been associated with cancer risk and reduced survival. As yet, little is known on NK cells in A-T. Here we studied the NK-cell compartment of A-T patients, focusing on the NKG2D activating receptor that potently triggers cytotoxicity upon engagement by ligands (NKG2DLs) typically induced via the DDR pathway on infected, transformed, and variously stressed cells.

METHODS: The phenotype and function of NK cells of 6 A-T patients were analyzed by flow cytometry. NKG2D expression was evaluated also by western blotting and qPCR. NKG2DLs were analyzed on T cells and fibroblasts exposed to antigen and DNA damaging agent, respectively. Plasma soluble NKG2DLs (sNKG2DLs) were measured by ELISA.

RESULTS: A-T NK cells were skewed towards the CD56neg anergic phenotype and displayed decreased expression of NKG2D and perforin. NKG2D was reduced at the protein level and resulted in lower NKG2D-mediated cytotoxicity. Compared with controls, stress-induced NKG2DL up-modulation was higher on A-T cells. Moreover, two sNKG2DLs, sMICA and sULBP1, accumulated in A-T plasma and inversely correlated with NKG2D expression.

DISCUSSION: Recurrent infections and pathologic inflammation typically associated with A-T might drive exhaustion of NK cells. NKG2D down-regulation on A-T NK cells could result from continued engagement with NKG2DL, these latter being induced by persistent DDR signaling in the ATM-deficient context.

CONCLUSIONS: We provided further insight into immune disorders in A-T showing an altered NKG2D/NKG2DL axis that may contribute to susceptibility to cancer and infections and represents a novel therapeutic target.

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P11.03

CTLA-4 HAPLOINSUFFICIENCY: CLINICAL AND IMMUNOLOGICAL DATA FROM A SINGLE CENTER COHORT OF PATIENTS

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PURPOSE: CTLA-4 is essential for the generation of tolerance among T cells, by competing with the costimulatory receptor CD28 for its ligand CD80 and CD86. Autosomal dominant mutations impair protein dimerization or ligand binding, leading to complex clinical phenotype characterized by immunodeficiency, immune dysregulation and autoimmunity.

METHODS: We report clinical and laboratory data on five patients affected by CTLA-4 haploinsufficiency, harboring 3 different mutations.

RESULTS: The most significant manifestations we observed were autoimmune cytopenias, severe enteropathy refractory to traditional therapy, recurrent respiratory infections with bronchiectasis, hepatosplenomegaly and arthritis. No malignant proliferation was observed. Three patients developed hypogammaglobulinemia, while four of them were put on antibiotic prophylaxis due to severe lymphopenia. When administered, specific CTLA-4–immunoglobulin fusion protein Abatacept and Vedolizumab led to good control of the clinical manifestations except for the gastrointestinal ones. One of the patients died during follow-up, while another patient underwent a successful allogenic hematopoietic stem cell transplantation (HSCT).

DISCUSSION: CTLA-4 haploinsufficiency may manifest extreme clinical and laboratory variability. The clinical presentation may range from complete health to severe multi-organ involvement. Though some of the patients present with normal laboratory examinations, many of them develop over time hypogammaglobulinemia and lymphopenia. Treatment options comprise support and prophylactic therapy; specific target therapy and, in the most severe cases, hematopoietic stem cell transplantation should be taken into consideration.[1]

CONCLUSIONS: Our data confirm the extreme variability reported in literature, even in sibling patients. Targeted treatments are available and may offer complete clinical response or may be used as bridge therapy before HSCT.

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P11.04

WHOLE-EXOME SEQUENCING IN COMMON VARIABLE IMMUNODEFICIENCY

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PURPOSE: Common variable immunodeficiency (CVID) is clinically, and genetically heterogeneous. Traditional candidate gene sequencing identified only few monogenic causes. Next Generation Sequencing (NGS) revolutionized the field of genetics. This study aimed to recruit a cohort of CVID patients and characterize them clinically, immunologically, and genetically using Whole-exome sequencing (WES).

METHODS: A cohort of 44 CVID patients and 23 relatives was recruited. Flow cytometry panel were used to characterize lymphocyte populations, including T cell subpopulations naïve, memory, Tfh and Treg cells and EUROClass B cells classification. WES was employed to search for genetic variants. Data pre-processing and variant calling were done according to GATK Best Practices. Variant annotation was done using ANNOVAR. An in-silico primary immunodeficiency associated genes panel was used to prioritize candidate variants. ExomeDepth was used to call Copy Number Variants (CNVs).

RESULTS: Patients had higher T CD8+ cells and lower NK cells. T CD4+ cells were reduced. B cells were comparable between patients and controls, but patients had markedly reduced switched memory B cells and plasmablasts. We identified candidate variants in 50% of patients, including common disease-associated variants in TACI and BAFF-R genes. We described two novel pathogenic variants in CTLA-4 and one frameshift deletion of NFKB1. Variants of unknown significance were found in CTLA-4, CARD11, NFKB2, CD40LG, PIK3CD, PTEN, and TCF3. The CNV study identified a CTLA-4/ICOS/CD28 deletion.

DISCUSSION: An unbiased genetic approach allows for unexpected discoveries tackling the problem of genetic and phenotypic heterogeneity. Moreover, In-silico CNV prediction can increase the diagnostic yield of WES.

CONCLUSIONS: The genetic dissection of CVID is contributing to the understanding of the immune system. Moreover, a genetic diagnosis can impact the clinical management by improving the prognostic stratification, inform on the hereditability of the disease and help tailoring personalized therapies.

12. T-CELLS

P12.02

miR-21 SUSTAINS CD28-SIGNALLING AND FACILITATES LOW AFFINITY T-CELL RESPONSES AT THE EXPENSE OF SELF-TOLERANCE

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PURPOSE: We sought to investigate the role of miR-21 in the development and functions of T and iNKT cells, representing adaptive and innate-like populations, respectively, given its high expression in iNKT (1) and activated T cells and its broad relevance for the cellular immune response (2), but poor definition of its T-cell autonomous functions.

METHODS: We investigated mice with a conditional deletion of miR-21 in all mature T lymphocytes, starting from DP thymocytes, but with a normal miR-21 expression in any other immune cells and tissues.

RESULTS: Thymic and peripheral T and iNKT compartments were normal in miR-21 KO mice. However, upon activation in vitro, miR-21 depletion reduced T-cell survival, TH17 polarization and, remarkably, T- and iNKT-cell ability to respond to a low-affinity antigen stimulation, without altering their response to high-affinity ones. Mechanistically, miR-21 sustained CD28-dependent costimulation pathways required to lower the T-cell activation threshold, via inhibition of its repressors in a positive feedback circuit, in turn increasing T cell sensitivity to antigenic stimulation and survival. Upon immunization with the low-affinity self-epitope MOG35–55, miR-21 KO mice were indeed less susceptible than WT animals to the induction of the prototypical autoimmune pathology EAE, whereas they mounted normal T-cell responses against high-affinity viral epitopes generated upon LCMV infection.

DISCUSSION: The induction of T cell responses to weak antigens (signal 1) depends on CD28 costimulation (signal 2). We found that miR-21 plays a new role in this mechanism by sustaining CD28 costimulation. This decreases the T-cell activation threshold and increases their sensitivity to antigenic stimulation and survival, broadening the immune surveillance range. However, this occurs at the cost of unleashing autoimmunity, resulting from the recognition of weak self-antigens by autoreactive immune responses.

CONCLUSIONS: miR-21 fine-tunes T cell response and self/non-self discrimination.

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P12.03

CD28-ASSOCIATED CLASS 1A PI3K REGULATES IL-22–MEDIATED EPITHELIAL BARRIER FUNCTIONS IN HUMAN T LYMPHOCYTES

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PURPOSE: IL-22 is a member of the IL-10 cytokine family involved in host protection against extracellular pathogens, by promoting epithelial cell regeneration and barrier functions. Dysregulation of IL-22 production has also frequently been observed in several chronic inflammatory and autoimmune diseases. We have previously described that human CD28, crucial co-stimulatory receptor necessary for full T cell activation, is able to deliver TCR independent signals and to induce the expression of inflammatory cytokines related to Th17 cells (1,2) including IL-22 (3). Here we characterized the mechanisms and functional effects of CD28-mediated up-regulation of IL-22 in human CD4+ T cells.

METHODS: Primary CD4+ T cells isolated from the peripheral blood of healthy donors were stimulated with agonistic anti-CD28 antibodies and both transcription and production of IL-22 was measured (RT PCR, ELISA). The specific recruitment of transcription factors (pSTAT3, RelA/NF-kB) on the human IL-22 proximal promoter was analysed by chromatin immunoprecipitation. Co-cultures of CACO-2 and CD4+ T cells were performed in trans-well plates and the expression of antimicrobial peptides and metalloproteases was analysed (RT PCR, ELISA, WB).

RESULTS and DISCUSSION: We found that CD28 stimulation in the absence of TCR strongly up-regulates IL-22 gene expression and secretion. As recently observed for IL-17A, we also found that CD28-mediated regulation of IL-22 transcription requires the cooperative activities of both IL-6-activated STAT3 and RelA/NF-kB transcription factors. CD28-mediated IL-22 production also promotes the barrier functions of epithelial cells by inducing mucin and metalloprotease expression. Finally, we also evidence a pivotal role of CD28-associated class 1A phosphatidylinositol 3-kinase (PI3K) in regulating both IL-22 expression and IL-22–dependent epithelial barrier functions.

CONCLUSIONS: Our results provide novel insights on the role of CD28 and associated signalling mediators in IL-22 regulation in human CD4+ T cells and may provide the biological bases for the development of new therapeutic strategies to dampen inflammatory and autoimmune disorders.

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P12.04

IDENTIFICATION OF FUNCTIONAL SUBSETS OF T LYMPHOCYTES BASED ON THE EXPRESSION OF IMMUNE-CHECKPOINTS, IN JUVENILE IDIOPATHIC ARTHRITIS

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PURPOSE
Juvenile idiopathic arthritis (JIA) is a persistent arthritis of unknown cause in children (1). T cells are certainly involved in its pathogenesis (2), but functional analyses of T cells subset of JIA patients are needed to better characterize the role of these cells in disease development.

METHODS
T cells gene expression in JIA samples was investigated trough single-cell RNA sequencing (scRNA-seq) analysis. Peripheral blood (PB) and Synovial Fluid (SF) T cells from 12 children with JIA were analyzed by flowcytometry for the expression of immune-checkpoint (IC) molecules TIGIT and PD1. T cells cytokine production profile was analyzed as well.

RESULTS
Data obtained from scRNA-seq analysis showed that T cells of PB and SF samples clustered separately and among the genes differentially expressed between clusters emerged TIGIT and PD1. These two ICs identified four different subsets. The expression of ICs was correlated with T cells functionality checking each subsets for cytokines genes expression. The TIGIT-PD1+ subset shows the highest expression of cytokines, followed by TIGIT+PD1+, TIGIT+PD1- and TIGIT-PD1-. In order to study the temporal relation between the different subsets, it was made a trajectory analysis, focusing on SF samples. Data showed that PD1 is more expressed at the beginning and next slightly reduce. TIGIT is less expressed at the beginning but next increase more than PD1 and then undergoes to reduction. ScRNA-seq data were confirmed by flowcytometry analysis, which showed that both TIGIT and PD1 expression was higher in SF compere to PB. Moreover, among the different subsets the maximum production of all cytokines was observed in TIGIT-PD1+ subset.

DISCUSSION
Cytokines and scRNA-seq analysis confirm the hypothesis that in SF recently activated T cells after repeated stimulation acquire an higher effector function and express PD1. The persisting stimulation, due to pro-inflammatory molecules, induces on T cells the expression of other inhibitory molecules such as TIGIT and decreases T cells effector capacity.

CONCLUSIONS
The results confirmed that PD1 is one of the mainly expressed molecules by active cells in SF, suggesting that it can be considered as a valid therapeutic target in chronic inflammation.

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P12.05

THE INTRAFLAGELLAR TRANSPORT PROTEIN IFT20 RECRUITS ATG16L1 TO EARLY ENDOSOMES TO PROMOTE AUTOPHAGOSOME FORMATION IN T CELLS

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PURPOSE: Lymphocyte homeostasis, activation and differentiation crucially rely on basal autophagy. The fine-tuning of this process depends on autophagy-related (ATG) proteins and their interaction with the trafficking machinery that orchestrates the membrane rearrangements leading to autophagosome biogenesis1. The underlying mechanisms are as yet not fully understood. The intraflagellar transport (IFT) system, known for its role in cargo transport along the axonemal microtubules of the primary cilium, has emerged as a regulator of autophagy in ciliated cells. Growing evidence indicates that ciliogenesis proteins participate in cilia-independent processes, including autophagy, in non-ciliated T cell2. Here we investigate the mechanism by which IFT20, an integral component of the IFT system, regulates basal T cell autophagy.

METHODS: Immunofluorescence analysis, *in vitro* binding and immunoprecipitation assays have been performed on Jurkat T cells knocked down for IFT20 or GMAP210, expressing IFT20-GFP or a deletion mutant of IFT20 lacking the coiled-coil domain.

RESULTS: We show that IFT20 interacts with the core autophagy protein ATG16L1 and is required for its association with the Golgi complex and early endosomes, both known membrane sources for phagophore elongation. We provide evidence that this involves the ability of IFT20 to interact with proteins that are resident at these subcellular localizations, namely GMAP210 at the Golgi apparatus and Rab5 at early endosomes. Unthethering IFT20 from the Golgi through GMAP210 depletion, while leading to a dispersion of ATG16L1 from the Golgi, did not affect basal autophagy, suggesting that this organelle is not a major source of autophagosomes in T cells. Conversely, IFT20 was found to recruit ATG16L1 to early endosomes tagged for autophagosome formation by the BECLIN 1/VPS34/Rab5 complex, which resulted in the local accumulation of LC3.

DISCUSSION: IFT20 participates in autophagosome biogenesis under basal conditions by regulating the localization of ATG16L1 at early endosomes to promote autophagosome biogenesis.

CONCLUSIONS: These data identify IFT20 as a new regulator of an early step of basal autophagy in T cells.

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P12.06

CD8+ T STEM CELL MEMORY CELLS IN TUBERCULOSIS

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PURPOSE: We aimed to determine if Mycobacterium tuberculosis (Mtb) infection generates antigen-specific and HLA-E-restricted CD8+ TSCM and to characterize their function.

METHODS: Direct ex vivo binding of HLA-E tetramers (TM) loaded with Mtb-peptides to CD8+ T cells was studied, by FACS analysis, in peripheral blood mononuclear cells (PBMCs) of 42 enrolled patients divided in four groups according to their TB status: latently-infected subjects (LTBI), active TB before therapy (aTB) and after 3 and 6 months of therapy. The phenotype of HLA-E/Mtb-peptide TM+CD8+ T cells was defined based on the expression of CCR7, CD45RA, CD95 and CD62L. TSCM phenotypically resemble naive T cells, but co-expression of memory markers distinguishes them from naive T cells. Analysis of data was performed with FlowJo 10.5.3 and Graphpad 5.0 softwares.

RESULTS: HLA-E/Mtb-specific CD8+ T cells frequency was significantly higher in aTB and active TB after 3 months of therapy compared to LTBI. Conversely, the frequency of HLA-E TM+ CD8+ TSCM was higher in LTBI compared to the other groups. Furthermore, low proliferation and survival of TSCM in different disease stages have been revealed. Those observation confirmed by gene expression analysis.

DISCUSSION: The frequency of Mtb-specific CD8+ TSCM cells decreases significantly in TB patients during and even after therapy, supporting the recently discovered role of TSCM population in inducing and maintaining long-lasting T cell immunity capable of replenishing all T cell memory subsets. Analysis of the CD8+ memory subsets revealed a reduction of Mtb-specific CD8+ TSCM population and linked to an increase of CD8+ T effector populations with exhausted phenotype.

CONCLUSIONS: The clarification of Mtb-specific CD8+ TSCM could be useful for new therapeutic strategies related against tuberculosis.

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13. TUMOR IMMUNOLOGY

P13.01

PD-1 SIGNALING REGULATES DRP1-DEPENDENT MITOCHONDRIAL FISSION IN TUMOR-INFILTRATING T CELLS

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PURPOSE: Drp1-dependent mitochondrial fission tightly regulates T cell response upon activation1,2,3, motility2 and proliferation2. Interestingly, all these aspects are down-regulated in tumor-infiltrating PD-1pos T cells4. However, no information is currently available on the changes to which mitochondria morphology undergoes during exhaustion and whether they may be partially responsible for such a dysfunctional state. We aimed at understanding how mitochondria morphology is affected in exhausted T cells within the tumor-microenvironment.

METHODS: MC38-derived murine tumor model was used to perform *in vivo* and *ex vivo* studies on tumor-infiltrating T lymphocytes. Furthermore, functional *in vitro* studies were performed using both murine and human primary T cells.

RESULTS: We found a specific down-regulation in Drp1 activity (the main mitochondria pro-fission protein) in both murine and human tumor-infiltrating PD-1pos exhausted CD8 T cells. In addition, we demonstrated that PD-1 signaling directly down-regulates Drp1 by inhibiting mTOR and ERK kinases, which control the Drp1 activity through phosphorylation on Ser616 residue. Last, we demonstrated that anti-PD-1 therapy fails in reducing tumor growth in mice lacking Drp1 in T cells, due to an inability to rescue T cell motility and proliferation.

DISCUSSION: Our data indicate that PD-1 signaling down-regulate Drp1-dependent mitochondrial fission in tumor-infiltrating T cells. Interestingly, such down-regulation is a mechanism exploited by PD-1 signaling to impair tumor-infiltrating T cell motility and proliferation, so reducing the anti-tumor response.

CONCLUSIONS: PD-1 signaling modulates Drp1 activity in CD8+ TILs to prevent an efficient anti-tumor response.

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P13.02

HUMAN INTESTINAL AND CIRCULATING INVARIANT NATURAL KILLER T CELLS ARE CYTOTOXIC AGAINST COLORECTAL CANCER CELLS VIA THE PERFORIN/ GRANZYME PATHWAY

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PURPOSE: The aim of this study was to evaluate invariant Natural Killer T (iNKT) cell cytotoxicity against human colorectal cancer (CRC) and the mechanisms underlying this activity.

METHODS: Stable human iNKT cell lines were generated from peripheral blood and from colon samples. In vitro cytotoxicity assays were performed using a panel of five different human CRC cell lines as target cells; a stable natural killer (NK) cell line and NK cells freshly isolated from peripheral blood were used as cell-mediated cytotoxicity controls. The cellular requirements for iNKT cell recognition and killing of CRC cells were also analyzed.

RESULTS: We showed that intestinal and circulating human iNKT cells were capable of exerting specific tumor-directed cytotoxic functions against the whole panel of CRC lines, similarly to NK cells. Furthermore, intestinal iNKT cell-mediated killing was substantially impaired by perforin inhibition, whereas TCR/CD1d signaling seemed to be a less stringent requirement for efficient killing.

DISCUSSION: In this model, iNKT cells exerted killing activities via granzyme B/perforin, which can be explained by the fast nature of this mechanism compared to the death receptor mechanisms. Additionally, intestinal iNKT cells might have recognized CRC cells via innate signals rather than TCR usage, in a way similar to natural killer cells.

CONCLUSIONS: This study elucidates key aspects of the functional requirements for human intestinal iNKT cell cytotoxicity, providing important insights for the use of iNKT cells antitumor activities against human colorectal cancer.

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P13.03

CD8+ T CELL DYNAMICS WITHIN HEPATOCELLULAR CARCINOMA

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PURPOSE: CD8+ T cells play a crucial role in controlling liver tumors, such as hepatocellular carcinoma (HCC) however we have only limited knowledge of the precise dynamics of their interactions with hepatic parenchymal and non-parenchymal cells at the single-cell level. We established a murine model of HCC in which the tumorigenic hepatocytes express a nominal antigen (TAg) and a fluorescent protein to follow the transformed hepatocytes. After TAg-specific CD8+ effector T cells (TE) adoptive transfer in tumor-bearing mice, just some of them respond to the TE treatment, yet we established a therapeutic threshold of 10mm3 lesion volume dividing lesions between responders (volume <10mm3) and non-responders (>10mm3). Our goal is to address the determinants that confer a therapeutic activity to the TAg-specific TE in responders and the ones that dampen the activity in the non-responders HCC lesions.

METHODS: In order to understand whether the volume-related therapeutic capacity of TAg-antigen-specific TE is due to an infiltration defect, we analyzed how these cells home and get activated within responders and non-responders HCC lesions by confocal microscopy and mRNA analysis. Then, taking advantage of intravital multiphoton microscopy (MP-IVM) we evaluated their different motility and localization within the lesions.

RESULTS: We analyzed how TAg-specific TE home to HCC lesions, we observe a lower accumulation in non-responder once, compared to the responders. We then quantified TAg-specific TE activation within responders and non-responders and we reveal a higher IFNg mRNA expression and protein production in responders HCC lesions. Finally, TAg-specific TE displayed a higher motility inside the responders HCC lesions.

DISCUSSION: In our experimental setting we observed a homing and activation impairment, a reduced single cell motility of TAg-specific TE in the non-responder lesions compared to the responders. Our results suggest that the hemodynamical and environmental features be founded in different HCC lesion size could impair TAg-specific TE effector ability.

CONCLUSIONS: The innovative nature of our work will elucidate new mechanisms whereby CD8+ T cells exert immune responses within the tumorigenic liver.

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P13.04

INFLAMMATORY HMGB1 MODULATION IN PRIMARY AND METASTATIC BREAST CANCER PATIENTS TREATED WITH DIFFERENT THERAPY

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PURPOSE: High mobility group box 1 (HMGB1) is a ubiquitous and highly conserved nuclear protein. When released, it is recognized as a damage-associated molecular pattern contributing to the inflammatory response. In cancer, HMGB1 has been shown to be crucial in immunogenic cell death (ICD) as well as in mediating immune evasion by promoting myeloid-driven suppressor cells and regulatory T cells, but its role in cancer patients remains unclear.
Here, we investigated the levels of HMGB1 in breast cancer patients (BCP) undergoing different therapies to assess its possible role as immune biomarker.

METHODS: 60 BCPs were enrolled in the study: 29 neoadjuvant patients (TNBC or luminal B or HER2+) treated with 5-fluorouracil-epirubicin-cyclophosphamide and 31 HR+/HER2− metastatic BCP in cyclin-dependent-kinase 4/6 inhibitors (CDK4/6i) treatment. BCP sera was isolated from blood at baseline (T0) and during therapy. HMGB1 levels were assessed by an enzyme-linked immunosorbent assay (ELISA) kit.

RESULTS: HMGB1 appears to be differently modulated in the two BCP cohorts. HMGB1 levels are significantly reduced during CDK4/6i therapy (p0,001), in particular in the responder patients (RP) (p0,05). RPs to CDK4/6i treatment show higher HMGB1 levels at T0 than not-responders (NR) (p0,05). In patients undergoing neoadjuvant therapy, HMGB1 is lower in RPs at T0 than NRs (p0,05), but after treatment, biomarker levels increased significantly (p0,01).

DISCUSSION: The different HMGB1 kinetics in primary and metastatic (CDK4/6i) treated BCPs, in particular in the RP subgroups may be due to the different off-target effects exerted by the two distinct therapeutic regimens. During neoadjuvant treatment, HMBG1 increase observed in RP could be associated to the induction of ICD, while in metastatic RP the HMGB1 downmodulation of the inflammatory response may be the result of an off target CDK4/6i effect on the inflammatory tumor microenvironment.

CONCLUSIONS: HMGB1 different modulation may correspond to a distinct effect of standard therapies on the immunological tumor microenvironment. The investigation of such off-target effects can contribute to design therapeutic schemes combining target and immune therapy in order to maximize patient benefit.

P13.05

CIRCULATING IMMUNE PROFILE OF UVEAL MELANOMA PATIENTS: IMPACT ON CLINICAL OUTCOME

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PURPOSE: Uveal Melanoma (UM) is the most common tumor with origin in the eye, highly metastatic and with a dismal prognosis for the majority of patients. To date no standard treatment has been yet established and immune checkpoint inhibitors (ICIs) represent one of the therapeutic options. ICIs are improving the survival of cancer patients, nevertheless only a very small number of UM patients respond to immunotherapy. This research study aims to evaluate several circulating immune molecules in order to investigate their prognostic role in metastatic UM (mUM).

METHODS: Serum of 12 mUM patients treated with anti-PD1 (Pembrolizumab) was collected at baseline to evaluate the levels of 20 cytokines and 14 soluble immune checkpoint molecules by multiplex assay. Indoleamine 2,3-dioxygenase (IDO) activity was measured as kyn/trp ratio by HPLC-MS/MS method. The level of all circulating molecules was correlated with patient survival. ROC curve analysis was performed to determine the optimal cut-off of each molecules related to survival. A score for each molecule and an overall score were calculated (range 0-3).

RESULTS: mUM patients were divided in two groups based on survival: < 6 months and ≥ 6 months. Among the molecules analyzed the values of sHVEM, kyn/trp ratio and IL8 resulted significantly higher in patients with survival <6 months compared to those with survival ≥ 6 months ($p<0.05$). ROC curve analysis showed the following cut-off value: sHVEM ^{3} 50 pg/ml; kyn/trp ratio ^{3} 0,024; IL8 ^{3}50 pg/ml. A score equal to 3 resulted correlated to the worst prognosis and was associated with survival < 6 months. ROC analysis assigned 2 as value for worst survival prediction, suggesting that the presence of at least two serum parameters with values above cut-off is associated with a worst prognosis.

DISCUSSION: IDO and sHVEM have a suppressive role in immune system, the first inhibiting T-cell proliferation and the second promoting Treg function. Moreover, the presence of high levels of IL-8 in serum seems to be associated with a worst outcome during anti-PD1 therapy.

CONCLUSIONS: The limited response to anti-PD1 therapy could be explained by a poor immune activation. sHVEM, IDO and IL-8 represent the three immune molecules able to generate a score that could be used to predict mUM patients' prognosis.

P13.06

REGULATORY T CELLS CAPTURE IRON THROUGH THE TRANSFERRIN RECEPTOR DURING PHYSIOLOGICAL AND TUMOR-ASSOCIATED EXPANSION

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PURPOSE: Regulatory T cells (Tregs) are involved in the inhibition of protective anti-tumor responses. Our previous data demonstrated that Tregs expressing OX40, a member of TNF receptor superfamily, accumulate in human hepatocellular carcinoma (HCC) and display a peculiar metabolic reprogramming, involving iron-transferrin capture (1-2). We aim to study whether activated Tregs show a preferential uptake of extracellular iron that favor their proliferation in tumor.

METHODS: We analyzed CD71 expression and fluorescence Transferrin (TF) uptake by multiparametric flow cytometry in different settings: ex vivo, in Tregs from HCC patients and from a mouse model of HCV- and steatosis-related HCC; in vitro, in proliferation assays using stimulated Tregs from PBMC of healthy donors, in presence or not of an anti-CD71 blocking antibody. We explored the functions of this pathway in a mouse model of Treg-restricted CD71 deletion.

RESULTS: Ex vivo in human HCC samples, OX40+ Tregs preferentially express CD71, and in the HCC mouse model OX40+ Tregs accumulate at the tumor site and show a tendency of enhanced TF uptake. In vitro activation induces high level of CD71 in human OX40+ Tregs leading to an improved TF capture, and CD71 blockade profoundly inhibits Treg expansion. In mice, Treg-restricted CD71 deficiency results in a scurfy-like disease due to a significantly low Treg frequency and a pronounced CD8 and CD4 T cell infiltration and activation in several organs.

DISCUSSION: Iron uptake via CD71 is critical for T cell functions. The strong CD71 induction observed in highly activated OX40+ Tregs in vitro and ex vivo and the effects of CD71 deficiency suggest a connection between Treg activation and the ability to exploit metabolic sources.

CONCLUSIONS: In tumor context, the pronounced capacity of OX40+ Tregs to sequester extracellular iron may represent a novel mechanism that foster Treg expansion and this may contribute to the impairment of effector T cell functions.

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P13.07

STEADY IFNα THERAPY PREVENTS THE DEVELOPMENT OF COLORECTAL LIVER METASTASIS THROUGH THE ACTIVATION OF LIVER SINUSOIDAL ENDOTHELIAL CELLS

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PURPOSE: Colorectal cancer (CRC) liver metastases are a frequent complication and a leading cause of death of CRC patients (1, 2). Thus, strategies aimed at reducing the risk of hepatic CRC colonization or able to reduce residual disease after surgery are of great interest.

METHODS: To test the efficacy and mode of action of steady intraperitoneal IFNα therapy, we have used mouse models of CRC metastatic spreading to the liver, either through intravascular seeding in the portal circulation of CT26 or MC38 CRC cell lines or by orthotopically implanting in the cecal wall the recently developed CT26LM3 cell line that spontaneously generates liver metastases (3).

RESULTS: We show that steady intraperitoneal administration of IFNα in a neo-adjuvant setting, before CRC portal circulation intravascular seeding, or in an adjuvant setting, after cecal wall orthotopic implantation, impairs CRC cell extravasation and hepatic tumor spreading by activating liver sinusoidal endothelial cells (LSECs). IFNα antitumor activity requires a direct action on LSECs, while does not require a direct effect on tumor cells, hepatocytes or dendritic cells. Mechanistically, while IFNα does not alter the number of CRC cells that reach the liver, it reduces CRC cell extravasation, promoting CRC cell death and antitumor immune activation and thus, IFNα treatment increases overall survival.

DISCUSSION: The identification of steady intraperitoneal administration of IFNα as an effective way to activate the liver microenvironment represents the starting point for the design of effective IFNα-based therapies. Furthermore, IFNα-mediated hindrance of liver metastatic colonization by directly modifying LSECs may also promote tumor-specific adaptive immune responses, which might have important clinical implications.

CONCLUSIONS: These results, pave the road for the design of neo-adjuvant or early adjuvant interferon-based approaches for patients undergoing CRC resections.

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P13.08

ROLE OF CD36 IN MULTIPLE MYELOMA PROGRESSION

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PURPOSE: Multiple myeloma (MM) is a lethal plasma cell malignancy characterized by the proliferation of malignant cells within the bone marrow (BM). MM pathophysiology encompasses a multistage evolution through asymptomatic stages such as monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM to the development of symptomatic full blown disease. Recent reports have highlighted the role of alterations in lipid metabolism and lipid signaling pathways in tumor growth and progression. Moreover, there is growing evidence that CD36, a multifunctional transmembrane glycoprotein that enhances cellular lipid uptake, have great impact on tumor growth, progression and metastasis. As BM is an adipose-rich tissues with abundance of exogenous fats, we examined the role of CD36 in MGUS to MM progression.

METHODS: We used a range of cell biology techniques to investigate the expression and the role of CD36 within BM microenvironment in 20 patients with MGUS and 20 patients with MM.

RESULTS: We found that BM myeloid-derived suppressor cells (MDSC) highly expressed CD36 and its expression progressively increased moving from MGUS to MM patients. CD36 enhanced their ability to uptake and accumulate long-chain fatty acids and triggered a metabolic and functional reprogramming to become highly immunosuppressive cells. An opposite pattern was observed for CD36 expression in T cells. Compared with CD8 T cells from MGUS patients, CD8 T cells from MM patients exhibited greater levels of CD36, an impaired mitochondrial function and underwent to apoptosis.

DISCUSSION and CONCLUSIONS: CD36 triggered a lipid metabolic reprogramming of BM MDSC and CD8 T cells favouring an immunosuppressive microenvironment and MM progression.

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P13.09

INTERLEUKIN-9 SECRETED BY LEUKEMIC CELLS FROM CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS PROMOTES CYTOTOXIC T CELL EXHAUSTION

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PURPOSE: The microenvironment of lymphoid organs is central to the pathogenesis of chronic lymphocytic leukemia (CLL).1 Within it, tumor cells escape from immunosurveillance by suppressing the killing activity of cytotoxic T lymphocytes (CTLs), which are unable to build a lytic immune synapse (IS).2 We previously identified IL-9 as a soluble factor secreted by leukemic cells from CLL patients with aggressive disease, which stimulates stromal cells of lymphoid organs to produce prosurvival factors.3 Our aim is to understand the implication of leukemic cell-secreted IL-9 on CTL suppression.

METHODS: Culture media conditioned by leukemic cells isolated from CLL patients or Em-TCL1 mice, the mouse model of human CLL, were used to culture healthy CTLs, in the presence of anti-IL-9 (0.1 mg/ml) or isotype control antibodies. The efficiency of lytic IS formation was assessed by confocal microscopy of CTL/B cell conjugates stained with fluorescent antibodies. PD-1 expression was assessed by qRT-PCR and flow cytometry.

RESULTS: Media conditioned by leukemic cells suppress the ability of healthy CTLs to build a productive IS. CTL suppression was enhanced when we used media conditioned by CLL cells isolated from patients or from mice with aggressive disease. Furthermore, CTLs cultured in the presence of CLL cell supernatants express high levels of the inhibitory receptor PD-1. These effects were reverted by IL-9 neutralizing antibodies.

DISCUSSION: Our results demonstrate that IL-9 secreted by leukemic cells suppresses the ability of healthy CTLs to build a productive IS, at least in part by promoting surface expression of PD-1, and provide new insights into the mechanisms exploited by leukemic cells to shape the microenvironment.

CONCLUSIONS: IL-9 localization is a new potential therapeutical approach to fight against CLL.

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P13.10

C1q-HYALURONIC ACID MATRIX AS AN IN VITRO MODEL FOR MIMICKING THE RESPONSE TO CHEMOTHERAPEUTIC DRUGS IN MALIGNANT PLEURAL MESOTHELIOMA

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PURPOSE: The development of personalized therapies for malignant pleural mesothelioma (MPM), a rare and aggressive tumor of the pleura, is still hampered by several limitations [1]. We have tried to recreate the characteristics of MPM microenvironment *in vitro* in order to set up a prognostic test to predict MPM patients' response to chemotherapy. In particular, we evaluated the effect of the complement protein C1q and hyaluronic acid (HA), two well-recognized players in MPM microenvironment [2,3], on the response to cisplatin, the gold-standard treatment in MPM.

METHODS: After isolation of seven primary MPM cells from biopsies and cell characterization, we performed cisplatin-based cell viability assays on cells seeded onto C1q-HA matrix, HA alone or fibronectin. Killing assay data were correlated with patients' clinical information.

RESULTS: We observed that sensitivity to cisplatin-induced killing of primary MPM cells was modified by different ECM components. Moreover, we detected a strong and statistically significant correlation (Pearson's $R=0.82$, $P<0.05$) between the percentage of cell viability on C1q-HA matrix and a response score based on the Response Evaluation Criteria in Solid Tumors.

DISCUSSION: Since C1q has also been previously demonstrated to be highly expressed in MPM microenvironment, exerting complement-independent pro-tumorigenic functions, also by virtue of its binding to HA (2,4), we have reasons to think that C1q-HA matrix is the optimal culture condition to mimic MPM tumor cell behavior and, on the basis of the obtained results, to predict patients' response to chemotherapy.

CONCLUSIONS: We set up an easy, reproducible and standardized testing method which may be applied in the future not only as a predictive tool for the individual patient but also as a translational model to test new therapeutic targets and promising treatments.

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P13.11

ABERRANT WNT/β-CATENIN SIGNALING AS A THERAPEUTIC TARGET IN HEPATOCELLULAR CARCINOMA

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PURPOSE: Hepatocellular carcinoma (HCC) is characterized by aberrant chromosomal status and rearrangements involving the oncogenes and oncosuppressors as pathogenetic drivers associated with the acquisition of copy number abnormalities (CNAs) and complex structural variants during the disease evolution. We examined the functional impact of the mutational status, CNAs, and chromosomal rearrangements (CRs) in HCC.

METHODS: Using a bioinformatic multiomic approach we dissected the gene expression deregulation caused by the most common mutations, CNAs and CRs from 25 public datasets. Using bioinformatics, we investigated WNT pathways and we tested them in vitro regarding epithelial mesenchymal transition (EMT), invasion and HCC dissemination.

RESULTS: CNAs and CRs analysis revealed a higher number of deregulated transcripts than gene mutations. Among the latter, the genes β-CATENIN (CTNNB1), H/K/NRAS and CSF-R were associated with the major impact on transcriptome. Oncosuppressors such as axis inhibition protein (AXIN1/2), TP53, HNF1A, RB1, SMAD2-4, PTEN and IGF2R were inactivated in HCC. By in-silico study of 386 resected HCC, we uncovered a gene signature predictive of OS and PFS and recurrence in patients whose tumors was < 5 cm diameter. Finally, we found that high-risk HCC with EMT and CTNNB1/WNT expression displayed a unique gene-expression signature. HCC patients clustering according to CTNNB1/WNT expression levels revealed a clear link to the EMT and focal adhesion pathways. In vitro functional knockdown of CTNNB1/WNT reduced cell viability and HCC invasiveness. Furthermore, WNT in vitro inhibition re-educated a tumor-permissive immune system.

DISCUSSION and CONCLUSIONS: We identified gene signatures for prognosis, vascular invasion and metastases in HCC and identification of HCC subjects susceptible to novel strategies. Moreover, we proposed a novel immune-based therapeutic strategy targeting WNT to enhance HCC cytotoxicity and restore anti-HCC immunity.

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P13.12

STEAROYL-COENZYME A DESATURASE 1 AND TREGS METABOLIC ADAPTATIONS IN HEPATOCELLULAR CARCINOMA

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PURPOSE: Tumor infiltrating regulatory T cells (T-Tregs) have evolved metabolic adaptations to cope with nutrient shortage in the tumor microenvironment. Here we investigate the role of stearoyl-coenzyme A desaturase 1 (SCD1), a monounsaturated fatty acid synthetizing enzyme, in T-Treg biology.

METHODS: Subcutaneous and orthotopic hepatocellular carcinomas (HCC) were induced in C57BL/6J mice and phenotypical analysis (including SCD1 mRNA expression) of Tregs and conventional T cells (Tconvs) in tumors and lymphoid organs was performed by multiparametric flow cytometry. SCD1 mRNA was also analysed in splenic Tregs and Tconvs isolated from healthy mice and stimulated in vitro with αCD3 and interleukin-2 with or without the SCD1 inhibitor A939572

RESULTS: The levels of SCD1 mRNA were higher in T-Tregs as compared to both T-Tconvs (49% vs 25%) and to splenic Tregs (49% vs 13%). In T-Tregs SCD1 was coexpressed with the activation marker OX40 and the proliferation marker Ki67, suggesting a link with Tregs expansion at the tumor site. Accordingly, in vitro experiments showed that SCD1 transcript was substantially upregulated in both Tregs and Tconvs after polyclonal stimulation. Specific inhibition of SCD1 by A939572 suppressed proliferation of both Tregs and Tconvs and was associated with a reduction of the mitochondrial mass, whose biogenesis has been linked to SCD1 activity1.

DISCUSSION: Rearrangements in fatty acid and mitochondrial metabolism play important roles in Treg protumoral activity impinging on their expansion and suppressive functions1,2. We have shown that SCD1 gene expression is increased in Tregs isolated from human hepatocellular carcinoma (HCC) as compared to both intratumoral Tconvs and to their circulating counterpart3.

CONCLUSIONS: Our results support the hypothesis that SCD1 is involved in Treg proliferation and expansion at the tumor site in HCC. Ongoing experiments focusing on additional aspects of T-Tregs biology will further clarify the reported observations.

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P13.13

P53 REACTIVATION BY PEG-PLA COPOLYMER NANOFORMULATION IMPROVES NATURAL KILLER CELL TARGETING OF MELANOMA CELLS

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PURPOSE: RITA is a small molecule able to reactivate p53 thus preventing the binding of MDM2/MDM4 to WT p53. Pharmacological reactivation of p53 by RITA enhanced the susceptibility of melanoma cells to NK cell-mediated killing (1). A very promising approach in anti-cancer therapy is the application of drug loaded nanocarriers. To improve the pharmacological effect of RITA on tumor cells, we generated RITA loaded PEG-PLA copolymers. The aim of this study was to increase drug solubility and transport efficiency within cells, thus developing a new pharmacological approach able to facilitate melanoma cells recognition and elimination by NK cells.

METHODS: The human melanoma A375 p53 WT cell line was grown in DMEM with 10% FBS, 100 UI/ml penicillin and 100 mg/ml streptomycin. Cells were cultured with empty nanoparticles (NPs), RITA loaded NPs and free RITA at the concentration of 5μM for 3 and 5 days. For cytotoxicity assays, healthy donors' PBMCs were separated with Biocoll separating solution and NK cells were obtained by immunomagnetic separation. These experiments were acquired by FACS. The mPEG2K-(PD,LLA)2 NPs were prepared according to previous procedure (2).

RESULTS: The analysis in solution of the mPEG2K-(PD,LLA)2 NPs loaded with RITA confirmed a micelle-type shape of the NPs, with the hydrophilic shell constituted by the PEG and the inner core constituted by the PD,LLA and loaded with hydrophobic molecule RITA. NPs directly deliver RITA inside melanoma tumor cells, enhancing melanoma recognition and killing by NK cells.

DISCUSSION: We hypothesize that the use of nanotechnology-based delivery of RITA in melanomas could increase their susceptibility to NK cells recognition thus improving the anti-immune checkpoint therapy beneficial effects.

CONCLUSIONS: We propose a new pharmacological strategy that could modulate the susceptibility to NK cell-mediated recognition, amplifying the antitumor immunity in melanoma patients.

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P13.14

DUAL TARGETING OF CANCER AND SUPPRESSIVE MYELOID CELLS BY TUMOR-REDIRECTED INKT CELLS AND ANTIGEN-CARRYING MICROPARTICLES

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BACKGROUND: Adoptive immunotherapy with T cells engineered with tumor-specific TCRs or CARs hold promise for the treatment of hematological and solid malignancies. However, suppressive cues generated by the tumor microenvironment (TME) can dampen the efficacy of engineered T cells. Hence, reprograming the TME is considered critical to optimize the current cell therapy approaches. CD1d-restricted invariant natural killer T (iNKT) cells are active component of the TME and participate in the tumor immunosurveillace by restraining cancer-supporting myeloid populations. Retargeting iNKT cells against cancer cells, by transducing tumor-specific TCR genes, may produce enhanced effectors able to concurrently kill malignant cells and modulate detrimental myeloid cells in TME.

METHODS: Mouse iNKT cells were expanded *in vitro*, engineered with TCRs specific for MHC-restricted tumor-associate peptide antigens and assessed either *in vitro* or upon transfer *in vivo* against tumors expressing the nominal tumor associate antigens. Moreover, the adoptive iNKT cell transfer was combined with their local restimulation with the strong agonist aGalactosylCeramide (αGalCer) delivered using porous silicon microparticle-based nanotherapeutics, which sequentially overcome biological barriers and accumulate at the tumor site.

RESULTS: iNKT cells engineered with MHC-restricted TCRs specific for tumor-associate peptide antigens are indeed bi-specific for CD1d- and MHC-restricted antigens *in vitro*. Upon adoptive transfer *in vivo*, TCR-engineered iNKT cells effectively delay the progression of tumors expressing the cognate antigens and remodel the local myeloid components. These dual anti-tumor functions are further sustained by delivering *in vivo* αGalCer using porous silicon microparticles resulting in enhanced tumor control.

CONCLUSIONS: Collectively, these results support the use of tumor-retargeted iNKT cells plus local restimulation to enhance adoptive cell transfer efficacy, suggesting a rational for future therapeutic strategies in cancer patients.

P13.15

IN PANCREATIC CANCER PATIENTS CHEMOTHERAPY, BUT NOT IMMUNE CHECKPOINT BLOCKADE, RESCUES EFFECTOR T CELL RESPONSE TO TUMOR ASSOCIATED ANTIGENS

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PURPOSE: In Pancreatic Ductal Adenocarcinoma (PDA) immune checkpoint blockade (ICB) failed to increase patient survival. We demonstrated that chemotherapy (CT) enhances the adaptive immune response of PDA patients to tumor associated antigens (TAA)1. Understanding how to better induce an effector response following TAA stimulation is critical to set up efficient precision immunotherapy in PDA.

METHODS: PBMCs from 18 PDA patients, before and after CT, were stimulated in vitro with TAA (ENO1, FUBP1, K2C8, G3P) in the presence or absence of anti-CTLA4 and anti-PD1 to evaluate proliferative T cell response, IFNγ and IL10 production. The V-J rearrangement of TCRb repertoire was profiled with next-generation sequencing.

RESULTS: ICB abolished high proliferative responses to TAA before and after CT, while the same were maintained after CT with an increased number of TAA recognized correlating with patient survival. Before and after CT, ICB decreased the number of high IFNγ responses, whereas these were increased after CT. Clustering analysis of IFNγ/IL10 ratio identified 3 patient groups: in "CT responder" the effector tone was increased or maintained after CT; in "exhausted" the effector tone was lost after CT and in "non-responder" the regulatory tone was predominant before and after CT. The expansion of TAA-specific T cell clones was observed by TCRb repertoire sequencing; the percentage of these TAA-specific V-J rearrangements was to a larger extent after CT.

DISCUSSION: Better than ICB, CT alone shifted the immunological tone toward an effector phenotype, with a significant gain of TAA-induced effector T cell response. The expansion of V-J rearrangements due to TAA stimulation was enhanced by CT, suggesting a stronger reactivity of precise TAA-specific T cell clones after CT.

CONCLUSIONS: These data suggest a strategy based on CT in combination with precision immunotherapy might be considered in selected responder PDA patients.

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P13.16

NEUTROPHIL PD-L1 EXPRESSION AS A NOVEL PREDICTIVE BIOMARKER FOR IV STAGE MELANOMA PATIENTS

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PURPOSE: Stage IV melanoma is a fatal cancer with 5-year survival rates <10%1. Monoclonal antibodies (mAbs) that block programmed death (PD-1) and PD-Ligand 1 (PD-L1) network have revolutioned cancer immunotherapy2. PD-L1 is expressed on several immune cells and evidence indicates that can be also expressed on human neutrophils (PMNs)3. In addition to Normal Density Neutrophils (NDNs), a population of Low Density neutrophils (LDNs) increases in certain cancer patients and correlates with cancer progression. The role of peripheral blood PMNs as predictive biomarkers in anti-PD-1 therapy is largely unknown.

METHODS: 60 stage IV melanoma patients were prospectively recruited. PMNs were isolated from peripheral blood, before and during anti-PD-1 therapy, to evaluate their activation status and PD-L1 expression.

RESULTS: melanoma patient NDNs displayed an activated phenotype (increased percentages CD16+Cd62L− cells) and increased PD-L1 levels compared to healthy controls (HCs). Melanoma patients had increased percentages of LDNs, which displayed higher levels of PDL-1 compared to autologous NDNs. PD-L1 expression levels on NDNs and LDNs did not change during immunotherapy. Patients with low NDN PD-L1 expression displayed better progression free survival (PFS) and overall survival (OS) compared with patients with high NDN PD-L1 expression.

DISCUSSION: PMNs play important roles in immune-mediated clinical conditions such as infection, autoimmunity, and cancer4. The activation status and PDL-1 expression of stage IV melanoma patient NDNs were modified compared to HCs and significantly predicted anti-PD-1 immunotherapy response.

CONCLUSIONS: NDN PD-L1 expression emerges as a novel predictive biomarker in stage IV melanoma patients undergoing anti-PD-1 immunotherapy. Our findings call for a rigorous assessment of neutrophil subsets role in melanoma immunotherapy.

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P13.17

DONOR-UNRESTRICTED TARGETING OF CD1c-EXPRESSING LEUKEMIA BY T CELLS ENGINEERED WITH LIPID-SPECIFIC TCR

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PURPOSE: Primary acute myelogenous and B-lymphoblastic leukemia blasts express the CD1c molecule, and a group of human CD1c-restricted, self-reactive T cell clones them by recognizing the leukemia-associated lipid antigen methyl-lysophosphatidic acid (mLPA)1. Because CD1c is identical in all individuals and expressed only by mature leukocytes, these results suggest a donor-unrestricted adoptive cell therapy (ACT) strategy with T cells redirected against CD1c+ acute leukemia.

METHODS: To assess the feasibility of ACT for acute leukemia with mLPA-specific T cells, we generated a library of lentiviral vectors encoding a panel of human mLPA-specific TCRs and we transduced Jurkat 76 cells and human primary T cells to identify the lead mLPA-specific TCR. Then, we tested the efficacy and the safety of mLPA-specific ACT *in vitro* and *in vivo* in immunodeficient mouse xenografts. Moreover, to gain further insight into the efficacy and safety of mLPA-specific ACT, we generated transgenic mice expreesing CD1c with a pattern similar to human one.

RESULTS: Upon TCR transduction, either Jurkat T cells or human primary T cells are specifically retargeted against CD1c-expressing malignant cells *in vitro*, defining a lead mLPA-specific TCR suitable for adoptive immunotherapy. Indeed, we can engineer total T lymphocytes from any donor with the lead mLPA-specific TCR to kill any CD1c-expressing leukemia cell *in vitro* and in immunodeficient mouse xenografts. CD1c transgenic mice harbored functional APCs recognized by mLPA-specific T cells and selected a CD1c self-reactive peripheral T cell repertoire.

DISCUSSION: The obtained results highlight a novel approach for ACT of acute leukemia with T cells engineered to recognize malignant cells by the transfer of a lipid-specific TCR that works across MHC-barriers like a CAR. We generated a unique pre-clinical model suitable to assess the role of these intriguing cells in the pathophysiology of the immune response.

CONCLUSIONS: Human primary T cells can be efficiently redirected against CD1c+ acute leukemia *in vitro* and *in vivo* by engineering with a mLPA-specific TCR.

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P13.18

TARGETING TOLL-LIKE RECEPTOR 2 TO IMPAIR BREAST CANCER PROGRESSION AND RESISTANCE TO CHEMOTHERAPY

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PURPOSE: Toll-Like Receptor (TLR)2 acts as a double-edge sword in cancer. Besides its role in immune responses, TLR2 is expressed on breast cancer (BC) cells and is associated with poor prognosis in patients1. TLR2 is overexpressed in breast cancer stem cells (CSCs), responsible for cancer progression, therapy resistance and invasion, and promotes their self-renewal through an autocrine loop initiated by high mobility group box (HMGB)12. The dual role of TLR2 in BC needs to be dissected to develop new anti-cancer therapies.

METHODS: Rat HER2-neu transgenic mice developing BC were crossed with TLR2 WT or KO mice (TLR2WT-neuT and TLR2KO-neuT). Tumor progression, CSCs and immune cells were compared. TLR2-mediated cancer cell intrinsic and extrinsic pro-tumor activities and the effect of TLR2 inhibitors in combination with chemotherapy on BC cells were analyzed.

RESULTS: TLR2KO-neuT mice showed a delayed tumor onset, increased survival, reduction of CSCs and T regulatory cells. Transplantation experiments showed that TLR2 acts mainly through cancer cell intrinsic mechanisms, although TLR2 expressed on immune cells also contributed to tumor promotion. TLR2 increased cancer cell proliferation and CSC self-renewal, and conferred resistance to chemotherapy. This was mediated by chemotherapy-induced release of TLR2 ligands, such as HMGB1. Treatment with TLR2 inhibitors impaired viability and induced apoptosis of BC cells, and a synergistic effect was observed when administered with chemotherapy.

DISCUSSION: We demontrated that TLR2 promotes BC progression and reprersents a mechanism of chemoresistance, since chemotherapy induces the release of its activatory ligands. TLR2 silencing or inhibition impair BC progression and restores sensitivity to chemotherapy.

CONCLUSIONS: The use of TLR2 inhibitors in association with chemotherapy opens new perspectives in the treatment of BC patients.

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P13.19

ROLE OF CALCINEURIN IN AFFECTING TUMOR GROWTH AND THE FORMATION OF THE TUMOR MICROENVIRONMENT

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PURPOSE: The purpose of this project is to investigate the role of the Ca++-dependent phosphatase Calcineurin (CN) in promoting tumor growth and shaping the immune tumor microenvironment. In this study we aim to characterize the phenotypical and functional changes induced by the constitutive inhibition of calcineurin interaction with its substrates.

METHODS: A series of murine tumor cell lines, such as 4T1 and B16, have been genetically engineered to constitutively block the substrate docking site of calcineurin. The growth and tumor-formation capacity of these cells have been evaluated *in vivo*, and the immune infiltrating populations have been analyzed by flow cytometry. Their transcriptional profile has been characterized by qPCR and bulk RNA sequencing.

RESULTS: There was no difference observed between the growth rates of controls and mutant B16 tumors, despite the latter having a significantly larger precursor exhausted CD8+ T cell infiltrate. By analyzing the RNAseq data obtained in 4T1 cells, CXCL11/10/9 have been pinpointed as differentially expressed cytokines with CD8+ T cell chemotactic activity. This has been confirmed *in vivo* in tumors generated by mutant B16 cells. According with the accumulation of CD8+ T cells, the administration of an anti-immune checkpoint inhibitors resulted in a greatly diminished tumor growth.

DISCUSSION: Our studies highlight the role of calcineurin in tumor development and how the modulation of its activity can lead to the differential recruitment of tumor-infiltrating immune populations and particularly of CD8+ T cells. Despite the apparent exhausted phenotype, it can be effectively reversed by immune checkpoint inhibition.

CONCLUSIONS: While more studies are necessary to further validate the data, calcineurin emerges as a potential target for novel therapeutic approaches, particularly as a potent aid to already existing immune checkpoint blockade therapies.

P13.20

TARGETING THE CYSTINE/GLUTAMATE ANTIPORTER XCT AND MUTANT-P53 SYNERGISTICALLY HINDERS BREAST CANCER PROGRESSION

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PURPOSE: Breast cancer is the most frequent cancer in women and, despite the recent advances, new therapeutic approaches are still required. It has been recently reported that mutations of the tumor suppressor gene *TP53* downregulate the expression of *SLC7A11*, encoding for the cystine/glutamate antiporter xCT, a tumor-associated antigen crucial for the maintenance of intracellular redox balance [1], rendering mutant-p53 tumours susceptible to oxidative damage. Thus, on one hand xCT expression levels may be predictive of the response to APR-246, a drug used in clinical trials that reactivates mutant p53, while on the other hand xCT targeting may enhance the efficacy of APR-246 treatment. Here, we propose the combination of anti-xCT DNA vaccination and APR-246 administration as a new strategy for tackling breast cancer from different angles.

METHODS: We performed viability and apoptosis assays on murine and human breast cancer cell lines cultured in 2D or as cancer stem cell (CSC)-enriched tumorspheres, treated with APR-246 and the xCT inhibitor sulfasalazine (SAS). BALB/c mice were orthotopically injected with murine mammary cancer cell lines, then tumor-bearing mice were treated with APR-246 and two boosts of anti-xCT DNA vaccination or the control plasmid [2].

RESULTS: *In vitro*, the combination of APR-246 and SAS significantly reduced cancer viability, induced apoptosis and affected CSC self-renewal if compared to the single treatments.
In vivo, APR-246 treatment and xCT immunotargeting synergistically hindered tumor progression and reduced lung metastasis.

DISCUSSION: APR-246 restores the wild-type conformation of mutated p53, however, it upregulates the expression of xCT, which contributes to CSC self-renewal and therapy resistance. Here we demonstrated that a vaccination-based anti-xCT immunization is able to synergize with APR-246 treatment and enhance its therapeutic effect.

CONCLUSIONS: Overall, our results suggest that the combination of APR-246 administration and anti-xCT vaccination may represent a valid strategy for breast cancer treatment

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P13.21

PROGNOSTIC VALUE OF CIRCULATING IMMUNOLOGIC PARAMETERS IN NSCLC PATIENTS TREATED WITH NIVOLUMAB: A PILOT STUDY

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PURPOSE: The aim of this observational study is the identification of predictive biomarkers of response to nivolumab in advanced NSCLC patients' peripheral blood (PB) correlating phenotypical and functional laboratory immunological parameters with patients survival.

METHODS: 30 patients before treatment with nivolumab, from February 2016 to May 2018 at University Hospital of Florence, were enrolled. Circulating T and NK cells were evaluated by flow cytometry for subpopulations frequencies, immune checkpoints' and cytotoxic molecules' expression and cytokines production profile after in vitro polyclonal stimulation.

RESULTS: PD-1 expression was higher in CD4+T lymphocytes and CD16+cells from patients than from healthy donors. The same trend was present for CTLA-4 expression. The intracellular expression of granzyme A and perforin was lower in patients' NK cells whereas was higher in CD8+cells of patients, compared to healthy donors. CD4+ cells producing IL-2 and TNF-alfa were significantly higher in patients compared to controls, whereas the frequency of IFN-gamma producing cells was strongly downregulated in CD4+ T cells and in NK cells. PD-L1 expression on both CD4+T cells and NK lymphocytes negatively correlated with OS while only CD4+T cells negatively correlated with PFS.

DISCUSSION: Here we identify relevant changes in the ICs and cytotoxic molecules expression and in cytokines production, in NSCLC patients, confirming that the assessment of a large panel of biomarkers is desirable in order to obtain an exhaustive picture of the patients' immune-ecosystem.

CONCLUSIONS: Results of this study are exploratory in nature referring to a small series of patients but suggest that the assessment of an immune signature on PB of NSCLC patients may be helpful for personalize therapeutic decisions, identifying patients who could benefit from ICI.

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P13.22

COMPLEMENT ACTIVATION PROMOTED BY THE LECTIN PATHWAY MEDIATES C3AR-DEPENDENT SARCOMA PROGRESSION AND IMMUNOSUPPRESSION

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PURPOSE: Complement has emerged as a component of tumor promoting inflammation (1). We conducted a systematic assessment of the role of complement activation and effector pathways in sarcomas.

METHODS: Experiments of mesenchymal 3-methycolanthrene (3-MCA)-induced carcinogenesis and two transplantable sarcoma models were performed in mice deficient for the key complement molecule C3, for molecules selectively necessary for the activation of each complement activating pathway (classical, lectin and alternative) and for the receptors of C3a and C5a produced downstream C3 cleavage. Tumor infiltrating immune cells were characterized by flow cytometry and RNA-sequencing. Treatment with anti-PD1 antibodies and/or with a C3aR antagonist in mice were performed to evaluate potential combination of immunotherapy with complement deficiency/inhibition. Public databases of RNA-seq data derived from human sarcomas were used for computational analysis aimed at clarifying the prognostic potential of C3 deficiency-associated signatures.

RESULTS and DISCUSSION: *C3*-/-, *MBL1/2*-/- and *C4*-/- mice showed reduced susceptibility to 3-methylcholanthrene sarcomagenesis and transplanted sarcomas, whereas C1q and factor B deficiency had marginal effects. Complement 3a receptor (C3aR), but not C5aR1 and C5aR2, deficiency mirrored the phenotype of *C3*-/- mice. C3 and C3aR deficiency were associated with reduced accumulation and functional skewing of tumor-associated macrophages, increased T cell activation and response to anti-PD-1 therapy. Transcriptional profiling of sarcoma infiltrating macrophages and monocytes revealed the enrichment of MHC II-dependent antigen presentation pathway in C3-deficient cells. In patients, computational analysis revealed that C3aR expression correlated with a macrophage population signature and C3 deficiency-associated signatures predicted better clinical outcome.

CONCLUSIONS: These results suggest that the lectin pathway and the C3a/C3aR axis are key components of complement and macrophage mediated sarcoma progression and immunosuppression.

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P13.23

INVESTIGATING THE CYSTINE/GLUTAMATE ANTIPORTER XCT IN THE INTERACTION BETWEEN MAMMARY CANCER AND THE IMMUNE SYSTEM

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PURPOSE: The transmembrane protein xCT is upregulated by cancer cells to afford protection against oxidative stress and represents a relevant target for cancer treatment. xCT expression is also needed for the activation and function of immune cells. However, whether the overall outcome of xCT depletion in the tumor and in the immune system results in promotion or suppression of tumor growth is unknown. The aims of this project are to investigate how xCT depletion in tumor cells affects tumor progression in an immunocompetent mouse model and if xCT depletion in the immune system affects tumor growth.

METHODS: We generated xCT-KO mouse mammary cancer cells (4T1) and tested their malignant properties in vitro and in vivo. We generated xCTnull BALB/c mice to investigate the role of xCT in the immune system and xCTnull / ErbB2-transgenic BALB-neuT mice to study the role of xCT in a mammary cancer-prone model.

RESULTS: xCT depletion in 4T1 cells decreased clonogenic potential, sensitized cells to oxidative stress, reduced migration in vitro and lung metastasization in vivo. Depletion of xCT in the immune system prevented activation/polarization of immune cells cultured ex vivo. Nevertheless, lack of xCT in the immune system did not impair the proper mounting of both humoral and cellular immune response in vivo. Finally, xCT depletion in BALB-neuT mice did not impair autochthonous tumor initiation. However, cancer cells isolated from these mice showed proliferation and redox balance defects in vitro.

DISCUSSION: While xCT is required for both cancer cell malignancy and immune system functionality in vitro, it is partially dispensable in vivo. The mechanisms at the basis of this discrepancy need to be identified.

CONCLUSIONS: While xCT depletion does not interfere with tumor initiation, it could sensitize otherwise normally growing tumors to oxidative stress-inducing therapies in vivo, opening up possibilities for a better design of combinatorial approaches involving xCT targeting.

P13.24

IDENTIFICATION OF TENEURIN 4 AS A NOVEL CANCER STEM CELL-ASSOCIATED ANTIGEN AND POTENTIAL BIOMARKER

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PURPOSE: Triple negative breast cancer (TNBC) is one of the most aggressive type of human breast cancer with a high incidence of recurrences and metastasis. This is due to a lower efficacy of current therapies to eliminate cancer stem cells (CSC). For this reason, the objective of this work is to identify novel functional targets expressed on CSC suitable for immunotherapy.

METHODS: RNA-seq was used to identify differences in gene expression between tumorspheres and epithelial cells. TNBC cells were silenced through RNAi, and knock-out (KO) mouse TNBC cells were obtained with the CRISPR/Cas9 technology. TENM4 role in self-renewal and migration, was tested in vitro performing tumorsphere-forming ability assay and transwell invasion assay. To evaluate TENM4 as TNBC biomarker TENM4 presence in the sera and in the exosomes of tumor bearing mice and of breast cancer patients was tested through ELISA and Western blot, respectively.

RESULTS: TENM4 was selected among the transmembrane proteins overexpressed in mouse and human TNBC stem cells-enriched tumorspheres. TENM4 silencing significantly impairs the tumorsphere-forming potential, lowering also the expression of some CSC markers, and their ability to migrate. Although injection of KO or wild-type cells in BALB/C mice didn't affect tumor growth, a lower number of lung metastasis was observed in mice injected with KO cells. Furthermore, the presence of TENM4 in the sera of tumor bearing mice, as well as in the plasma of some breast cancer patients and in exosomes of TNBC cells supernatants was observed. Finally, data mining of publicly available data sets showed a trend of correlation between TENM4 higher expression in TNBC and shorter patients' relapse-free survival.

DISCUSSION: Our *in vitro* results suggest a possible link between TENM4 expression and cancer stem-like features and tumor-initiating potential. Moreover, *in vitro* and *in vivo* experiments show that TENM4 can impair tumor cell invasion affecting also metastasis formation and suggesting an involvement of TENM4 in cell migration. Furthermore, the presence of TENM4 in the supernatant of TNBC cells and in the sera of breast cancer patients, suggest its potential use as biomarker.

CONCLUSIONS: Overall, our results show that TENM4 could be identified as a novel immune-target for TNBC treatment and as a novel biomarker.

P13.25

EMERGING IMMUNOLOGICAL CHECKPOINTS PUT THE BRAKES ON MELANOMA INFILTRATING γδ T CELLS

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PURPOSE: Melanoma is an immunogenic tumor and patients' survival is improved by immune checkpoint inhibitors therapy1. gd T cells have showed antitumoral properties in several tumors including melanoma and they are optimal candidates to immunotherapy2. Indeed, supposing a new combined immunotherapeutical approach, we have analysed if melanoma infiltrating gd T cells could be influenced by immunological checkpoint molecules expressed on tumoral cells.

METHODS: Using GEPIA 2.0 website, we first investigated the relative expression of some checkpoint's ligands in melanoma and corresponding normal tissue and the correlation between ligand and receptor genes and gene signature of TCR gd in the tumor. Moreover, we analysed by flow cytometry the frequency of melanoma infiltrating gd T cells and their expression of checkpoint receptors paired with ligands present in the tumor.

RESULTS: Bioinformatics analysis of checkpoint ligands expression in melanoma showed higher levels of LGALS3, LGALS9 and CD274 compared to healthy tissue, even though the relative expression of the latter was lower than others. Moreover, Spearman correlation analysis between immune checkpoint ligand genes and gd T cells gene signature was significantly positive only for CD274 and LGALS9 whilst LGALS3 expression was negatively correlated. Correlation between PD1, LAG3 and TIM3 and gd T cells gene signature was significantly positive. Flow cytometry analysis showed that gd T cells were more represented in melanoma compared to normal tissue and their expression of immunological checkpoint receptors, confirming the bioinformatics analysis.

DISCUSSION: These preliminary results identify in emerging melanoma immunological checkpoints that could affect the effector functions of tumor infiltrating gd T cells.

CONCLUSIONS: Combining immune checkpoint inhibitors with gd T cells based immunotherapy could improve the clinical response and prognosis of melanoma patients.

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P13.26

MATERNAL IMMUNIZATION AGAINST ANAPLASTIC LYMPHOMA KINASE: A NEW WEAPON AGAINST NEUROBLASTOMA

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PURPOSE: Neuroblastoma (NB) is the most common extracranial solid tumor in infancy that can occur in the early postnatal age or even during fetal life. Given our previous findings attesting the efficacy of maternal immunization (MI) against a cancer-associated antigen in delaying breast cancer development in genetically predestined offspring [1], a pre-birth immunotherapy approach against the anaplastic lymphoma kinase (ALK) oncoantigen, involved in NB, has been evaluated. To this aim, we exploited a NB mouse model (ALK/MYCN), which spontaneously develops early-onset multifocal lesions.

METHODS: MYCN transgenic females underwent immunization against ALK trough DNA electrovaccination with the ALK-ECTM plasmid, prior to mating with ALKF1174L transgenic males. In ALK/MYCN offspring, abdominal, cervical and paraspinal tumors have been quantified by Magnetic Resonance Imaging. The humoral immune response induced against ALK in mothers and offspring, as well as the presence of immune complexes (IC), have been evaluated by ELISA. ALK expression in tumor tissue, and in human NB cells treated with anti-ALK serum, was assessed by Western blot.

RESULTS: Pre-birth immunization against ALK leads to an extended survival time and to a lower tumor growth kinetic in ALK/MYCN offspring born to ALK-ECTM-vaccinated mothers (ALK offspring) as compared to controls, born from mothers. Maternally derived anti-ALK antibodies were successfully transferred from mothers to newborns together with IC containing ALK; moreover, anti-ALK IgM were found in the sera of ALK offspring. MI against ALK decreased ALK expression in tumor tissue of ALK offspring. Finally, a decreased expression of ALK was revealed in ALK-positive human NB cells, following incubation with sera of ALK-ECTM-vaccinated mothers and ALK offspring.

DISCUSSION: MI against ALK is able to induce an active immunization against this oncoantigen in ALK offspring, probably due to the breast milk-mediated transfer of specific IC. This phenomenon, coupled with the transfer of maternal immunity, impairs tumor development, enhancing survival time in a preclinical model of high-risk NB.

CONCLUSIONS: These results indicate that MI against ALK could be a viable option for childhood cancer management in clinical practice.

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P13.27

A CHIMERIC HUMAN/DOG DNA VACCINE AGAINST THE CHONDROITIN SULFATE PROTEOGLYCAN 4 REVEALS POTENTIAL THERAPEUTIC EFFECTS FOR THE TREATMENT OF MELANOMA

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PURPOSE: Among the most interesting targets for immunotherapeutic approaches, the Chondroitin Sulfate Proteoglycan (CSPG)4 stands out, with low expression in healthy tissues, high expression in several tumors and a key role in cancer progression [1]. Thanks to the CSPG4 over-expression by both human and canine malignant melanoma (MM), we demonstrated the safety and the clinical effectiveness of a xenogeneic human (Hu)-CSPG4 DNA vaccine in prolonging the overall survival of client-owned canine patients with stage II-III surgically resected CSPG4+ MM [2]. However, Hu-CSPG4 vaccine was barely effective in activating dog and human T cells in vitro. Based on these results, we aimed to increase the effectiveness and the translational power of our approach.

METHODS: We generated a hybrid plasmid, derived in part from the Hu- and in part from the dog (Do)-CSPG4 sequence (HuDo-CSPG4). We tested the safety, immunogenicity and anti-tumor potential of HuDo-CSPG4 DNA vaccine in mice and in dogs with stage II-IV surgically resected CSPG4+ MM and in a human setting in vitro.

RESULTS: HuDo-CSPG4 vaccination is immunogenic and endowed with an anti-tumor potential in mice. In canine patients, the procedure is safe and clinically effective. HuDo-CSPG4 significantly increased the overall survival of vaccinated dogs as compared to controls treated with conventional therapies alone. These clinical results were related to the induction of antibodies against both the Hu- and Do-CSPG4, with a higher affinity as compared to those induced by the Hu-CSPG4 DNA vaccine. Moreover, a cytotoxic response against canine MM CSPG4+ cells was detected. Preliminary results obtained in vitro with T cells from human subjects suggested HuDo-CSPG4 could be also immunogenic.

DISCUSSION: HuDo-CSPG4 DNA vaccination is a safe and effective way to break immune tolerance against the self-antigen, providing the rationale to propose this strategy for the treatment of canine CSPG4+ tumors.

CONCLUSIONS: Thanks to the power of naturally occurring cancers in dogs as valuable predictive models for cancer immunotherapy response, these data represent a solid basis to stimulate the translation of this approach in a human clinical setting.

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P13.28

CSPG4 IMMUNE-TARGETING AS NOVEL STRATEGY FOR THE TREATMENT OF OSTEOSARCOMA

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PURPOSE: Osteosarcoma (OSA) is a fatal paediatric tumour characterized by high frequency of metastasis and resistance to therapies. The chondroitin sulfate proteoglycan (CSPG)4 is overexpressed by several tumours that fail to respond to conventional therapeutic regimens, including OSA. Therefore, it could be an ideal target for innovative therapeutic approaches such as immunotherapy. Since the rarity of OSA, canine OSA represents a valuable translational pre-clinical model for testing new treatments. We investigated the impact of CSPG4 immune-targeting and its role in sustaining tumour-related processes. Based on our previous results [1] the pre-clinical efficacy of an anti-CSPG4 immunotherapy in dogs is being tested.

METHODS: We treated cells with anti-CSPG4 monoclonal antibodies (mAbs), alone or combined with chemotherapy, and sera from canine patients [1]. We downregulated or overexpressed CSPG4 and functional assays were performed to evaluate cell proliferation, migration, chemoresistance and tumorspheres viability. Naturally-occurring OSA-bearing dogs are being enrolled in a clinical veterinary trial and adjuvantly treated with an anti-CSPG4 DNA vaccine. FACS analysis was performed to evaluate the vaccine-induced antibody response.

RESULTS: MAbs and vaccine-induced antibodies [1], inhibited canine OSA cells tumorigenic potential, potentiating the effect of chemotherapy *in vitro*. CSPG4 down-modulation in OSA cells reduced CSPG4 dependent tumour potential *in vitro* and its overexpression conferred resistance to chemotherapy. Adjuvant anti-CSPG4 vaccination in dogs is demonstrating to be safe and able to induce a humoral immune response.

DISCUSSION: These results provide the rationale for testing anti-CSPG4 immunotherapy *in vivo*. Further investigations could provide more insights toward the understanding of the immune response triggered by vaccination and its possible effect on prolonging the survival of canine patients.

CONCLUSIONS: In the future, these findings could be translated in a human clinical setting, hopefully improving life expectancy of OSA patients that cannot benefit from present therapies.

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P13.29

NUTLIN-3A ENHANCES NATURAL KILLER CELL-MEDIATED KILLING OF NEUROBLASTOMA BY RESTORING P53-DEPENDENT EXPRESSION OF LIGANDS FOR NKG2D AND DNAM-1 RECEPTORS

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PURPOSE: In this study we explored whether Nutlin-3a, a well-known nontoxic small-molecule compound antagonizing the inhibitory interaction of MDM2 with the tumor suppressor p53, may restore ligands for Natural Killer (NK) cell-activating receptors (NK-ARs) on neuroblastoma (NB) cells, and ultimately enhance the NK cell-mediated killing of NB.

METHODS: NB cell lines were treated with Nutlin-3a and the expression of ligands for NKG2D and DNAM-1 NK-ARs and the NB susceptibility to NK cells were evaluated. Adoptive transfer of human NK cells in a xenograft NB-bearing NSG murine model was assessed. Two datasets of NB patients were explored to correlate p53 expression with ligands. Luciferase assay and chromatin immunoprecipitation (ChIP) analysis of p53 functional binding on *PVR* promoter were performed. Primary NB cells were treated with Nutlin-3a and NB spheroids obtained from one high-risk patient, essayed for NK cell cytotoxicity.

RESULTS: We provide evidence showing that the Nutlin-3a-dependent rescue of p53 function in NB cells results in: (*i*) increased surface expression of ligands for NK-ARs, thus rendering NB cell lines significantly more susceptible to NK cell-mediated killing; (*ii*) shrinkage of human NB tumor masses that correlates with overall survival, upon adoptive transfer of NK cells in NB-bearing mice; (*iii*) increased expression of ligands in primary NB cells and boosting of NK cell-mediated disaggregation of NB spheroids. In addition, we found that p53 is a direct transcription factor regulating the expression of PVR ligand recognized by DNAM-1.

DISCUSSION: Nutlin-3a is non-toxic for normal cells, thus appearing as a suitable tool for tumor therapy. Interestingly, pediatric tumors are very often p53-wt at the diagnosis, and therefore potential therapeutic targets for Nutlin-3a-based treatment. The immunomodulatory activity of Nutlin-3a at low doses had never been reported before.

CONCLUSIONS: Our findings demonstrate a previously undescribed immunomodulatory role of Nutlin-3a, which might be prospectively employed for a novel NK cell-based immunotherapy of NB.

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P13.30

MACROPHAGE-DRIVEN INFLAMMATION IN MALIGNANT PLEURAL MESOTHELIOMA: ROLE OF OSTEOPONTIN AND GPNMB IN TUMOR PROGRESSION

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PURPOSE: In a gene expression analysis of human mesothelioma surgical samples, we identified two highly upregulated genes: GPNMB, coding for glycoprotein non metastatic B (GPNMB), and SPP1, coding for osteopontin (OPN). Both proteins were found by our group upregulated also in tumor-conditioned macrophages [1-2]. The aim of the project is to investigate the biological role of OPN and GPNMB in Malignant Pleural Mesothelioma (MPM) tumor growth.

METHODS: We used MPM biological samples (tumors, plasma) and a murine mesothelioma model with three cell lines AB1, AB12, and AB22, mimicking the different histotype of human MPM: sarcomatoid, biphasic, epithelioid [3].

RESULTS: OPN and GPNMB were found to be expressed at higher levels (ELISA) in MPM patients than in healthy donors. OPN was strongly expressed by the three cell lines while GPNMB levels were very low, unlike human MPMs; accordingly, we silenced OPN and overexpressed GPNMB in AB1, AB12, and AB22 cells to study their role in vivo. We demonstrated that OPN silencing strongly decreases tumor growth, while GPNMB overexpression increased tumor progression. Injecting MPM non-engineered cells in GPNMB knock-out and wildtype mice we further revealed that GPNMB plays a pro-tumoral role also when expressed by macrophages in the tumor microenvironment.

DISCUSSION: MPM is an aggressive cancer characterized by chronic inflammation driven by the presence of non-degradable asbestos fibers. Our study highlighted two interesting proteins, OPN and GPNMB, that play a pro-tumoral role in MPM development. The identification of new molecular pathways that can be targeted for therapeutic interventions is of critical importance to lead to more effective treatments.

CONCLUSIONS: Overall, the results indicate that OPN and GPNMB have a pro-tumoral role in MPM and may be considered as molecular targets for therapeutic interventions.

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P13.31

A DISINTEGRIN AND METALLOPROTEINASES INHIBITORS DIRECT EFFECTS AND BRENTUXIMAB VEDOTIN ENHANCEMENT IN HODGKIN'S LYMPHOMA 3-DIMENSIONAL MODELS

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PURPOSE: Shedding of A Disintegrin And Metalloproteinases (ADAM10) substrates, like TNFα or CD30, can affect both anti-tumor immune response and antibody-drug-conjugate (ADC)-based immunotherapy.1 We reported two new ADAM10 inhibitors, LT4 and MN8 able to prevent such shedding in Hodgkin lymphoma (HL).2 As tumor tissue architecture can influence the outcome of anti-cancer treatments,3 we checked the anti-lymphoma potential of ADAM10 inhibitors in three-dimensional (3D) systems.

METHODS: Two 3D models were set up: mixed spheroids of HL lymph node (LN) mesenchymal stromal cells (MSC) and Hodgkin lymphoma cells (HL cells) or LN-derived matrices and collagen sponges repopulated with both LN-MSC and HL cells.

RESULTS: In these 3D systems LT4 and MN8 reduced intracellular ATP, glucose consumption, cell proliferation, and increased lactate dehydrogenase release as a marker of cell damage. In addition, mixed spheroids decreased in size and CD30 and TNFα shedding was limited by ADAM10 inhibitors; these effects could be reproduced in LN matrix or collagen scaffolds repopulated with LN-MSC and HL cells.

DISCUSSION: LT4 and MN8 not only interfered with HL cell growth, but also enhanced the anti-lymphoma effect of the anti-CD30 ADC brentuximab-vedotin (BtxVed) in 3D systems very similar to those approved as pre-clinical models.4 This was evident at low ineffective doses of the ADC, indicating a possible combined scheme to potentiate ADC-based lymphoma therapy.

CONCLUSIONS: Both direct and combined anti-lymphoma effect of ADAM10 inhibitors with BtxVed could be demonstrated in 3D models recapitulating features of LN microenvironment, leading to ADC effects improvement. Starting from this evidence, repopulated scaffolds may represent elementary structures to reconstitute the whole LN composition, useful to understand the complexity of lymphoma microenvironment and test drug effects.

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P13.32

IMMUNE MICROENVIRONMENT PROFILING OF BREAST CANCER BRAIN METASTASES USING MULTIPLEX IMMUNOFLUORESCENCE

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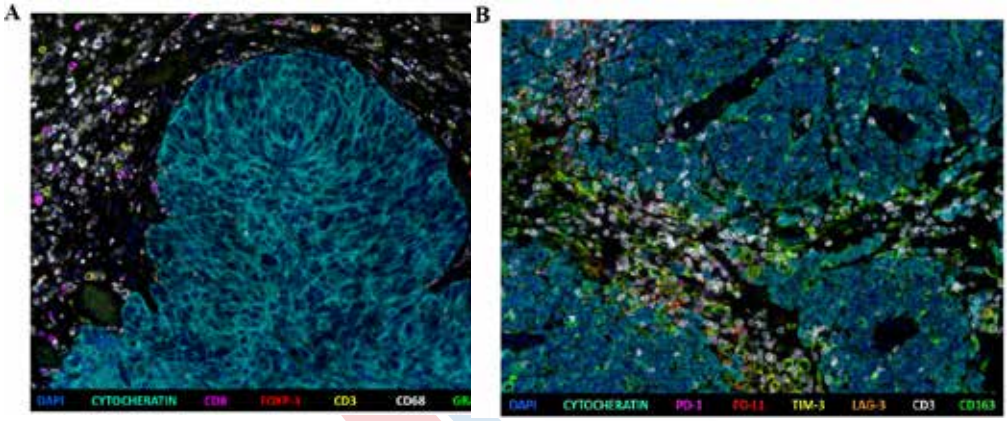
PURPOSE: Despite potential clinical implications, the complexity of immune microenvironment in breast cancer (BC) brain metastases (BM) is still poorly understood. Multiplex immunofluorescence (mIF) allows simultaneous visualization and quantification of several markers while maintaining spatial information.

METHODS: BM samples were collected for 60 BC patients undergoing neurosurgery (2003-2018) at three institutions. BCBM immune contexture was characterized using two custom mIF panels, including cell subtyping (CD4, CD8, FoxP3, CD68), activation (Granzyme B), immune checkpoint and co-inhibitory molecules (PD1, PD-L1, TIM3, LAG3, CD163) and localization (cytokeratin for tumor recognition) markers (Figure).



RESULTS: Sixty BCBM samples were analyzed: 32% HR+/HER2-, 38% HER2+, 30% HR-/HER2-. At a median follow-up of 43 months, the only clinical variable associated with OS was BC subtype. In the tumor area, HR+/HER2- tumors showed higher density of CD68+ cells compared to other subtypes, while a higher percentage of CD8+Granzyme B+ lymphocytes was observed in HR-/HER2-. Higher CD163+ macrophage density was significantly associated with worse OS, even after correction by BC subtype. In the HR-/HER2- BCBM subgroup, high intra-tumoral infiltration of T lymphocytes, high percentage of tumor cells within a 25 µm radius from CD8+ cells and low percentage of CD3+ cells within a 10 µm radius from CD163+ macrophages, were associated with longer OS. In HR+/HER2- BCBM, high TIM3+ cell density in the stroma was associated with longer OS. Conversely, low density of CD3+PD-1+LAG3+ T cells and low percentage of PD-1+ cells within a 15 µm radius from PD-L1+ cells were associated with increased OS in HER2+ BCBM group.

DISCUSSION: mIF can be used to comprehensively describe BCBM immune microenvironment, potentially providing useful information to guide novel therapeutic approaches. In BCBM, immune infiltrate differs according to BC subtype. M2 macrophage polarization is consistently associated with worse OS across all BC subtypes, and checkpoint expression is correlated with patient outcome.

CONCLUSIONS: A more tolerogenic immune microenvironment is associated with worse OS and might represent a target for optimization of immunotherapy for these patients.

P13.33

TUMOR MICROENVIRONMENT CHARACTERIZATION OF HUMAN PAPILLOMAVIRUS-POSITIVE AND -NEGATIVE OROPHARYNGEAL SQUAMOUS CELL CARCINOMAS

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PURPOSE: The Human papilloma virus (HPV) infection is the cause of a subset of oropharyngeal squamous cell carcinoma (OPSCC). We characterized density and spatial distribution of immune cells in HPV+ and HPV- OPSCC microenvironment, in primary tumors and in the corresponding neck metastases.

METHODS: Ten HPV+ and 9 HPV- OPSCC samples were analysed with a 9-color multiplex immunofluorescence (mIF) panel including markers for cell subtyping (CD68, CD8, CD103), functionality (FoxP3, CD163), immune checkpoint molecule (PD-1, PD-L1) and localization (pan-cytokeratin for tumor recognition; Figure). Cell densities, percentages and cell distances were evaluated for each sample.

RESULTS: No significant difference in immune cell densities was observed between primary tumors and the corresponding lymph node metastases, either in HPV+ or HPV- patients. HPV+ primary tumors and metastases showed a higher infiltration of CD8+ T cell, CD8+CD103-, CD8+CD103+, CD8+PD-1+ cells, and CD8+CD103+PD-1+ T compared to HPV- samples. A shorter mean distance between tumor cells and CD8+, CD8+CD103+ and CD8+PD-1+ T and an increased percentage of tumor cells within a 15 µm radius from CD8+ T lymphocytes were observed in HPV+ primary tumors and metastases. Moreover, CD8+ T lymphocytes in HPV+ specimens were closer to CD163+ macrophages and to FoxP3+ cells. In both HPV+ primary tumors and metastases, the density of PD-L1+ cells was higher, with most abundant PD-L1 expression on cancer cells, but also on infiltrating macrophages. PD-L1+ tumor cells and CD8+PD-1+ T cells resulted closer in HPV+ samples. Independently from HPV status, a better disease-free survival (DFS) was associated with higher densities of CD8+CD103+PD-1+, FoxP3+ cells and a lower density of CD163+PD-L1+ in primary tumors. Patients with a major density of CD8+PD-1+ lymphocytes in metastasis showed a better DFS.

DISCUSSION: mIF technique allowed the characterization of close interactions between cells expressing immunotherapy-targeted molecules within OPSCC microenvironment. Indeed, we observed that HPV+ OPSCC samples were more infiltrated, but also enriched in cell populations susceptible to inhibition.

CONCLUSIONS: With these findings is possible to consider the checkpoint therapy for OPSCC patients as a first line treatment.

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CASPASE-11 IS AT THE CROSSROAD BETWEEN SMOKE-INDUCED INFLAMMATION AND LUNG CANCER IN MICE

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PURPOSE: Smoking is the major risk factor for chronic obstructive pulmonary disease (COPD), and is associated with a higher likelihood of lung cancer establishment (1). Because we demonstrated that caspase-11promotes lung carcinogenesis in mice (2), we aim to investigate whether cigarette smoke (CS) exposure promotes a lung inflammatory pattern associated to caspase-11 activation which, in turn, drives lung cancer onset.

METHODS: To mimic the inhalation profile as in smokers, C57Bl/6N and 129Sv mice were exposed to first-hand smoking for 4-8-16 weeks. Murine data were compared to human samples from smoker adenocarcinoma patients.

RESULTS: The exposure to CS induced air space enlargement, and was associated to fibrotic deposits along the bronchi, mucus production and a state of inflammation (IL-1-like cytokines) in C57Bl/6N mice. We found that 129Sv mice, which carry a caspase-11 mutation showed a decreased deposition of collagen, mucus production, percentage of recruited dendritic cells and IL-1-like cytokines levels, which release is well-known to be correlated to cancer progression. By comparing above results to human smoker lung cancer-derived lung specimens, we found that high levels of tumor-tissue caspase-4, the human analogue of the murine caspase-11, were associated to lower survival.

DISCUSSION: Chronic inflammation typical of COPD and lung cancer patients reflects the site of deposition of inhaled irritants, like CS (1). Our data suggest that smoking mice as well as humans may be more susceptible to the caspase-11/-4-associated inflammatory processes involved in lung cancer development.

CONCLUSIONS: We found that caspase-11 in mice is associated to smoke-induced lung latent inflammation and could drive the establishment of lung cancer.

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P13.36

PATIENT-DERIVED COLON EPITHELIAL ORGANOIDS AS A THREE DIMENSIONAL MODEL TO STUDY Vδ2T CELL-MEDIATED IMMUNE RESPONSE IN COLORECTAL CARCINOMAS

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PURPOSE: Vδ2T cells, can be triggered to proliferate and exert anti-tumor effects by zoledronic acid (ZA) that is presented through BTN3A1 and BTN2A1 receptors (1). Human Vδ2T cells are different from their counterpart in animals, so that murine models of colorectal carcinomas (CRC) cannot fully resemble the features of this neoplastic disease. Thus, three dimensional culture systems where patient-derived immune cells and tumor cells could interact in appropriate tissue architecture and microenvironment, have been set up (2).

METHODS: Organoids were derived from CRC biopsies and cultured in medium with defined composition, without cell derived growth/differentiating factors. Patients' peripheral blood T cells were isolated and co-cultured with self-organoids and ZA to determine Vδ2T cells expansion. Then, self-organoids were challenged with activated self-Vδ2T cells to detect anti-tumor effects.

RESULTS: CRC organoids mutational status was similar to that of the original biopsies: in particular epithelial cells were Vil1+ MUC2+ BTN3A1+ BTN2A1+; usually CRC organoids did not express NKG2DL, ICAM1 and PDL1 but were ESA+or HLA-I+ and reacted with DNAM1 chimeric receptor. CRC organoids could stimulate self-Vδ2T cells expansion and killing in the presence of soluble ZA. IFNγ induced the expression of ICAM1, PDL1 and HLA-I on these organoids that retained the ability to trigger the Vδ2T cellexpansion and tumor killing.

DISCUSSION: We provide evidence that patient-derived organoids are suitable to study the interaction between Vδ2T cells and tumor epithelial cells.

CONCLUSIONS: We provided the proof of principle that CRC organoids, resembling original tumor specimens, can be used in vitro to expand self-Vδ2T cells and trigger their anti-tumor activity. This can be a reliable tool to reduce, refine or even replace the in vivo animal models to plan novel therapeutic approaches to CRC.

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P13.37

ROLE OF TUMOR-ASSOCIATED MACROPHAGES IN IMMUNE EVASION IN COLORECTAL CANCER

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PURPOSE: Tumor-associated macrophages (TAMs) are reported to adopt protumor, anti-inflammatory features in different types of solid tumors1,2. However, in the specific case of colorectal cancer (CRC), the role of TAMs is controversial, with some studies reporting a correlation between higher macrophage infiltration and an adverse prognosis3, and others concluding that the presence of TAMs is associated with an improved survival4. The lack of agreement on TAM function in CRC prompts a deeper characterization of macrophage subpopulations in CRC, and the investigation of the relationship between TAMs and colorectal cancer cells *in vivo*.

METHODS: Bone marrow (BM)-derived macrophages (BMDM) were differentiated from BM cells isolated from mouse femurs and tibias, and colon organoids were generated starting from colon crypts and cultured as previously described5. A mouse model of CRC was generated using azoxymethane (AOM)/dextran sodium sulfate (DSS) model of colitis-associated cancer.

RESULTS: A new coculture system between murine BMDM and colon organoids was established and optimized to study TAM-CRC interaction in a physiologically relevant system. After 48h of coculture in Matrigel, BMDM-organoid interaction was assessed by immunofluorescence and BMDM polarization profile was analyzed by flow cytometry. The coculture protocol was applied to both AOM/DSS treated and control mice.

DISCUSSION: A productive anti-tumor response requires an effective antigen presentation by TAMs to CD4 T lymphocytes via major histocompatibility complex (MHC)-II molecules. The latter are not expressed in about two-third of CRC and the loss of MHC-II expression correlates with an increase of the metastatic potential. This newly generated coculture system will be employed to unravel the molecular mechanisms leading to unsuccessful antigen presentation by TAMs in CRC.

CONCLUSIONS: Most of clinical trials based on immunotherapeutic approaches in CRC did not provide the desired results so far. In conclusion, this study may thus contribute to develop novel TAM-targeted immunotherapeutic options for CRC patients.

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P13.38

hMENA11a LOSS ACTIVATES TYPE I IFN AND INFLAMMATORY PATHWAYS, AND IN TURN PD-L1 EXPRESSION, BY ACTIVATING THE VIRAL SENSOR RIG-I

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The actin cytoskeleton regulatory protein hMENA and its splicing-derived isoforms, hMENA11a and hMENAΔV6, participate to non-small-cell lung cancer (NSCLC) progression. Low hMENA11a expression, along with high hMENA/hMENAΔV6, identify early NSCLC patients with poor prognosis [1-3].

PURPOSE: To gain insights into molecular mechanisms accounting for the poor prognostic value of low hMENA11a expression.

METHODS: We analyzed the transcriptome by RNA-Seq and the secretome by Bio-Plex and ELISA of NSCLC cells, depleted of hMENA11a (si-11a) or total hMENA(si-hMENA(t)). ATAC-Seq was performed to identify epigenetic changes.

RESULTS: We observed the increase of different transcripts related to IFN signaling, such as STAT1, PD-L1 and IFNB1 in both freshly explanted and stabilized NSCLC si-11a cells. The increase of IFNβ secretion was also evidenced. We found that only in si-11a, but not in si-hMENA(t) cells, the activation of JAK/STAT1/IRF1 axis occurs. Moreover, ATAC-Seq revealed unknown regulatory regions in *CD274* (PD-L1) locus in si-11a cells. We found that si-11a cells secrete different inflammatory molecules, including CXCL1, IL6 and IL8, and display higher activation of NF-kB. We demonstrated that hMENA11a silencing, which induces a critical cytoskeleton remodeling, increases the expression of the actin cytoskeleton-linked viral sensor RIG-I, which sustains both anti-viral and inflammatory responses.

DISCUSSION: These data support the role of hMENA11a isoform in restraining anti-viral and inflammatory pathways, by counteracting RIG-I. This may have crucial effects in patient prognosis and response to therapy.

CONCLUSIONS: Type I IFN pathway has been recently reported as crucial in resistance to immunotherapy with or without radiotherapy (4-6), thus we are evaluating the role of hMENA11a in response to ICB in NSCLC.

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P13.39

A NOVEL AND EFFECTIVE HYALURONIC ACID-BASED VACCINATION STRATEGY FOR THE PREVENTION AND TREATMENT OF HER2/NEU-OVEREXPRESSING BREAST CANCERS

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PURPOSE: The use of proteins as immunogens is attractive for the development of vaccines, but requires efficient adjuvants to overcome their weak immunogenicity. Recently, we investigated the potential of the TLR2/4 agonist hyaluronan (HA) as an immunological adjuvant for protein-based vaccines1,2. Conjugation of HA to antigens promoted their rapid translocation to draining lymph nodes, resulting in robust and long-lasting humoral responses1. Here, we investigated the potentiality of HA-based technology in the design of cancer vaccines by conjugating HA to the extracellular domain of rat HER2/neu (rHER2/neu).

METHODS: Female BALB/c or BALB-neuT mice were immunized with rHER2/neu-HA. Depletion of CD4+, CD8+ T and B cells was performed, sera and spleens were collected to characterize antigen-specific humoral and cellular responses. Vaccinated BALB/c mice were challenged and re-challenged with TUBO cells to assess the protective/therapeutic activity and the induction of immunological memory.

RESULTS: HA performed efficiently as robust and long-lasting humoral and cellular responses were detected using very low antigen doses. At 1-year post-vaccination, anti-rHER2/neu specific antibodies showed even improved effector functions. HA vaccination turned out effective in both the prophylactic (100% mice survived) and therapeutic (tumor regression in 2/12 mice) settings, and broke tolerance against rHER2/neu, delaying spontaneous tumor growth in BALB-neuT mice. Both humoral and cellular responses contributed to the success of HA-based vaccination, but CD8+ T cells played only a marginal role.

DISCUSSION: Cancer vaccines have not yet achieved significant clinical efficacy due to their poor immunogenicity, and the validation of more effective adjuvants occurred sometimes at the expense of safety. HA combines the unique immunomodulatory features of a TLR agonist with the tolerability of a fully natural polymer, proving to be a promising adjuvant for the creation of effective and safe cancer vaccines.

CONCLUSIONS: HA can be easily exploited for the design of cancer vaccines with the potential for rapid clinical translation.

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