1 (PD-L1) to suppress effector immune cells activity via PD-L1-PD-1 axis. We developed a novel vaccine using recombinant MUC4 fragments and exploiting adjuvant-like properties of amphiphilic polyanhydride-based nanoparticle delivery system. The strong involvement of MUC4 in disease aggressiveness and PD-L1 in immunosuppression makes a compelling case for their combined targeting. We hypothesize that combined overexpression of MUC4 and PD-L1 expression in pancreatic tumor microenvironment contributes to immunosuppressive and aggressive tumor behavior. Methods: Recombinant MUC4β fragment was encapsulated into polyanhydride nanoparticles (20:80 CPTEG:CPH) via nanoprecipitation. Murine PC cell lines (KCT960) derived from spontaneous pancreatic cancer mouse model (KPC mice) were transfected with human MUC4 expressing construct known as KCT960-mini-MUC4 cells for in-vivo studies. Flow cytometry, immunoblotting, PCR and immunofluorescence techniques were utilized to perform to characterize the immune response and target expression. Results: MUC4β nanovaccine immunized mice exhibited slower tumor growth kinetics than unimmunized control mice. We investigated tumor infiltrating lymphocytes (TILs) and necrosis in the tumor bed. We observed a positive correlation between TILs and tumor regression. Accumulation of infiltrating $CD8^+$ and $CD4^+$ T cells was greater in mice receiving the MUC4-Nanovaccine compared to soluble MUC4 delivered with blank nanoparticles, indicating the benefit of sustained availability of antigen via encapsulation. However, we did not observe complete tumor regression and ex vivo studies indicated induction of IFN-gamma by MUC4 vaccine. Based on this, we rationalized that PD-L1 expression by MUC4-expressing tumor cells suppressed and inhibited the therapeutic benefits of the nanovaccine in-vivo. We observed differential surface expression of PD-L1 on endogenously MUC4 expressing and negative PC cell lines. Paired analysis of MUC4 CRISPR knockdown and scrambled Capan-1 cell lines further suggested similar positive correlation. Conclusion: MUC4 nanovaccine suppressed tumor progression in-vivo indicating its potential for immunotherapy of PC. Positive correlation observed between MUC4 and PD-L1 in PC cell lines suggests an underlying molecular mechanism for establishing an immunosuppressive and aggressive PC tumor microenvironment and provides a strong rationale for evaluating MUC4 vaccine in combination with immune checkpoint blockade agents.

#3679 VEGF-A is increased and correlates with MDSC-driven immune suppression, systemic inflammation, nutritional impairment and poor prognosis in patients with cancer. Masahiko Shibata, Kenji Gonda, Takahiro Nakajima, Koji Kono, Hiroyuki Suzuki, Seiichi Takenoshita. *Fukushima Medical University, Fukushima, Japan.*

Vascular Endothelial Growth Factor (VEGF) is a key factor for tumor progression through induction of angiogenesis and other actions including immune alteration. Myeloid-derived suppressor cells (MDSC) are a major type of immune-suppressing cell that appear in cancer or inflammation and that have been reported to express the VEGF-receptor and to be activated by VEGF. To study the clinical importance of VEGF and MDSC for cancer, peripheral blood was collected from patients with gastrointestinal, ovarian, breast, thyroid and pulmonary cancers. Peripheral blood mononuclear cells (PBMC) were separated using a Ficoll-density gradient and were used for the detection of MDSC (CD11b+CD14-CD33+) using flow cytometry and for cytokine-production assays. For these assays, PBMC were stimulated with PHA and the production of cytokines including IL-12 (Th1 inducer), IL-10, (anti-inflammatory cytokine) and IL-17, (pro-inflammatory cytokine), were measured over 24 h using ELISAs. Serum concentrations of IL-10 and VEGF were also measured using ELISAs. The serum levels of both VEGF and MDSC were significantly increased in almost all types of cancer tested and significantly correlated with each other. Their levels also significantly correlated with neutrophil/lymphocyte ratios (NLR) (inflammation marker) and CRP levels, and were inversely correlated with the PHA-stimulation index (SI) (cell mediated immune responses marker) and serum concentrations of rapid-turnover protein (RTP) (nutrition marker). VEGF levels also correlated with serum concentrations of IL-10 and VEGF, and production of IL-17, and inversely correlated with production of IL-12. The prognosis of stage IV colorectal cancer with high VEGF was significantly worse than that with low VEGF. In thyroid cancer, the number of MDSC was significantly higher, NLR and CRP levels were higher, and RTP levels were lower in patients with undifferentiated carcinoma than in those with differentiated carcinoma including papillary and follicular carcinomas. Thus VEGF was increased in cancer and correlated with immune suppression driven by MDSC, inflammation and malnutrition. Although cancer immunotherapy is currently in use for a number of cancers, MDSC have been reported to be a major inhibitor of cancer immunotherapy even in cases in which an immune checkpoint inhibitor was used. An anti-VEGF treatment strategy has now been established in combination with chemotherapy for many types of cancer. Among various types of anti-MDSC trials, anti-VEGF treatment seems to be an effective adjuvant therapy of cancer immunotherapy.

#3680 Cancer-killing viruses combined with tumor-targeting immune checkpoint modulation elicits an *in situ* vaccination effect and expansion of tumor-specific T cells responsible for efficacious systemic anti-cancer activity. Hong Jiang, Andrew Dong, Yisel Rivera-Molina, Karen Clise-Dwyer, Xuejun Fan, Francisco W. Martinez, Teresa Nguyen, Verlene Henry, Caroline Carrillo, Candelaria Gomez-Manzano, Juan Fueyo. *UT MD Anderson Cancer Ctr., Houston, TX.*

Oncolytic viruses are cancer-selective and disrupt immunosuppression within the tumor, but they show suboptimal efficacy in patients. Immune checkpoint modulation is efficacious in a variety of cancers but is associated with nonspecific T-cell activation and a limited effect in tumors with a nonimmunogenic microenvironment. We hypothesized that combining these two strategies likely resulted in both efficacious and specific cancer therapy. Therefore, we constructed oncolytic adenovirus Delta-24-RGDOX expressing the immune costimulator OX40L and tested its activity in orthotopic GL261-C57BL/6 glioma and B16-C57BL/6 melanoma mouse models. Compared to its predecessor Delta-24-RGD, Delta-24-RGDOX was more effective to induce inflammatory activation within the tumors, enhanced the capability of the tumor cells to directly activate cancer-specific T cells and the proliferation of the cell population through OX40L expression on the cell surface, resulting in specific anti-tumor immunity. To track the expansion and migration of tumor-specific T cells during virotherapy, we first injected OVA-specific CD8+ T cells from OT-I/Luc transgenic mice in the first tumor derived from B16-OVA cells, followed by Delta-24-RGDOX injection in the same tumor. Monitoring the T cells with bioluminescent imaging revealed that the viral injection greatly augmented the T cell population than the PBS treatment within the tumor, and promoted the T cell migration to distant B16-OVA tumor but not to B16 tumor, suggesting local viral treatment enhanced the expansion of tumor-specific T cells and the migration of these cells to a distant tumor with the same tumor antigen. Consistently, flow cytometry analysis with OVA-tetramer staining showed that virus treatment greatly increased the frequency of OVA-specific CD8+ T cells in the local and distant tumors, peripheral blood and spleen (from high to low frequency). 70-80% cells of this cell population were CD44+ CD62L+ that are markers for central memory T cells. Hence, this new virus was efficacious to inhibit the virus-injected tumor and distant tumor, prolong the survival of the treated mice and induce immune memory specific to the virus-injected tumor type. Importantly, intratumoral injection of Delta-24-RGDOX and an anti-PD-L1 antibody synergized to reject gliomas and significantly increased survival in mice. Our data demonstrate that combining an oncolytic virus with tumor-targeting immune checkpoint modulation elicits potent in situ cancer vaccination and skews the injected tumor microenvironment from tumorigenic to immunogenic, resulting in a local expansion of the tumor-specific T cells. Moreover, this local effect is capable to extend to distant tumors, achieving specific and long-lasting systemic therapeutic efficacy.

#3681 A patient derived *ex vivo* platform CANScript™ predicts distinct therapeutic outcomes to multiple PD-1 checkpoint inhibitors in single tumor biopsies. Padhma Radhakrishnan,[1] Vasanthakumar Sekar,[2] Nilesh Brijwani,[2] Priyanka Chevour,[3] Babu Balakrishnan,[2] Dency D Pinto,[2] Muthusami Oliyarasi,[2] Debapriya G. Mehrotra,[2] Manjusha Biswas,[2] Sabitha K S,[4] Kodaganur S. Gopinath,[5] Arkasubhra Ghosh,[3] M s Ganesh,[6] Ashok M. Shenoy,[4] Saravanan Thiyagarajan,[2] Biswanath Majumder,[2] Aaron Goldman[1]. *[1]Mitra Biotech, Woburn, MA; [2]Mitra Biotech, Bangalore, India; [3]Grow Research Lab, Bangalore, India; [4]Kidwai Memorial Institute of Oncology, Bangalore, India; [5]Bangalore Institute of Oncology, Bangalore, India; [6]Vydehi Institute of Oncology and Research Centre, Bangalore, India.*

Background: Emerging clinical evidence using immunotherapy in recent years has demonstrated its power to suppress tumor growth by releasing the brakes on the immune system. For example, blockade of immune checkpoints, such as PD-1, has revolutionized treatment options for patients with aggressive cancers such as head and neck squamous cell carcinoma (HNSCC). However, clinical responses to PD-1 inhibition vary widely among patients while majority of them do not show any anti-tumor response. Multiple FDA-approved drugs against the same immune checkpoints have resulted in globally distinct outcomes in the clinic. There is a huge unmet need to understand these disparities at the individual patient level and to maximize the clinical benefits of these agents. Methods: Here, we employed a patient-derived ex vivo model, CANScript™ (Majumder B et al. Nature Commun 2015 Feb 27;6:6169 and Goldman A et al. Nature Commun

2015 Feb 11;6:6139), which recreates the native 3D tumor microenvironment, autocrine-paracrine dynamic and response to therapy by incorporating fresh tumor tissue and autologous immune cells with immunotherapy agents. Utilizing late stage HNSCC (N=50) we interrogated phenotypic response to two FDA-approved PD-1 inhibitors, Pembrolizumab (KEYTRUDA) and Nivolumab (OPDIVO). To do this, we used a comprehensive panel of immunological assays to evaluate changes in the immune compartments by flowcytometry and immunohistochemistry (primarily CD8, CD45, FOXP3, CXCR4, CD68, PDL1, PD1), multiplex cytokine profiling (IL6, IL8, IFN-g, IL10, IL12, Perforin, GranzymeB), along with functional/phenotypic effects including tumor proliferation, histological changes and cell death. Results: The data demonstrated that CANScript™ preserves the tumor-immune contexture and native heterogeneity across different clinical stages and patients. Importantly, we observed that PD-1 blockade resulted in patient-specific therapeutic response, which was characterized by differential distribution and maintenance of infiltrating CD8+ and CD4+ lymphocytes, distinct patterning of cytokines linked to functional dysregulation, and changes in tumor proliferation and apoptosis. Interestingly, data suggest that both Pembrolizumab and Nivolumab act on the same immune network axis but trigger functionally diverse phenotypes in the tumor immune compartment and distinct antitumor effects within an individual patient tumor. Conclusion: Together, these findings demonstrate the utility of CANScript™ as an ex vivo platform to predict therapeutic response of immune checkpoint inhibitors at the individual patient level. It also highlights mechanistic variations that could impact clinical outcome of these agents having the same molecular target. Such information can re-shape our understanding of patient selection and rational combinations for novel immune checkpoint inhibitors.

#3682 Synergistic immunostimulatory effects and therapeutic benefit of combined histone deacetylase and bromodomain inhibition in non-small cell lung cancer. Dennis O. Adeegbe,[1] Yan Liu,[1] Patrick Lizotte,[2] Yusuke Kamihara,[1] Mark Awad,[3] David Barbie,[3] Jerome Ritz,[1] Simon Jones,[4] Steven Quayle,[5] Peter Hammerman,[1] Kwok-Kin Wong[1]. *[1]Dana Farber Cancer Institute, Boston, MA; [2]Belfer Institute for Applied Cancer Science, Boston, MA; [3]Brigham and Women's Hospital, Boston, MA; [4]Acetylon Pharmaceuticals, Inc, Boston, MA; [5]Acetylon Pharmaceuticals Inc, Boston, MA.*

Effective therapies for non-small cell lung cancer (NSCLC) remain challenging despite an increasingly comprehensive understanding of somatically altered oncogenic pathways. It is now clear that therapeutic agents with potential to impact the tumor immune microenvironment potentiate immune-orchestrated therapeutic benefit. This study evaluated the immunoregulatory properties of two classes of drugs that modulate the epigenome, histone deacetylase (HDAC) and bromodomain inhibitors with a focus on key cell subsets that are engaged in an immune response. By evaluating human peripheral blood and NSCLC tumors, we show that the selective HDAC6 inhibitor ricolinostat promotes phenotypic changes associated with enhanced T-cell priming and function of antigen presenting cells. The bromodomain inhibitor JQ1 attenuated CD4+Foxp3+ T regulatory cell suppressive function and synergized with ricolinostat to facilitate immune-mediated tumor growth arrest, leading to prolonged survival of mice with lung adenocarcinomas. Collectively, our findings highlight immunomodulatory effects of two epigenetic modifiers that together promote T-cell-mediated anti-tumor immunity and demonstrate their therapeutic potential for NSCLC treatment.

#3683 Melphalan stimulates dendritic cell and CD8$^+$ T cell expansion by inducing immunogenic cell death in melanoma cells. Junko Johansson, Roberta Kiffin, Per Lindnér, Peter Naredi, Roger Olofsson Bagge, Anna Martner. *University of Gothenburg, Gothenburg, Sweden.*

Background: Regional hyperthermic perfusion with the alkylating agent melphalan is a treatment option for patients with metastatic melanoma confined to the limbs or the liver. Following a single perfusion, tumors often decrease gradually in size during several months, suggesting an immune-mediated mechanism of action, in addition to the direct cytotoxic effects of melphalan. This study was designed to characterize the immunogenic effects of melphalan. Materials and methods: We have established an in vitro model of regional hyperthermic perfusion where human melanoma cell lines are exposed to melphalan at 40°C for 1 h, thus mimicking the currently employed clinical protocol. The melphalan-exposed melanoma cells were analyzed for markers of immunogenic cell death and were co-cultured with peripheral blood mononuclear cells (PBMCs) in the presence or absence of IL-2. The number and activation status of various immune populations were analyzed by flow cytometry. Results: Melphalan exposure triggered the expression of several immune-related markers on melanoma cells, including calreticulin, MHC class I, Hsp70 and PD-L1. Melphalan-treated, but not untreated melanoma cells, triggered an increase in dendritic cell (DC) numbers along with a dramatic expansion of CD8$^+$ T cells in co-cultured PBMCs. The expanded CD8$^+$ T cells showed an activated phenotype with the majority of cells belonging to the effector memory subtype. Conclusions: Melanoma cells exposed to melphalan undergo immunogenic cell death and trigger DC expansion with subsequent expansion and activation of CD8$^+$ T cells. We propose that these events may contribute to the anti-tumor efficacy of regional hyperthermic perfusion with melphalan in metastatic melanoma.

#3684 Inhibition of STAT3 by antisense oligonucleotide treatment decreases the immune suppressive tumor microenvironment in syngeneic and GEM tumor models. Rich Woessner,[1] Vasu Sah,[1] Patricia McCoon,[1] Shaun Grosskurth,[1] Nanhua Deng,[1] Rachel DuPont,[1] Deborah Lawson,[1] Lourdes Pablo,[1] Corinne Reimer,[1] Marco A. De Velasco,[2] Hirotsugu Uemura,[3] Juliana Candido,[4] Paul Lyne[1]. *[1]AstraZeneca Pharmaceuticals LP, Waltham, MA; [2]Kindai University Faculty of Medicine, Osakasayama, MA; [3]Kindai University Faculty of Medicine, Osakasayama, Japan; [4]Barts Cancer Institute, Queen Mary University, London, MA.*

AZD9150, a gen2.5 antisense oligonucleotide (ASO) targeting human STAT3, has improved drug-like properties compared to previous generation ASO therapeutics, including increased stability and resistance to nucleases, reduced proinflammatory effects, and enhanced potency. We have previously reported that in tumors, STAT3 ASOs are taken up preferentially in stromal and immune cells of the tumor microenvironment (TME). Since AZD9150 is selective for human STAT3, we used a surrogate ASO (muSTAT3 ASO) to explore the pharmacodynamics of ASO-mediated STAT3 inhibition in syngeneic and genetically engineered mouse (GEM) tumor models, focusing on effects in the TME. In mice bearing subcutaneous CT-26 tumors, treatment with muSTAT3 ASO at 50 mg/kg, s.c., on a qdx5/wk schedule decreased STAT3 levels in immune cell subsets in the tumor and in circulating leukocytes by 40 - 60%, similar to the decrease in STAT3 achievable in circulating leukocytes in human patients after AZD9150 treatment. In a Nanostring analysis (nCounter mouse immunology panel) of CT-26 tumors from muSTAT3 ASO treated mice, CD163 (M2 immune suppressive macrophage marker) was the gene most consistently and significantly downregulated, by an average of 84% in three independent experiments, and was confirmed by immunohistochemistry (IHC). Flow cytometry analysis of myeloid subpopulations - tumor associated macrophages (F4/80+ TAMs), monocytic myeloid derived suppressor cells, and granulocytic cells - showed a decrease in TAMs averaging 69% across three independent experiments. The analysis was extended to include IHC for arginase (Arg, a marker of functional immune suppression activity). Subpopulations of cells identified included Arg+, CD163+, and Arg+CD163+. Treatment with muSTAT3 ASO decreased these populations by 79%, 88% and 97% respectively, compared to control treatment. These populations were also analyzed in two GEM tumor models - the KPC pancreatic cancer model, and a PTEN -/- prostate cancer model - which have a TME more representative of that found in tumors in the clinic. While the specific changes varied across the models, likely reflecting differences in TME makeup, a reduction in immune suppressive cell populations was present in both GEM models, including a decrease in CD163+ cells of 79% (along with modest antitumor activity) in the PTEN -/- prostate model after muSTAT3 ASO treatment. These results indicate that selective STAT3 inhibition can reduce immune suppressive cell populations in the TME, and suggest that STAT3 inhibition has the potential to enhance the antitumor activity of T-cell targeted therapies, such as those targeting the PD1-PDL1 axis. In support of this hypothesis, we observed that addition of muSTAT3 ASO to anti-PD-L1 Ab treatment significantly enhanced the antitumor activity of PD-L1 Ab treatment in two subcutaneous syngeneic tumor models, CT-26 and A20.

#3685 Antigen-capturing nanoparticles improve the abscopal effect and cancer immunotherapy. Yuanzeng Min. *UNC Chapel Hill, Chapel Hill, NC.*

Introduction: Cancer immunotherapy, the utilization of patients' own immune system to treat cancer, has emerged as a powerful new strategy in cancer treatment. The main limitation of this strategy is the low long-term durable response rate. Therefore, there has been high interest in developing strategies to further improve cancer immunotherapy. We hypothesized that antigen-capturing nanoparticles (AC-NPs) could improve immune responses to checkpoint inhibitor. The NPs can induce the abscopal effect by capturing tumor antigens released during radiotherapy and improve the presentation of antigens to professional antigen presenting cells (APCs). Methods: We developed several types of antigen-capturing NPs (AC-NPs) using poly (lactic-co-glycolic acid) (PLGA), a biocompatible and biodegradable polymer. The surfaces of nanoparticles were