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 amphiophobic 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 MUC4 fragment was encapsulated into polyanhydride nanoparticles (2080 CPTeG:CPhE) 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 immunohistochemistry techniques were utilized to perform to characterize the immune response and target expression. Results: MUC4 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 a correlation between tumor regression and ex vivo measured induced 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. The discrepancy between PD-L1 in PC in vitro and in vivo suggests an underlying molecular mechanism for establishing an immunosuppressive and aggressive 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 prognostic factors in head and neck cancer. Masahiko Shiga, Kenji Genda, 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, 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. We employed a patient-derived ex vivo platform CANScript predict distinct therapeutic outcomes to multiple PD-1 checkpoint inhibitors in single tumor biopsies. Padhma Ravikrishnan, 1 Vasanthakumari Sekar, 2 Nilesh Brijwani, 2 Priyanka Chevvari, 2 Babu Balakrishnan, 2 Dency D Pinto, 2 Mithusmany Oliyariyazhi, 2 Debapriya G. Mehrotra, 1 Manjusha Biswas, 1 Sabitha K. S. 1 Kodanagaru S. Gopinath, 1 Arkasubhra Ghosh, 1 M’s Ganesh, 2 Ashok M. Shenoy, 1 Saravan Thiyagarajan, 2 Biswanath Majumder, 2 Aaron Goldmian, 1 Mitra Biotech, Woburn, MA; 1 Mitra Biotech, Bangalore, India; 2 Grow Research Lab, Bangalore, India; 3 Kidwai Memorial Institute of Oncology, Bangalore, India; 4Yedhi 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 knowledge gap among these disparities in the clinical outcomes 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...
pressive function and synergized with ricolinostat to facilitate immune-modomain inhibitor JQ1 attenuated CD4 enhanced T-cell priming and function of antigen presenting cells. The bro-regulatory properties of two classes of drugs that modulate the epigenome, immune-orchestrated therapeutic benefıt. This study evaluated the immuno-potential to impact the tumor immune microenvironment potentiate im altered oncogenic pathways. It is now clear that therapeutic agents with cell lung cancer. Dennis O. Adeegbe, Yan Liu, Patrick Lizotte, Yusuke Kami-hara, Mark Awad, David Barbie, Jerome Ritz, Simon Jones, Steven Quayle, Peter Hammersman, Kwok Kin Wong, Dana Farber Cancer Institute, Boston, MA; Belfer Institute for Applied Cancer Science, Boston, MA; Brigham and Women’s Hospital, Boston, MA; Aetelyon Pharmaceuticals, Inc, Boston, MA; Aetelyon Pharmaceuticals Inc, Boston, MA. Effective therapies for non-small cell lung cancer (NSCLC) remain chal-lenging 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 im-mune-orchestrated therapeutic benefit. This study evaluated the immuno-regulatory properties of two classes of drugs that modulate the epigenome, histone deacetylase (HDAC) and bromodomain inhibitors with a focus on specific 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 bro-modomain inhibitor JQ1 attenuated CD4+ Foxp3+ T regulatory cell sup-pressive function and synergized with ricolinostat to facilitate immune-me-diation in tumors leading to profound changes in tumor immune suppressive cell populations. 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.

#3682 Synergistic immunostimulatory effects and therapeutic benefit of combined histone deacetylase and bromodomain inhibition in non-small cell lung cancer. Dennis O. Adeegbe, Yan Liu, Patrick Lizotte, Yusuke Kami-hara, Mark Awad, David Barbie, Jerome Ritz, Simon Jones, Steven Quayle, Peter Hammersman, Kwok Kin Wong, Dana Farber Cancer Institute, Boston, MA; Belfer Institute for Applied Cancer Science, Boston, MA; Brigham and Women’s Hospital, Boston, MA; Aetelyon Pharmaceuticals, Inc, Boston, MA; Aetelyon Pharmaceuticals Inc, Boston, MA. Effective therapies for non-small cell lung cancer (NSCLC) remain chal-lenging 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 im-mune-orchestrated therapeutic benefit. This study evaluated the immuno-regulatory properties of two classes of drugs that modulate the epigenome, histone deacetylase (HDAC) and bromodomain inhibitors with a focus on specific 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 bro-modomain inhibitor JQ1 attenuated CD4+ Foxp3+ T regulatory cell sup-pressive function and synergized with ricolinostat to facilitate immune-me-diation in tumors leading to profound changes in tumor immune suppressive cell populations. 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 Ardin, Per Lindner, Peter Naredi, Roger Olofsson Bagge, Anna Martinsson, 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 grad-u ally in size during several months, suggesting an immune-mediated mecha-nism of action, in addition to the direct cytotoxic effects of melphalan. This study was designed to characterize the immunogenic effects of melphalan. Ma-terials and methods: We have established an in vitro model of regional hyper-thermic 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 as well as examined during radiotherapy with peripheral blood mononuclear cells (PB-MCs) 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. Mel-phalan-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 pheno-type with a significantly higher proportion of CD107a+ TNF-α+ cells compared with the effector memory subset. Inclusion: 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 melano-na.

#3684 Inhibition of STAT3 by antisense oligonucleotide treatment decreases the immune suppressive tumor microenvironment in syngeneic and GEM tumor models. Rich Woesner, Vau Sah, Patricia McGoom, Shaun Grosskurth, Nanhua Deng, Rachel DuPont, Deborah Lawson, Lourdes Pablo, Corinne Reimer, Marco A. De Velasco, Hirotsugu Uemura, Juliana Carlos-de-Crato, Paul Lynne, AstaZeneca Pharmaceuticals, Wallingford, MA; Kin-dai University Faculty of Medicine, Okayasayama, MA; Barts Cancer Institute, Queen Mary University, London, MA. AZD9156, a gen2.5 antisense oligonucleotide (ASO) targeting human STAT3, has improved drug-like properties compared to previous generation ASO therapeutic敝, including increased stability and resistance to nucleases, re-duced proinflammatory effects, and enhanced potency. We have previously re-ported that in tumors, STAT3 ASO can be taken up by tumor immune cells of the tumor microenvironment (TME). Since AZD9156 is selec-tive 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 patients receiving 150 mg treatment. In a Nanostring analysis (mCounter 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 exper-iments, 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 experi-ments. The analysis was extended to include HIC for arginase (Arg, a marker of functional immune suppression activity). Subpopulations of cells identified in-cluded 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 dif-ferences 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 treatment with muSTAT3 ASO. These results indicate that selective STAT3 inhibi-tion 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-PD1L 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. Yuanshen Min, UNC Chapel Hill, Chapel Hill, NC. Introduction: Cancer immunotherapy, the utilization of patients’ own im-mune 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-captur-ing nanoparticles (AC-NPs) could improve immune responses to checkpoint inhibitor. The NPs can induce the abscopal effect by capturing tumor antigens during the process of 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...