Programmed Cell Death and Cancer Biology Research Literatures

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Abstract: Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries. This article introduces recent research reports as references in the related studies.

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1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries. This article introduces recent research reports as references in the related studies.

The following introduces recent reports as references in the related studies.

Aghajani, M., et al. (2018). "Clinicopathologic and Prognostic Significance of Programmed Cell Death Ligand 1 Expression in Patients with Non-Medullary Thyroid Cancer: A Systematic Review and Meta-Analysis." Thyroid **28**(3): 349-361.

Background: Evidence has shown that programmed cell death ligand 1 (PD-L1) overexpression is associated with poor prognosis and resistance to immune therapies in several human cancers. However, data on the prognostic significance of PD-L1 expression in thyroid cancer are limited and remain controversial. This systematic review and meta-analysis aimed to evaluate comprehensively the clinicopathologic significance and prognostic value of PD-L1 expression in non-medullary thyroid cancers. METHODS: Electronic databases, including Medline/PubMed, EMBASE, and the Cochrane Library, were searched up until July 5, 2017. In total, seven comparisons (from six articles) comprising 1421 patients were included in the pooled analysis. RESULTS: There was moderate quality evidence from four studies (n = 721) that shows positive PD-L1 expression was significantly associated with poor survival among thyroid cancer patients (pooled hazard ratio = 3.73 [confidence interval (CI) 2.75-5.06]). Increased PD-L1 expression was also found to be significantly associated with disease recurrence (odds ratio = 1.95 [CI 1.15-3.32]) and concurrent thyroiditis (odds ratio = 1.65 [CI 1.09-2.51]). CONCLUSIONS: The results confirm the prognostic significance of PD-L1 expression in thyroid cancer patients. PD-L1 expression has the potential to be implemented as a prognostic biomarker used to guide clinicians in identifying patients with more aggressive cancers, and for the selection of individuals that would derive durable clinical benefit from anti-PD-1/PD-L1 immunotherapy. Prospective clinical trials will be useful to support these findings.

Aghajani, M. J., et al. (2018). "Predictive relevance of programmed cell death protein 1 and tumor-infiltrating lymphocyte expression in papillary thyroid cancer." Surgery **163**(1): 130-136.

BACKGROUND: Co-signaling molecule programmed cell death 1 ligand 1 has been shown to induce potent inhibition of T cell-mediated antitumoral immunity. Our study aimed to investigate the prognostic value of programmed cell death 1 ligand 1 expression and tumor-infiltrating lymphocyte density as biomarkers in specimens from patients with papillary thyroid cancer. METHODS: We retrospectively analyzed the data and tissue samples of 75 patients with papillary thyroid cancer. Stained cells were counted manually and analyzed for clinical and histopathologic correlations and disease-free survival. RESULTS: Programmed cell death 1 ligand 1 expression was significantly correlated with increased incidence of lymphovascular invasion (P =.038), extrathyroidal extension (P =.026), and concurrent lymphocytic thyroiditis (P =.003). Patients with low CD8+ and CD3+ expression presented with a

significantly higher incidence of lymph node metastasis (P =.042) and extrathyroidal extension (P =.015). The subgroup of cases with positive programmed cell death 1 ligand 1 expression and low CD8+ T cell infiltration demonstrated a significantly increased incidence of lymph node metastasis (P =.031). Univariate and multivariate analysis confirmed that a high CD8+ T cell density was significantly associated with favorable disease-free survival (P =.017). Subanalysis revealed that the shortest disease-free survival was evident in the programmed cell death 1 ligand 1(+)/CD8(low) group (P =.004). CONCLUSION: Our findings indicate that CD8+ tumor-infiltrating lymphocyte density and programmed cell death 1 ligand 1 expression may serve as valuable predictive biomarkers in patients with papillary thyroid cancer.

Arbour, K. C., et al. (2018). "Impact of Baseline Steroids on Efficacy of Programmed Cell Death-1 and Programmed Death-Ligand 1 Blockade in Patients With Non-Small-Cell Lung Cancer." J Clin Oncol **36**(28): 2872-2878.

PURPOSE: Treatment with programmed cell death-1 or programmed death ligand 1 (PD-(L)1) inhibitors is now standard therapy for patients with lung cancer. The immunosuppressive effect of corticosteroids may reduce efficacy of PD-(L)1 blockade. On-treatment corticosteroids for treatment of immune-related adverse events do not seem to affect efficacy, but the potential impact of baseline corticosteroids at the time of treatment initiation is unknown. Clinical trials typically excluded patients who received baseline corticosteroids, which led us to use real-world data to examine the effect of corticosteroids at treatment initiation. METHODS: We identified patients who were PD-(L)1-naive with advanced non-small-cell lung cancer from two institutions-Memorial Sloan Kettering Cancer Center and Gustave Roussy Cancer Center-who were treated with single-agent PD-(L)1 blockade. Clinical and pharmacy records were reviewed to identify corticosteroid use at the time of beginning anti-PD-(L)1 therapy. We performed multivariable analyses using Cox proportional hazards regression model and logistic regression. RESULTS: Ninety (14%) of 640 patients treated with single-agent PD-(L)1 blockade received corticosteroids of >/= 10 mg of prednisone equivalent daily at the start of the PD-(L)1 blockade. Common indications for corticosteroids were dyspnea (33%), fatigue (21%), and brain metastases (19%). In both independent cohorts, Memorial Sloan Kettering Cancer Center (n = 455) and Gustave Roussy Cancer Center (n = 185), baseline corticosteroids were associated with decreased overall response rate, progression-free survival, and overall survival with PD-(L)1 blockade. In a multivariable analysis of the pooled population, adjusting for smoking history, performance status, and history of brain metastases, baseline corticosteroids remained significantly associated with decreased progression-free survival (hazard ratio, 1.3; P =.03), and overall survival (hazard ratio, 1.7; P <.001). CONCLUSION: Baseline corticosteroid use of >/= 10 mg of prednisone equivalent was associated with poorer outcome in patients with non-small-cell lung cancer who were treated with PD-(L)1 blockade. Prudent use of corticosteroids at the time of initiating PD-(L)1 blockade is recommended.

Armstrong, D. K., et al. (1992). "Programmed cell death in an estrogen-independent human breast cancer cell line, MDA-MB-468." Cancer Res **52**(12): 3418-3424.

Previous studies have demonstrated that estrogen-responsive human breast cancer cells can be induced to undergo an energy-dependent, genetically programmed series of biochemical changes that result in the active suicide of the cells following estrogen ablation. In contrast, estrogen-independent human breast cancer cells do not activate this programmed cell death pathway following estrogen ablation. This could be due either to the absence of the cellular machinery required for programmed cell death or simply to the inability of estrogen ablation to activate this machinery. To discriminate between these two possibilities, the MDA-MB-468 estrogen-independent human mammary adenocarcinoma cell line was used as a model system to study the mechanism of cell death following cytotoxic drug treatment. Exposure of these cells to the fluorinated pyrimidines, 5-fluoro-2'-deoxyuridine or trifluorothymidine, resulted in growth inhibition and loss of proliferative capacity within 24 h. These changes occurred while cell membrane integrity was intact as measured by either cellular morphology or trypan blue exclusion. After 48 h of drug treatment, loss of cell membrane integrity was followed by cell lysis and a rapid decline in cell number. The addition of 16 microM thymidine prior to drug treatment prevented cell death, but thymidine did not rescue these cells once drug treatment was initiated. Analysis of DNA revealed the characteristic fragmentation into nucleosomal oligomers that is a hallmark of programmed cell death. Associated with this death pathway was a 15-fold induction of transforming growth factor beta 1 gene expression that has been previously observed in a variety of cellular systems undergoing programmed cell death. These results indicate that MDA-MB-468 estrogen-independent human mammary carcinoma cells retain the ability to undergo programmed cell death after treatment with cytotoxic drugs that induce a "thymineless" state.

Austin, L. A., et al. (2011). "Plasmonic imaging of human oral cancer cell communities during programmed cell death by nuclear-targeting silver nanoparticles." J Am Chem Soc **133**(44): 17594-17597.

Plasmonic nanoparticles (NPs) have become a useful platform in medicine for potential uses in disease diagnosis and treatment. Recently, it has been reported that plasmonic NPs conjugated to nuclear-targeting peptides cause DNA damage and apoptotic populations in cancer cells. In the present work, we utilized the plasmonic scattering property and the ability of nuclear-targeted silver nanoparticles (NLS/RGD-AgNPs) to induce programmed cell death in order to image in real-time the behavior of human oral squamous carcinoma (HSC-3) cell communities during and after the induction of apoptosis. Plasmonic live-cell imaging revealed that HSC-3 cells behave as nonprofessional phagocytes. The induction of apoptosis in some cells led to attraction of and their subsequent engulfment by neighboring cells. Attraction to apoptotic cells resulted in clustering of the cellular community. Live-cell imaging also revealed that, as the initial concentration of NLS/RGD-AgNPs increases, the rate of self-killing increases and the degree of attraction and clustering decreases. These results are discussed in terms of the proposed mechanism of cells undergoing programmed cell death.

Bae, S. U., et al. (2018). "Prognostic impact of programmed cell death ligand 1 expression on long-term oncologic outcomes in colorectal cancer." Oncol Lett **16**(4): 5214-5222.

The present study evaluated the association between programmed cell death ligand-1 (PD-L1) expression and long-term oncologic outcomes in colorectal cancer (CRC). PD-L1 expression was evaluated using immunohistochemistry in 175 patients who underwent surgical resection for CRC between September 1999 and August 2004. Patients were grouped according to PD-L1 expression, with 82 (46.9%) and 93 (53.1%) in the low and high PD-L1 expression groups, respectively. The overall survival (OS) and disease-free survival (DFS) rates were significantly better in the high expression group compared with in the low expression group (OS: 48.2 vs. 32.9%, P=0.047; DFS: 43.3 vs. 32.9%, P=0.021). According to the Tumor-Node-Metastasis stage subgroups, the OS rates in the low and high expression groups, respectively, were 66.7 and 60.0% in stage I (P=0.715), 51.8 and 46.7% in stage II (P=0.789), 19.6 and 51.1% in stage III (P=0.011) and 9.1 and 0% in stage IV (P=0.005). The DFS rates in the low and high expression groups, respectively, were 66.7 and 60.0% in stage I (P=0.715), 51.8 and 46.7% in stage II (P=0.857), 19.6 and 38.3% in stage III (P=0.006) and 9.1 and 0% in stage IV (P=0.700). The systemic recurrence rate was significantly higher in the low expression group compared with in the high expression group (42.7 vs. 12.9%, respectively, P=0.030). Low PD-L1 expression was significantly associated with tumor relapse and poor prognosis in stage III CRC.

Baird, S. K., et al. (2008). "Oncolytic adenoviral mutants induce a novel mode of programmed cell death in ovarian cancer." Oncogene **27**(22): 3081-3090.

Oncolytic adenoviral mutants have considerable activity in ovarian cancer. However, the mechanisms by which they induce cell death remain uncertain. dl922-947, which contains a 24 bp deletion in E1A CR2, is more potent than both E1A wild-type adenoviruses and the E1B-55K deletion mutant dl1520 (Onyx-015). We investigated the mode of death induced by three E1A CR2-deleted replicating adenoviruses in models of ovarian cancer and also the importance of E3 11.6 (adenovirus death protein) in determining this mode of death. Ovarian cancer cells were infected with dl922-947 (E3 11.6+) and dlCR2 (E3 11.6-). We also generated dlCR2 tSmac, which also encodes the gene for processed Smac/DIABLO. Classical apoptosis does not occur in adenoviral cell death and there is no role for mitochondria. Expression of Smac/DIABLO does not enhance cytotoxicity nor increase apoptotic features. A role for cathepsins and lysosomal membrane permeability was excluded. Autophagy is induced, but is not the mode of death and may act as a cell survival mechanism. There is no evidence of pure necrosis, while the presence of E3 11.6 does not modulate the mode or extent of cell death. Thus, E1A CR2-deleted oncolytic adenoviral cytotoxicity in ovarian cancer may define a novel mode of programmed cell death.

Banerjee, M., et al. (2016). "Cytotoxicity and cell cycle arrest induced by andrographolide lead to programmed cell death of MDA-MB-231 breast cancer cell line." J Biomed Sci **23**: 40.

BACKGROUND: Breast cancer is considered as an increasing major life-threatening concern among the malignancies encountered globally in females. Traditional therapy is far from satisfactory due to drug resistance and various side effects, thus a search for complementary/alternative medicines from natural sources with lesser side effects is being emphasized. Andrographis paniculata, an oriental, traditional medicinal herb commonly available in Asian countries, has a long history of treating a variety of diseases, such as respiratory infection, fever, bacterial dysentery, diarrhea, inflammation etc. Extracts of this plant showed a wide spectrum of therapeutic effects, such as

anti-bacterial, anti-malarial, anti-viral and anti-carcinogenic properties. Andrographolide, a diterpenoid lactone, is the major active component of this plant. This study reports on andrographolide induced apoptosis and its possible mechanism in highly proliferative, invasive breast cancer cells, MDA-MB-231 lacking a functional p53 and estrogen receptor (ER). Furthermore, the pharmacokinetic properties of andrographolide have also been studied in mice following intravenous and oral administration. RESULTS: Andrographolide showed a time- and concentration- dependent inhibitory effect on MDA-MB-231 breast cancer cell proliferation, but the treatment did not affect normal breast epithelial cells, MCF-10A (>80 %). The number of cells in S as well as G2/M phase was increased after 36 h of treatment. Elevated reactive oxygen species (ROS) production with concomitant decrease in Mitochondrial Membrane Potential (MMP) and externalization of phosphatidyl serine were observed. Flow cytometry with Annexin V revealed that the population of apoptotic cells increased with prolonged exposure to andrographolide. Activation of caspase-3 and caspase-9 were also noted. Bax and Apaf-1 expression were notably increased with decreased Bcl-2 and Bcl-xL expression in andrographolide-treated cells. Pharmacokinetic study with andrographolide showed the bioavailability of 9.27 +/- 1.69 % with a Cmax, of 0.73 +/- 0.17 mumol/L and Tmax of 0.42 +/- 0.14 h following oral administration. AG showed rapid clearance and moderate terminal half lives (T1/2) of 1.86 +/- 0.21 and 3.30 +/- 0.35 h following IV and oral administration respectively. CONCLUSION: This investigation indicates that andrographolide might be useful as a possible chemopreventive/chemotherapeutic agent for human breast cancers.

Berchtold, M. W. and A. Villalobo (2014). "The many faces of calmodulin in cell proliferation, programmed cell death, autophagy, and cancer." Biochim Biophys Acta **1843**(2): 398-435.

Calmodulin (CaM) is a ubiquitous Ca (2+) receptor protein mediating a large number of signaling processes in all eukaryotic cells. CaM plays a central role in regulating a myriad of cellular functions via interaction with multiple target proteins. This review focuses on the action of CaM and CaM-dependent signaling systems in the control of vertebrate cell proliferation, programmed cell death and autophagy. The significance of CaM and interconnected CaM-regulated systems for the physiology of cancer cells including tumor stem cells, and processes required for tumor progression such as growth, tumor-associated angiogenesis and metastasis are highlighted. Furthermore, the potential targeting of CaM-dependent signaling processes for therapeutic use is discussed.

Berntsson, J., et al. (2018). "Expression of programmed cell death protein 1 (PD-1) and its ligand PD-L1 in colorectal cancer: Relationship with sidedness and prognosis." Oncoimmunology **7**(8): e1465165.

Expression of programmed cell death protein 1 (PD-1) and its ligand PD-L1 has been demonstrated to confer a prognostic value in colorectal cancer (CRC), but no studies have investigated whether this association differs according to tumour location. In this study, immunohistochemical expression of PD-1 and PD-L1 was analysed in tissue microarrays with primary tumours from 557 incident CRC cases from a prospective population-based cohort. Univariable and multivariable Cox regression analyses, adjusted for age, sex, TNM stage, differentiation grade and vascular invasion, were applied to determine the impact of biomarker expression on 5-year overall survival (OS), in the entire cohort and in subgroup analysis of right colon, left colon, and rectum. High PD-L1 expression on tumour-infiltrating immune cells was an independent factor of a prolonged OS in the entire cohort (hazard ratio [HR] = 0.49; 95% confidence interval [CI] CI 0.35 - 0.68), and in tumours of the right colon (HR = 0.43; 95% CI 0.25 - 0.74) and the left colon (HR = 0.28; 95% CI 0.13 - 0.61), but not in rectal cancer. Tumour-specific PD-L1-expression was not prognostic, neither in the full cohort nor according to tumour location. High immune cell-specific PD-1 expression was associated with a prolonged OS in the entire cohort and in tumours of the right colon, but not in the left colon or rectum, and only in univariable analysis. In conclusion, these results demonstrate that immune cell-specific PD-L1 and PD-1 expression is prognostic in a site-dependent manner, whereas tumour-specific PD-L1-expression is not prognostic in CRC.

Biswas, A., et al. (2018). "Clinical performance of endobronchial ultrasound-guided transbronchial needle aspiration for assessing programmed death ligand-1 expression in nonsmall cell lung cancer." Diagn Cytopathol **46**(5): 378-383.

BACKGROUND: Pembrolizumab was recently approved as a first line agent for metastatic NSCLC in patients with high programmed death-ligand 1 (PD-L1) expression. OBJECTIVES: Since a significant portion of lung cancer is diagnosed by endobronchial ultrasound-guided transbronchial needle aspiration (EBUS TBNA); there is a need for PD-L1 testing in these specimens. However, to date few studies have evaluated performance of cytology specimens from EBUS TBNA for PD-L1 analysis. METHODS:

Patients who had a diagnosis of NSCLC and in whom ancillary testing, i.e., next generation sequencing (NGS), anaplastic lymphoma kinase (ALK), and PD-L1 expression was requested between January and May 2017 were reviewed. RESULTS: Fifty of the 112 patients reviewed had the diagnosis of NSCLC for which ancillary testing was requested. Twelve patients (24%) had squamous cell carcinoma, twenty-seven had adenocarcinoma (54%), five had NSCLC favor adenocarcinoma (10%), two had NSCLC favor squamous cell cancer (4%), and four had NSCLC not otherwise specified (NOS) (8%). Size of the lymph nodes or lesion sampled ranged from 10 to 50 mm. Four (8%) patients had insufficient number of tumor cells in the cell block for any of the ancillary molecular testing. Forty-one (82%) patients had an adequate sample for all three ancillary tests. Satisfactory results for PD-L1 expression for all cases was 86% with 14 (32%) patients having levels of PD-L1 expression >50%. CONCLUSION: EBUS TBNA is effective and has a high proportion of satisfactory results for testing PD-L1 expression on tumor cells in addition to NGS and ALK FISH.

Cartee, L. and G. L. Kucera (1998). "Gemcitabine induces programmed cell death and activates protein kinase C in BG-1 human ovarian cancer cells." Cancer Chemother Pharmacol **41**(5): 403-412.

PURPOSE: Cytosine arabinoside induces apoptosis and this cell death process is influenced by protein kinase C signaling events in leukemic cells. We present findings that extend these observations to include another deoxycytidine analog, gemcitabine, which is more potent in solid tumors. METHODS AND RESULTS: Gemcitabine induced programmed cell death in BG-1 human ovarian cancer cells based on biochemical and morphologic analyses. The DNA was fragmented in BG-1 cells exposed to gemcitabine (0.5 microM, 1.0 microM and 10 microM) for 8 h, but gemcitabine treatment did not induce internucleosomal DNA degradation. Scanning and transmission electron microscopy of BG-1 cells showed morphologic changes associated with apoptosis in response to gemcitabine: membrane blebbing, the formation of apoptotic bodies and chromatin condensation. Thus, BG-1 cells undergo programmed cell death in response to gemcitabine treatment without internucleosomal DNA fragmentation. Furthermore, gemcitabine (10 microM) activated protein kinase C in BG-1 cells and the phosphorylation of the endogenous protein kinase C substrate, myristoylated alanine-rich C kinase substrate, was increased following exposure of BG-1 cells to gemcitabine for up to 6 h. Clonogenicity studies with gemcitabine in combination with various protein kinase C-modulating agents demonstrated that gemcitabine cytotoxicity was influenced by protein kinase C signaling events in BG-1 cells. Short-term (1 h) exposure to TPA (1 or 10 nM) followed by gemcitabine (0.5 microM for 4 h) did not alter the response to gemcitabine. However, a 24-h exposure to TPA followed by gemcitabine resulted in synergistic cytotoxicity, while coincubation of TPA with a PKC inhibitor (e.g. bisindolylmaleimide or calphostin-C) in this regimen abrogated the synergistic response. CONCLUSIONS: Based on our findings, it is plausible that gemcitabine therapy could be improved by modulating PKC signaling events linked to drug-induced apoptosis/cytotoxicity.

Choi, Y. Y., et al. (2018). "Microsatellite Instability and Programmed Cell Death-Ligand 1 Expression in Stage II/III Gastric Cancer: Post Hoc Analysis of the CLASSIC Randomized Controlled study." Ann Surg.

OBJECTIVE: We investigated microsatellite instability (MSI) status and programed cell death ligand 1 (PD-L1) expression as predictors of prognosis and responsiveness to chemotherapy for stage II/III gastric cancer. BACKGROUND: The clinical implications of MSI status and PD-L1 expression in gastric cancer have not been well-elucidated. METHODS: Tumor specimens and clinical information were collected from patients enrolled in the CLASSIC trial-a randomized controlled study of capecitabine plus oxaliplatin-based adjuvant chemotherapy. Five quasi-monomorphic mononucleotide markers were used to assess tumor MSI status. PD-L1 expressions of tumor and stromal immune cells were evaluated using immunohistochemistry. RESULTS: Of 592 patients, 40 (6.8%) had MSI-high (MSI-H) tumors. Among 582 patients available for immunohistochemistry evaluation, PD-L1 was positive in tumor cells (tPD-L1) of 16 patients (2.7%) and stromal immune cells (sPD-L1) of 165 patients (28.4%). Multivariable analysis of disease-free survival (DFS) showed that MSI-H and sPD-L1-positivity were independent prognostic factors [hazard ratio 0.301 (0.123-0.736), 0.714 (0.514-0.991); P = 0.008, 0.044), as were receiving chemotherapy, age, tumor grade, and TNM stage. Although adjuvant chemotherapy improved DFS in the microsatellite-stable (MSS) group (5-year DFS: 66.8% vs 54.1%; P = 0.002); no benefit was observed in the MSI-H group (5-year DFS: 83.9% vs 85.7%; P = 0.931). In the MSS group, sPD-L1-negative patients, but not sPD-L1-positive patients, had significant survival benefit from adjuvant chemotherapy compared with surgery only (5-year DFS: 66.1% vs 50.7%; P = 0.001). CONCLUSION: MSI status and PD-L1 expression are clinically actionable biomarkers for stratifying patients

and predicting benefit from adjuvant chemotherapy after D2 gastrectomy for stage II/III gastric cancer.

Cincin, Z. B., et al. (2018). "Hesperidin promotes programmed cell death by downregulation of nongenomic estrogen receptor signalling pathway in endometrial cancer cells." Biomed Pharmacother **103**: 336-345.

Endometrial carcinoma (EC) is the most common malignant gynecologic tumor in women. EC is thought to be caused by increasing estrogen levels relative to progesterone in the body. Hesperidin (Hsd), a biologically active flavonoid, could be extracted from Citrus species. It has been recently shown that Hsd could exert anticarcinogenic properties in different cancer types. However, the effects of Hsd and its molecular mechanisms on EC remain unclear. In this study, the antiproliferative, apoptotic and genomic effects of Hsd in EC and its underlying mechanisms were identified. We found that Hsd significantly suppressed the proliferation of EC cells in dose and time dependent manner. Mechanistic studies showed that Hsd could contribute apoptosis by inducing externalization of phosphatidyl serine (PS), caspase-3 activity and loss of mitochondrial membrane (MMP). Furthermore, we examined that Hsd could also significantly upregulate the expression of proapoptotic Bax subgroup genes (Bax and Bik) while downregulating the anti-apoptotic protein Bcl-2 in EC cell lines. According to GO enrichment and KEGG pathway analysis of differentially expressed genes in Hsd treated EC cells, we identified that Hsd could promote cell death via downregulation of estrogen receptor I (ESRI) that was directly related to ERK/MAPK pathway. Taken together, our study first showed that Hsd could be an antiestrogenic compound that could modulate nongenomic estrogen receptor signaling through inhibition of EC cell growth. Our findings may provide us a novel growth inhibitory agent for EC treatment after verifying its molecular mechanism with in vivo studies.

Constantinidou, A., et al. (2018). "Targeting Programmed Cell Death -1 (PD-1) and Ligand (PD-L1): A new era in cancer active immunotherapy." Pharmacol Ther.

Improved understanding of the immune system and its role in cancer development and progression has led to impressive advances in the field of cancer immunotherapy over the last decade. Whilst the field is rapidly evolving and the list of drugs receiving regulatory approval for the treatment of various cancers is fast growing, the group of PD1- PDL-1 inhibitors is establishing a leading role amongst immunomodulatory agents. PD1- PDL-1 inhibitors act against pathways involved in adaptive immune suppression resulting in immune checkpoint blockade. Within the last four years two PD-1 and three PD-L1 inhibitors have been utilized in clinical practice against a variety of malignancies. Focus was initially placed on targeting cancers considered immunogenic such as melanoma, renal and lung cancers but subsequently the application expanded to include amongst others Hodgkin Lymphoma, urothelial as well as head and neck cancer. This article provides a comprehensive review of the early and late phase trials that led to the regulatory approval of all five PD1- PDL-1 inhibitors in the corresponding cancer types. It presents available data on the combinations of PD1- PDL-1 inhibitors with other therapies (immunotherapy, targeted therapy and chemotherapy), the toxicity profile of the PD1-PDL-1 inhibitors and ongoing trials testing the efficacy of these agents in cancer types beyond those that have been addressed already. Finally, current and future challenges in the application of PD-1 and PD-L1 inhibitors are discussed with emphasis on the role of predictive biomarkers.

Constantinou, C., et al. (2009). "Caspase-independent pathways of programmed cell death: the unraveling of new targets of cancer therapy?" Curr Cancer Drug Targets **9**(6): 717-728.

In the past few years, accumulating evidence in the literature supports the existence of pathways of caspase-independent programmed cell death (CI-PCD). These pathways are likely to be acting as 'death backup systems' that ensure effective removal of defective cells from the organism. Similar to classical apoptosis i.e. caspase-dependent programmed cell death (CD-PCD), the mitochondrion is the main organelle orchestrating the series of events which are required for the induction of CI-PCD. In addition, the pro-apoptotic proteins Bax and Bid are also key participants in CI-PCD. However, contrary to CD-PCD, CI-PCD involves executioners other than the caspases which include the cathepsins, the calpains and serine proteases. The protein AIF may also play an important role in the induction of CI-PCD. In this review we report current knowledge on CI-PCD and provide evidence for its regulation by chemotherapeutic agents currently used in the clinic and under investigation in clinical trials. Lastly, we discuss how the study of natural and synthetic agents triggering CI-PCD may help in the pharmacological design of a new generation of more effective chemotherapeutic drugs. The use of such drugs activating both CD-PCD and CI-PCD pathways should achieve a more successful eradication of carcinogenic cells and the attainment of lower levels of tumor resistance.

Denmeade, S. R., et al. (1996). "Role of programmed (apoptotic) cell death during the progression and therapy for prostate cancer." Prostate **28**(4): 251-265.

Cells possess within their epigenetic repertoire the ability to undergo an active process of cellular suicide termed programmed (or apoptotic) cell death. This programmed cell death process involves an epigenetic reprogramming of the cell that results in an energy-dependent cascade of biochemical and morphologic changes (also termed apoptosis) within the cell, resulting in its death and elimination. Although the final steps (i.e., DNA and cellular fragmentation) are common to cells undergoing programmed cell death, the activation of this death process is initiated either by sufficient injury to the cell induced by various exogenous damaging agents (e.g., radiation, chemicals, viruses) or by changes in the levels of a series of endogenous signals (e.g., hormones and growth/survival factors). Within the prostate, androgens are capable of both stimulating proliferation as well as inhibiting the rate of the glandular epithelial cell death. Androgen withdrawal triggers the programmed cell death pathway in both normal prostate glandular epithelia and androgen-dependent prostate cancer cells. Androgen-independent prostate cancer cells do not initiate the programmed cell death pathway upon androgen ablation; however, they do retain the cellular machinery necessary to activate the programmed cell death cascade when sufficiently damaged by exogenous agents. In the normal prostate epithelium, cell proliferation is balanced by a equal rate of programmed cell death, such that neither involution nor overgrowth normal occurs. In prostatic cancer, however, this balance is lost, such that there is greater proliferation than death producing continuous net growth. Thus, an imbalance in programmed cell death must occur during prostatic cancer progression. The goal of effective therapy for prostatic cancer, therefore, is to correct this imbalance. Unfortunately, this has not been achieved and metastatic prostatic cancer is still a lethal disease for which no curative therapy is currently available. In order to develop such effective therapy, an understanding of the programmed death pathway, and what controls it, is critical. Thus, a review of the present state of knowledge concerning programmed cell death of normal and malignant prostatic cells will be presented.

Dixit, M., et al. (1997). "Abrogation of cisplatin-induced programmed cell death in human breast cancer cells by epidermal growth factor antisense RNA." J Natl Cancer Inst **89**(5): 365-373.

BACKGROUND: Epidermal growth factor receptor (EGF-R) perturbation by receptor ligand (s), e.g., epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-alpha), or receptor-specific antibodies accentuates cisplatin-induced toxicity in tumor cells. This sensitization occurs only in tumor cells with high expression of EGF-R but not in those with low expression of EGF-R. PURPOSE: Therefore, we have studied the role of EGF-R expression on cisplatin-mediated cytotoxicity. METHODS: MDA-468 human breast cancer cells were stably transfected with a p-chloramphenicol acetyl transferase (pact [p]-CAT) vector containing a 4.1-kilobase full-length antisense EGF-R complementary DNA. EGF-R content was assessed by 125I-EGF binding and EGF-R immunoblot assays. Cisplatin sensitivity was evaluated by (a) colony-forming assay in vitro, (b) xenograft growth in nude mice, (c) cell cycle distribution of propidium iodide-labeled DNA, (d) DNA fragmentation in agarose gels, and (e) terminal deoxynucleotidyl transferase (Tdt) fluorescence in situ. Cisplatin uptake was measured by atomic absorption spectroscopy, and the levels of drug-DNA intrastrand adducts were determined by a dissociation-enhanced fluoroimmunoassay that utilizes an antibody against cisplatin-modified DNA. RESULTS: Selected clones (MDA-468/AS-EGFR) exhibited more than 90% loss of both 125I-EGF binding and receptor content determined by western blot analysis, whereas clones transfected with the vector alone (MDA-468/p-CAT) had EGF-R levels similar to those of the parent cells. By use of a colony-forming assay, the 1-hour IC50 (i.e., the concentration of drug required for 1 hour to achieve 50% cell kill) for cisplatin was 2 microM or less for parental and vector-transfected clones (n = 4), whereas it was 25 microM or more for all MDA-468/AS-EGFR clones (n = 3). MDA-468/p-CAT clones exhibited internucleosomal DNA fragmentation, enhanced Tdt-end labeling in situ, and G2 arrest 48 hours after a 1-hour incubation with 3-30 microM cisplatin. Under these conditions, apoptosis and G2 arrest were undetectable in all MDA-468/AS-EGFR clones. An MDA-468 subline selected after long-term treatment with a TGF-alpha-Pseudomonas exotoxin A fusion protein 40 lacked EGF binding and also exhibited cisplatin resistance (1-hour IC50: > 30 microM) compared with parental cells. This EGF-R-dependent difference in cisplatin response was confirmed in a nude mouse xenograft model by use of high- and low-EGF-R-expressing cell clones. Total intracellular drug accumulation after a 1-hour cisplatin exposure, as measured by atomic absorption spectroscopy, was identical in both groups of cells. Intrastrand drug-DNA adducts, however, were statistically higher in high EGF-R expressors than in low-EGF-R-expressing clones. CONCLUSIONS: These data indicate that a critical level of EGF-R signaling, which is amplified in some common human

cancers, is necessary for cisplatin-mediated apoptosis in tumor cells and suggest an inhibitory effect of this pathway on the repair of cisplatin-damaged DNA.

Dolled-Filhart, M., et al. (2016). "Development of a Companion Diagnostic for Pembrolizumab in Non-Small Cell Lung Cancer Using Immunohistochemistry for Programmed Death Ligand-1." Arch Pathol Lab Med.

Context.- Programmed death ligand-1 (PD-L1) expression by tumors may enable them to avoid immunosurveillance. Objective.- To develop a PD-L1 immunohistochemical assay using the 22C3 anti-PD-L1 murine monoclonal antibody on the Dako platform as a possible companion diagnostic for pembrolizumab in patients with non-small cell lung cancer. Design.- Tumor samples from 146 patients with non-small cell lung cancer treated with pembrolizumab in KEYNOTE-001 and for whom response data were available were scored according to their staining intensity by a single pathologist using 4 methods: percentage of tumor cells staining at any intensity (PS1), moderate/strong intensity (PS2), strong intensity (PS3), and H-score (PS1 + PS2 + PS3). The cutoff score for predicting response to pembrolizumab was determined using receiver operating characteristic analysis. Progression-free and overall survival were assessed in patients with measurable disease per Response Evaluation Criteria in Solid Tumors, version 1.1 (n = 146). Results.- The 4 scoring methods assessed performed similarly; PS1 with a 50% cutoff score is the simplest and easiest method to implement in practice. Response to pembrolizumab was observed in 19 of 44 patients (43%) with a PS1 score of 50% or higher and 8 of 102 patients (8%) with PS1 lower than 50% (odds ratio, 8.93). Median progression-free and overall survival was 4.0 months and not yet reached, respectively, for patients with a PS1 of 50% or higher, and 2.1 and 6.1 months, respectively, for those with PS1 lower than 50%. Conclusion.- The PD-L1 immunohistochemical assay shows the potential for enrichment of trial populations and as a companion diagnostic tool in non-small cell lung cancer.

Domblides, C., et al. (2018). "Nonsmall cell lung cancer from HIV-infected patients expressed programmed cell death-ligand 1 with marked inflammatory infiltrates." AIDS **32**(4): 461-468.

OBJECTIVE: Immunotherapies targeting the programmed cell death-1 (PD-1)/PD-ligand 1 (PD-L1) checkpoint improved prognosis in lung cancer. PD-1/PD-L1 status, however, has not been investigated in human immunodeficiency virus (HIV)-positive patients. This study assessed PD-L1 status and tumor immune-cell infiltration in nonsmall cell lung cancer (NSCLC) in HIV patients. METHODS: Consecutive HIV patients treated between 1996 and 2014 were enrolled. PD-L1 tumor expression was assessed using immunohistochemistry with two antibodies (clones 5H1 and E1L3N), and tumor immune-cell infiltration with CD3, CD4, CD8, CD20, CD163, and MPO. PD-L1 expression and immune infiltration results were compared with those of 54 NSCLCs from unknown HIV status patients. RESULTS: Thirty-four HIV-positive patients were evaluated: predominantly men (88.2%) (median age: 51.1 years) presenting stage IV (38.2%) adenocarcinomas (76.5%). The median blood CD4 count was 480 cells/muL (86-1120) and 64% exhibited undetectable viral load. The PD-L1 score (percentage of positive cells x intensity) was higher in HIV-positive than HIV-undetermined patients with the E1L3N clone [median (range) 0 (0-150) versus 0 (0-26.7), P = 0.047], yet not with the 5H1 clone [0 (0-120) versus 0 (0-26.7) P = 0.07, respectively]. PD-L1 expression frequency did not differ between both cohorts (18.7 versus 9.3% using E1L3N and 10 versus 5.6% using 5H1 clone, respectively). There were significantly greater cytotoxic T-cell (P < 0.001), B-lymphocyte (P = 0.005), and activated macrophage (P < 0.001) infiltrations in the HIV-positive patients, but no differences for CD4 T cells. CONCLUSION: Tumors in HIV-positive patients seem to express higher PD-L1 levels with increased immune infiltration, supporting their inclusion in clinical trials assessing immune checkpoint inhibitors.

Emens, L. A., et al. (2016). "Targeting the programmed cell death-1 pathway in breast and ovarian cancer." Curr Opin Obstet Gynecol **28**(2): 142-147.

PURPOSE OF REVIEW: Immune checkpoint blockade is changing cancer therapy. Targeting the programmed cell death-1 (PD-1) pathway releases T cells from inhibitory signals within the tumor microenvironment, thereby activating a latent antitumor immune response. Here, we review the biology underlying the activity of PD-1/programmed cell death-ligand 1 (PD-L1) antagonists, and data describing their clinical activity in breast and ovarian cancer. RECENT FINDINGS: Several antagonists of PD-1 and PD-L1 have been tested in breast and ovarian cancer. These drugs are generally well tolerated, with some immune-related adverse events that are typically easily managed. Objective response rates generally range from about 10 to 20% in both breast cancer and ovarian cancer, with durable responses noted in multiple trials. Selecting patients with PD-L1 expression by cells within the tumor microenvironment appears to enrich for responses. These agents are under accelerated development based on these promising early data. SUMMARY: Monoclonal antibody-based blockade of the PD-1

pathway results in objective and durable clinical responses in a subset of patients with breast or ovarian cancers, particularly those with PD-L1-positive cells within the tumor microenvironment. Current priorities are to refine biomarkers of therapeutic response, and to develop combination immunotherapy strategies that integrate PD-1/PD-L1 antagonists with both standard and immune-based cancer therapies to increase efficacy.

Enkhbat, T., et al. (2018). "Programmed Cell Death Ligand 1 Expression Is an Independent Prognostic Factor in Colorectal Cancer." Anticancer Res **38**(6): 3367-3373.

BACKGROUND/AIM: Programmed cell death protein 1 (PD-1)/ programmed cell death ligand 1(PD-L1) axis is associated with immune tolerance via inhibition of T cell activation. The aim of this study was to clarify the significance of PD-1 and PD-L1 expressions and analyze the relationships between PD-1, PD-L1, transforming growth factor-beta (TGF-beta) and Forkhead box P3 (Foxp3) expressions in colorectal cancer (CRC). PATIENTS AND METHODS: A total of 116 patients who underwent curative colectomy for stage II/III CRC were included in the study. PD-1, PD-L1, TGF-beta, and Foxp3 expressions were examined by immunohistochemistry and related to prognostic factors by Kaplan-Meier. RESULTS: PD-1 expression was correlated with PD-L1, TGF-beta, and Foxp3 expressions. Overall survival rates were significantly poorer in the PD-1 and PD-L1-positive groups. Multivariate analysis showed that PD-L1-positive is an independent risk factor. Disease-free survival (DFS) was tended in the PD-L1-positive group. The group with double-positive expression had significantly poorer prognosis. CONCLUSION: PD-1 and PD-L1 expressions were associated with a poor prognosis and correlated with TGF-beta and Foxp3 expressions in patients with CRC.

Eto, S., et al. (2016). "Programmed cell death protein 1 expression is an independent prognostic factor in gastric cancer after curative resection." Gastric Cancer **19**(2): 466-471.

BACKGROUND: Programmed cell death protein 1 (PD-1) and its ligand PD-L1 downregulate T cell activation and are related to immune tolerance. The aim of this study was to clarify the significance of PD-1 and PD-L1 expression and to analyze the relationships among PD-1, PD-L1, and Foxp3 expression in gastric cancer. METHODS: A total of 105 patients who underwent curative gastrectomy for stage II/III gastric cancer were included in this study. PD-1, PD-L1, and Foxp3 expression were examined by immunohistochemistry and related to prognostic factors by univariate and multivariate analyses. RESULTS: PD-1 expression was correlated with both PD-L1 and Foxp3 expression. Disease-free survival (DFS) was significantly poorer in PD-1-positive patients than in PD-1-negative patients (3-year DFS, 36.1 % vs. 64.7 %, respectively; $p < 0.05$). Overall survival also tended to be poorer in PD-L1-positive patients than in PD-L1-negative patients. Univariate analysis identified sex, T factor, lymphatic invasion, and PD-1 positivity as significant predictors of poor DFS. Multivariate analysis confirmed male sex, lymphatic invasion, and positive PD-1 expression as independent prognostic indicators. CONCLUSIONS: PD-1 expression is associated with a poor prognosis and is correlated with PD-L1 and Foxp3 expression in patients with gastric cancer.

Fassan, M., et al. (2010). "Programmed cell death 4 protein in esophageal cancer." Oncol Rep **24**(1): 135-139.

Screening for genes down-regulated in esophageal cancers (Oncomine database) pinpointed programmed cell death 4 (PDCD4) as one of the most consistently involved. PDCD4 is a new putative tumor suppressor gene implicated in cell transformation, tumorigenesis, and invasiveness. Based on such a biological rationale, the aim of the present study was to evaluate the prognostic value of PDCD4 in esophageal cancers. The immunohistochemical expression of PDCD4 protein was assessed in 111 consecutive esophageal cancers (63 adenocarcinomas and 48 squamous cell carcinomas) and paired non-cancerous samples. PDCD4 immunostaining was significantly lower in cancer samples than in non-cancerous mucosa ($p<0.001$). In all cases, the native esophageal epithelium consistently expressed nuclear PDCD4, which was significantly less expressed (37/111 cases) or completely lacking (31/111 cases) in the cancer samples. A significant inverse correlation emerged between nuclear PDCD4 expression and tumor stage ($p=0.002$), pT ($p<0.001$), nodal metastasis ($p=0.038$), and with both vascular ($p=0.005$) and perineural invasion ($p=0.004$). Nuclear PDCD4 expression was associated with a longer disease-free ($p=0.011$) and overall ($p=0.021$) survival. PDCD4 expression predicts the patient outcome in esophageal cancers. Additional functional studies should look into the role of PDCD4 in the multistep process of esophageal oncogenesis also inquiring on the clinical usefulness of the protein expression as prognostic marker in esophageal precancerous lesions.

Frankel, L. B., et al. (2008). "Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells." J Biol Chem **283**(2): 1026-1033.

MicroRNAs are emerging as important regulators of cancer-related processes. The miR-21 microRNA is overexpressed in a wide variety of cancers and has been causally linked to cellular proliferation, apoptosis, and migration. Inhibition of mir-21 in MCF-7 breast cancer cells causes reduced cell growth. Using array expression analysis of MCF-7 cells depleted of miR-21, we have identified mRNA targets of mir-21 and have shown a link between miR-21 and the p53 tumor suppressor protein. We furthermore found that the tumor suppressor protein Programmed Cell Death 4 (PDCD4) is regulated by miR-21 and demonstrated that PDCD4 is a functionally important target for miR-21 in breast cancer cells.

Fujimoto, D., et al. (2018). "Programmed Cell Death Ligand 1 Expression in Non-Small-cell Lung Cancer Patients With Interstitial Lung Disease: A Matched Case-control Study." Clin Lung Cancer **19**(5): e667-e673.

BACKGROUND: Programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) checkpoint inhibitors have demonstrated antitumor activity, and immunohistochemical analysis of PD-L1 expression has been used to identify the response in patients with non-small-cell lung cancer (NSCLC). Recently, considerable interest has ensued toward extending the benefit of these inhibitors to high-risk patients, such as those with NSCLC and interstitial lung disease (ILD). However, no studies have compared PD-L1 expression in NSCLC patients with and without ILD. Therefore, we conducted a case-control study to evaluate PD-L1 expression and stromal CD8(+) lymphocyte density in these patients. MATERIALS AND METHODS: The data from patients with pathologic stage I or II NSCLC who had undergone surgery from January 2007 to January 2016 were analyzed. RESULTS: We identified 62 patients with pathologic stage I or II NSCLC and ILD. We compared these patients with 1:1-matched cohort. In both groups with and without ILD, approximately 60% were PD-L1(+). Tumor cell PD-L1 expression was similar between the groups (median, 1%; interquartile range, 0%-5%; vs. median, 1%; interquartile range, 0%-5%; P =.49). The proportion of patients with positive (>/= 1%) and strongly positive (>/= 50%) PD-L1 expression was also similar between the 2 groups (P =.46 and P = 1.00, respectively). Additionally, the CD8(+) lymphocyte density did not differ between patients with and without ILD. CONCLUSION: PD-L1 expression and stromal CD8(+) lymphocyte density were comparable between the NSCLC patients with and without ILD. PD-1 axis inhibitors might be effective for NSCLC patients with ILD.

Fujimoto, D., et al. (2018). "Predictive Performance of Four Programmed Cell Death Ligand 1 Assay Systems on Nivolumab Response in Previously Treated Patients with Non-Small Cell Lung Cancer." J Thorac Oncol **13**(3): 377-386.

INTRODUCTION: Nivolumab has demonstrated efficacy against metastatic NSCLC. Four programmed cell death ligand 1 (PD-L1) immunohistochemistry (IHC) assay systems are available for identification of responders among patients with NSCLC, and these assays show some differing characteristics. Accordingly, in this study, we evaluated the ability of these assays to identify responders to nivolumab therapy. METHODS: We retrospectively analyzed patients with previously treated advanced NSCLC, who received nivolumab between January 2016 and September 2016. Specimens were stained using four PD-L1 IHC assays (28-8, 22C3, SP142, and SP263). We classified patients as having test results that were strongly positive (tumor proportion score [TPS] >/=50%), weakly positive (TPS 1%-49%), or negative (TPS <1%). RESULTS: A total of 40 patients with NSCLC and their specimens were analyzed. Analytical comparisons demonstrated good concordance of PD-L1-stained tumor cells among the 28-8, 22C3, and SP263 assays (weighted kappa coefficient 0.64-0.71), whereas the SP142 assay showed lower concordance with other assays (weighted kappa coefficient 0.39-0.55). Progression-free survival in patients showing strongly positive PD-L1 staining classified by 28-8, 22C3, and SP263 assays was significantly longer than that in patients with a negative result for PD-L1 staining. Predictive performance of response to nivolumab, as assessed by receiver operating characteristic analysis, was also equivalent among the 28-8, 22C3, and SP263 assays (area under the curve 0.75-0.82), whereas the SP142 assay exhibited lower predictive performance (area under the curve 0.68). CONCLUSIONS: The 28-8, 22C3, and SP263 PD-L1 IHC assays showed equivalent predictive performance, whereas the SP142 assay showed lower predictive performance.

Fukumoto, K., et al. (2018). "Clinical Role of Programmed Cell Death-1 Expression in Patients with Non-muscle-invasive Bladder Cancer Recurring After Initial Bacillus Calmette-Guerin Therapy." Ann Surg Oncol **25**(8): 2484-2491.

BACKGROUND: The programmed cell death-1 (PD-1) pathway has been suggested to play an important role in tumor immune escape. We evaluated changes in PD-1 expression before and after Bacillus Calmette-Guerin (BCG) therapy and its prognostic significance in non-muscle-invasive bladder cancer (NMIBC) patients. METHODS: We examined 78 paired tissue samples of NMIBC in tumors just before

BCG therapy and BCG-relapsing tumors, defined as recurrence after achieving disease-free status by initial BCG instillations for 6 months. We counted PD-1-positive cells, and PD-1 expression was defined as high when the number of PD-1-positive cells was more than 18 under x200 magnification. RESULTS: The median number of PD-1-positive cells in tumors just before BCG therapy was 3.5, significantly lower than that in BCG-relapsing tumors (17.0, $p < 0.001$). High PD-1 expression was observed in 20 tumors just before BCG therapy (25.6%) and 36 BCG-relapsing tumors (46.2%). Fifty-two cases (66.6%) showed an increase in the number of PD-1-positive cells in BCG-relapsing tumors. High PD-1 expression in BCG-relapsing tumors was independently associated with subsequent tumor recurrence ($p = 0.011$) and stage progression ($p = 0.033$). The 5-year recurrence-free and progression-free survival rates were 40.7 and 74.1% in patients with high PD-1 expression in BCG-relapsing tumors, significantly lower than those in their counterparts (72.9 and 94.1%, respectively). CONCLUSIONS: PD-1 was induced by BCG therapy, and its expression in BCG-relapsing tumors may be an important indicator for predicting worse clinical outcomes in NMIBC patients treated with BCG therapy.

Funaki, S., et al. (2017). "Chemotherapy enhances programmed cell death 1/ligand 1 expression via TGF-beta induced epithelial mesenchymal transition in non-small cell lung cancer." Oncol Rep **38**(4): 2277-2284.

In cancer immunology, the programmed cell death 1-programmed cell death 1/ligand 1 (PD-1/PD-L1) pathway plays a major role. Anti-PD-1 and anti-PD-L1 antibodies provide reliable immunotherapy when given as treatment for various types of malignancy including lung cancer. PD-L1 expression in cancer cells has been reported to be a predictive factor for the therapeutic effects of immunotherapy. However, the mechanism of PD-L1 expression remains unclear. Another key process in cancer progression is epithelial-mesenchymal transition (EMT). In the present study, we investigated the mechanism of PD-L1 expression as well as changes in its expression during the EMT process in non-small cell lung cancer (NSCLC). In this study, A549 cells underwent EMT by treatment with TGF-beta or chemotherapeutic agents and then PD-L1 expression was evaluated. The alterations of PD-L1 expression was also examined during the reverse EMT process; mesenchymal-epithelial transition (MET). The relationship between for PD-L1 expression and EMT status in clinical specimens with NSCLC after induction chemotherapy were analyzed by immunohistochemical staining. We found that PD-L1 expression was upregulated following TGF-beta induction; in contrast, it was downregulated by TGF-beta receptor-kinase inhibitors and the MET process. Furthermore, chemo-treatment increased TGF-beta expression and enhances PD-L1 expression via autocrine TGF-beta induced EMT. Analysis of clinical samples revealed a significant relationship between PD-L1 expression and EMT status ($P<0.05$). In conclusion, our results suggest that PD-L1 expression is regulated by TGF-beta induced EMT and enhanced by chemo-treatment via the chemo-induced TGF-beta signaling. The anti-PD-1/PD-L1 blockade may provide more effective anticancer activities in combination with chemotherapy in NSCLC.

Furuya, Y. and J. T. Isaacs (1994). "Proliferation-dependent vs. independent programmed cell death of prostatic cancer cells involves distinct gene regulation." Prostate **25**(6): 301-309.

Androgen-independent Dunning R-3327 AT-3 rat prostatic cancer cells can be induced to undergo programmed cell death in either a proliferation-dependent or independent manner depending upon the therapeutic agent used. In the present study, 5-fluorodeoxyuridine (5-FrdU) was used to induce proliferation-dependent death of the AT-3 cells via its ability to inhibit thymidylate synthetase. Ionomycin and thapsigargin were used to induce proliferation-independent death of these cells via their ability to sustain an elevation in intracellular free Ca2+. Based upon the temporal sequence of DNA fragmentation, morphologic changes, and loss of cell viability, each of the three test agents, at the doses used, induces the programmed death of AT-3 cells with essentially identical kinetics. Based upon these similarities, comparisons of the pattern of gene expression during the proliferation-dependent (i.e., 5-FrdU-induced) vs. proliferation-independent (i.e., ionomycin and thapsigargin-induced) programmed death of AT-3 cells allow identification of genes whose enhanced expression is involved in the initiation vs. completion of programmed cell death. Based upon this approach, enhanced H-ras and TRPM-2 expression is associated with initiation of proliferation-dependent programmed death of AT-3 cells while enhanced c-myc, calmodulin, and alpha-prothymosin expression is associated with initiation of proliferation-independent programmed death of these cells. In contrast, enhanced expression of glucose-regulated 78 kilodalton and tissue transglutaminase genes are associated with the completion of programmed cell death, since their expression is enhanced in both proliferation-dependent and independent programmed cell death of AT-3 cells.

Glinsky, G. V. and V. V. Glinsky (1996). "Apoptosis amd metastasis: a superior resistance of

metastatic cancer cells to programmed cell death." Cancer Lett **101**(1): 43-51.

We studied the response to different external signals leading to apoptosis of several poorly and highly metastatic cell lines employing a murine B16 melanoma experimental metastasis model. We found that highly metastatic cells exhibit a superior survival ability and resistance to apoptosis compared to poorly metastatic cells which would give the former an obvious selective growth advantage during tumor progression. Our results indicate that there is a genetic link between aggressive metastatic phenotype and resistance to apoptosis.

Gonzalez-Villasana, V., et al. (2012). "Programmed cell death 4 inhibits leptin-induced breast cancer cell invasion." Oncol Rep **27**(3): 861-866.

Obesity is a significant risk factor for post-menopausal women to develop and die from breast cancer. Leptin, an adipokine is produced in high levels in obese individuals, and its receptor is overexpressed in breast tumors and lymph node metastases. Previously, we demonstrated that leptin stimulates breast cancer cell invasion, which is correlated with breast cancer metastasis. Programmed cell death 4 (PDCD4) has been shown to block cancer cell invasion. However, whether PDCD4 blocks leptin-induced breast cancer cell invasion is not known. Here, we report the novel findings that leptin failed to induce invasion in MCF-7 breast cancer cells overexpressing PDCD4 (MCF-7/PDCD4). Tissue inhibitor of metalloproteinase-2 (TIMP-2) was essential to the anti-invasive effect of PDCD4, as leptin stimulated the invasion of MCF-7/PDCD4 cells pretreated with TIMP-2 siRNA. Furthermore, TIMP-2 knockdown allowed leptin to augment phosphorylation of extracellular signal-regulated kinases 1,2 and signal transducer and activator of transcription 3, but not that of Jun N-terminal kinases. These data indicate that PDCD4 utilizes TIMP-2 to exert its anti-invasive effect by suppressing leptin-induced activation of extracellular signal-regulated kinases 1,2 and signal transducer and activator of transcription 3. Novel therapeutic strategies aiming at enhancing PDCD4 expression in breast tumors may be able to stop obesity-related breast tumor progression and prolong the life of patients.

Gorka, M., et al. (2005). "Autophagy is the dominant type of programmed cell death in breast cancer MCF-7 cells exposed to AGS 115 and EFDAC, new sesquiterpene analogs of paclitaxel." Anticancer Drugs **16**(7): 777-788.

The molecular mechanism of cell death induced by AGS 115 and EFDAC, sesquiterpene analogs of paclitaxel, was investigated in human breast cancer MCF-7 cells. The study was carried out using laser scanning cytometry, homeostatic confocal microscopy, atomic force microscopy and electron microscopy. AGS 115 and EFDAC exhibited a microtubule-stabilizing effect as confirmed by a significant increase in alpha-tubulin aggregation. Both paclitaxel analogs also induced death in MCF-7 cells. Evaluation of biochemical and morphological features suggested that the major form of programmed cell death induced by AGS 115 and EFDAC was autophagy. This was confirmed by MAP I LC3 expression and the ultrastructural pattern revealed by electron microscopy. Surface images of cells undergoing autophagy showed that, unlike during apoptosis, the dimensions remained unchanged, but the surface of the cell was deformed. The occurrence of apoptosis was confirmed by the efflux of Smac/DIABLO from mitochondria, caspase-7 activation and DNA loss, and did not exceed 9.7%. Therefore, AGS 115 and EFDAC appear to be promising candidates for further investigation in anti-cancer therapy.

Govindarajan, R., et al. (2018). "Programmed Cell Death-Ligand 1 (PD-L1) Expression in Anal Cancer." Am J Clin Oncol **41**(7): 638-642.

OBJECTIVE: To evaluate the expression of programmed cell death-ligand 1 (PD-L1) in anal cancer. PATIENTS AND METHODS: In a retrospective cohort analysis, subjects with squamous cell carcinoma of the anal canal were tested for PD-L1 expression, then followed for recurrence and survival. Crude recurrence rates (CRRs), crude mortality rates (CMRs), and crude event rates (CERs) were assessed for PD-L1-dependent differences using Poisson regression. All 3 types of crude rate were expressed as the number that occurred per hundred person-years (hPY) of follow-up. RESULTS: Samples from 41 subjects were evaluated for PD-L1 expression; 23 (56%) were positive. Subjects with PD-L1-expressing versus PD-L1-negative tumors respectively had CRRs of 30.8 versus 12.1 recurrences/hPY (P=0.082), CMRs of 16.7 versus 12.0 deaths/hPY (P=0.47), and CERs of 39.2 versus 16.9 events/hPY (P=0.069). CONCLUSIONS: PD-L1 positivity was associated with worse CRR and CER, and marginally worse CMR. The effect on progression-free and overall survival needs to be validated in a study with a larger sample size.

Guan, Y. Q., et al. (2011). "Pathway of programmed cell death in HeLa cells induced by polymeric anti-cancer drugs." Biomaterials **32**(14): 3637-3646.

Synthesis of anticancer polymeric materials plus their biological applications is one of the most charming and active research areas in biological

functional materials. However, the predominant mechanisms for controlling cancer cell viability are not yet clear. In this work, cell culture polymeric materials co-immobilized with death signal proteins interferon-gamma (IFN-gamma)/tumor necrosis factor-alpha (TNF-alpha) on the surface were prepared by photochemical method to develop an anticancer polymeric drug model. Various characterizations on the microstructures and compositions, including the Fourier transform infrared spectroscopy, UV absorption spectroscopy, fluorescence measurement, atomic force microscopy, and electron spectroscopy for chemical analysis, were performed. For addressing the biological applications, we investigated systematically the death pathways of HeLa cells attached onto the drug model by means of a series of cell-biology techniques. It was demonstrated that the IFN-gamma plus TNF-alpha co-immobilized on the polymeric material surface exhibited more notable inhibitive effects than the free IFN-gamma plus TNF-alpha, and the induced HeLa cells were mainly along apoptosis-like PCD with the translocation of EndoG from the cytoplasm to the nucleus. These findings indicate that the polymeric drugs with the co-immobilized IFN-gamma plus TNF-alpha may offer significant potentials for therapeutic manipulation of human cervical cancer.

Hamada, T., et al. (2017). "Aspirin Use and Colorectal Cancer Survival According to Tumor CD274 (Programmed Cell Death 1 Ligand 1) Expression Status." J Clin Oncol **35**(16): 1836-1844.

Purpose Blockade of the programmed cell death 1 (PDCD1, PD-1) immune checkpoint pathway can improve clinical outcomes in various malignancies. Evidence suggests that aspirin (a widely used nonsteroidal anti-inflammatory drug) not only prolongs colorectal cancer survival, but can also activate T cell-mediated antitumor immunity and synergize with immunotherapy through inhibition of prostaglandin E2 production. We hypothesized that the survival benefit associated with aspirin might be stronger in colorectal carcinoma with a lower CD274 (PDCD1 ligand 1, PD-L1) expression level that resulted in lower signaling of the immune checkpoint pathway. Patients and Methods Using data from 617 patients with rectal and colon cancer in the Nurses' Health Study and the Health Professionals Follow-Up Study, we examined the association of postdiagnosis aspirin use with patient survival in strata of tumor CD274 expression status measured by immunohistochemistry. We used multivariable Cox proportional hazards regression models to control for potential confounders, including disease stage, microsatellite instability status, CpG island methylator phenotype, long interspersed nucleotide element-1 methylation, cyclooxygenase-2 (PTGS2), and CDX2 expression, and KRAS, BRAF, and PIK3CA mutations. Results The association of postdiagnosis aspirin use with colorectal cancer-specific survival differed by CD274 expression status (Pinteraction <.001); compared with aspirin nonusers; multivariable-adjusted hazard ratios for regular aspirin users were 0.16 (95% CI, 0.06 to 0.41) in patients with low CD274 and 1.01 (95% CI, 0.61 to 1.67) in patients with high CD274. This differential association seemed consistent in patients with microsatellite-stable or PIK3CA wild-type disease and in strata of PTGS2 expression, CDX2 expression, tumor-infiltrating lymphocytes, or prediagnosis aspirin use status. Conclusion The association of aspirin use with colorectal cancer survival is stronger in patients with CD274-low tumors than CD274-high tumors. Our findings suggest a differential antitumor effect of aspirin according to immune checkpoint status.

Hamanishi, J., et al. (2007). "Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer." Proc Natl Acad Sci U S A **104**(9): 3360-3365.

The ligands for programmed cell death 1 (PD-1), an immunoinhibitory receptor belonging to CD28/cytotoxic T lymphocyte antigen 4 family, are PD-1 ligand 1 and 2 (PD-Ls). Recent reports suggest that the aberrant expression of PD-Ls on tumor cells impairs antitumor immunity, resulting in the immune evasion of the tumor cells. Although an inverse correlation between the expression level of PD-Ls and patients' prognosis has been reported for several malignant tumors, the follow-up period was limited because of the lack of the antibody (Ab) applicable to paraffin-embedded specimens. Here we generated a new Ab against PD-1 ligand 1 (PD-L1) and analyzed the expression level of PD-Ls in human ovarian cancer using paraffin-embedded specimens. Patients with higher expression of PD-L1 had a significantly poorer prognosis than patients with lower expression. Although patients with higher expression of PD-1 ligand 2 also had a poorer prognosis, the difference was not statistically significant. A significant inverse correlation was observed between PD-L1 expression and the intraepithelial CD8(+) T lymphocyte count, suggesting that PD-L1 on tumor cells directly suppresses antitumor CD8(+) T cells. Multivariate analysis showed the expression of PD-L1 on tumor cells and intraepithelial CD8(+) T lymphocyte count are independent prognostic factors. The PD-1/PD-L pathway can be a good target for restoring antitumor immunity in ovarian cancer.

Hammer, M., et al. (2018). "Thoracic Imaging of Non-Small Cell Lung Cancer Treated With Anti-

programmed Death Receptor-1 Therapy." Curr Probl Diagn Radiol.

PURPOSE: Treatment with anti-programmed death receptor-1 (PD-1) therapeutics can lead to unconventional responses and side effect profiles due to their potentiating effects on the immune system. Here we evaluate the radiologic manifestations of anti-PD-1 therapy in the chest in patients with non-small cell lung cancer (NSCLC) receiving anti-PD-1 therapy. MATERIALS AND METHODS: A retrospective review of real-world clinical practice was conducted of all the patients with NSCLC receiving anti-PD-1 therapy at our institution between 2013 and 2016. All patients without adequate clinical or radiologic follow-up data in the electronic medical records were excluded. Imaging examinations for all patients deemed by their thoracic oncologists to have radiologic pseudoprogression or therapy-associated pneumonitis were reviewed by experienced thoracic radiologists. RESULTS: A total of 166 patients with NSCLC had available clinical and imaging data for retrospective review. Of these patients, 4 (2%) were considered to have radiologic pseudoprogression, 3 of which manifested as increased tumor size and 1 of which manifested with new lesions. A total of 5 patients (3%) were clinically deemed to have pneumonitis attributable to anti-PD-1 therapy, 4 of which had radiologic manifestations on computed tomography. CONCLUSION: Radiologic pseudoprogression and drug-induced pneumonitis are uncommon but important manifestations of anti-PD-1 therapy on thoracic imaging.

Hansen, C. M., et al. (2000). "Cyanoguanidine CHS 828 induces programmed cell death with apoptotic features in human breast cancer cells in vitro." Anticancer Res **20**(6B): 4211-4220.

The cyanoguanidine CHS 828 was recently shown to possess potent anti-tumour effects both in vitro and in vivo. The exact mechanism of action of CHS 828 is not known, but recent results have indicated that induction of programmed cell death may be one mechanism by which CHS 828 exerts its anti-tumour effects. To investigate this aspect in more detail, we studied the effect of CHS 828 and the reference compound Taxol beta on programmed cell death in human breast cancer cells in vitro. Both compounds were found to induce DNA fragmentation in the cells. However, microscopic examination of the cells demonstrated that CHS 828 and Taxol triggered different types of cell death. In the CHS 828-treated cultures most cells were found to be Annexin-V positive, indicating that these cells were early apoptotic cells, while no morphological characteristics of classical apoptosis were seen. In contrast, the cells in the Taxol-treated cultures displayed morphological features characteristic of classical apoptotic cells, but no Annexin-V positive cells could be observed. These findings together with the previously reported potent effects of CHS 828 on tumour cells, makes CHS 828 a promising new agent for the treatment of cancer patients.

Hashemi, M., et al. (2015). "Association between Programmed Cell Death 6 Interacting Protein Insertion/Deletion Polymorphism and the Risk of Breast Cancer in a Sample of Iranian Population." Dis Markers **2015**: 854621.

It has been suggested that genetic factors contribute to patients' vulnerability to breast cancer (BC). The programmed cell death 6 interacting protein (PDCD6IP) encodes for a protein that is known to bind to the products of the PDCD6 gene, which is involved in the apoptosis pathway. The aim of this case-control study is to investigate the relationship between the PDCD6IP 15 bp insertion/deletion (I/D) polymorphism (rs28381975) and BC risk in an Iranian population. A total of 491 females, including 266 BC patients and 225 control subjects without cancer, were enrolled into the study. Our findings revealed that the PDCD6IP 15 bp I/D polymorphism decreased the risk of BC in codominant (OR = 0.44, 95% CI = 0.31-0.65, $p < 0.0001$, I/D versus DD; OR = 0.39, 95% CI = 0.17-0.88, $p = 0.030$, I/I versus DD) and dominant (OR = 0.44, 95% CI = 0.30-0.63, $p < 0.0001$, D/I + I/I versus D/D) tested inheritance models. Also, the PDCD6IP I allele significantly decreased the risk of BC (OR = 0.59, 95% CI = 0.45-0.78, $p < 0.001$) compared to the D allele.

Hata, A., et al. (2017). "Programmed death-ligand 1 expression according to epidermal growth factor receptor mutation status in pretreated non-small cell lung cancer." Oncotarget **8**(69): 113807-113816.

Background: Current clinical trials have suggested poorer efficacies of anti-programmed death-1 (PD-1)/PD-ligand 1 (PD-L1) immunotherapies for non-small cell lung cancer (NSCLC) harboring epidermal growth factor receptor (EGFR) mutations, implying lower PD-L1 expression in EGFR-mutant NSCLC than in EGFR-wild type. Methods: We retrospectively analyzed correlation between PD-L1 expression and EGFR status in clinical samples of pretreated NSCLC. PD-L1 immunohistochemistry was performed using the 28-8 anti-PD-L1 antibody for tumor cell membrane staining. H-score was adopted to evaluate both percentage and intensity. We investigated H-scores >/=1, >/=5, and >/=10 as PD-L1+ cut-offs. H-score >/=10 was defined as strong PD-L1+. Results: We investigated 96 available histologic samples in 77 pretreated patients with NSCLC. Median H-score in EGFR-mutant samples

(n=65) was 3 (range, 0-150), whereas EGFR-wild-type (n=31) was 8 (range, 0-134) (p=0.0075). Using H-scores >/=1, >/=5, and >/=10 cut-offs, incidence of PD-L1+ in EGFR-mutant vs. EGFR-wild-type samples were: 85% (55/65) vs. 94% (29/31) (p=0.2159); 42% (27/65) vs. 74% (23/31) (p=0.0027); and 22% (14/65) vs. 48% (15/31) (p=0.0074), respectively. Patient-oriented (n=77) univariate analysis for strong PD-L1+ found age of sample (p=0.0226) and EGFR mutation status (p=0.0490) as significant factors. Multivariate analysis identified EGFR mutation status as the only significant factor (p=0.0121, odds ratio 2.99) for strong PD-L1+. H-scores of PD-L1 expression varied in all 11 cases receiving multiple rebiopsies, and categories of positivity migrated in 10 (91%) of 11 patients. Conclusions: PD-L1 expression was significantly lower in EGFR-mutant NSCLC samples than in EGFR wild-type samples. Its expression could be dynamic and affected by age of sample.

Hata, A., et al. (2017). "Programmed death-ligand 1 expression and T790M status in EGFR-mutant non-small cell lung cancer." Lung Cancer **111**: 182-189.

BACKGROUND: Differential biology and prognosis between T790M+ and T790M- populations imply immunological differences also. METHODS: We retrospectively analyzed programmed death-ligand 1 (PD-L1) expression and T790M status in rebiopsied samples of epidermal growth factor receptor (EGFR)-mutant non-small cell lung cancer (NSCLC). PD-L1 immunohistochemistry was performed using the SP142 antibody for tumour cell (TC) and tumour-infiltrating immune cell (IC) and the 28-8 antibody for TC. PD-L1+ was defined as TC or IC >/=1%. RESULTS: We investigated 67 available rebiopsied histologic samples in 47 patients. Using the SP142, prevalence of PD-L1 any+, moderate+, and strong+ in T790M+ vs. T790M- samples were 31% vs. 61%, 8% vs. 15%, and 0% vs. 2%, respectively, representing PD-L1+ prevalence of T790M+ samples was significantly lower than that of T790M- (p=0.0149). Prevalence of any TC+/IC+ in T790M+ vs. T790M- samples were TC: 31% vs. 51% (p=0.0997) and IC: 8% vs. 27% (p=0.0536), respectively. Using the 28-8, median percentage of PD-L1+ in T790M+ samples was 1.9 (range, 0-27.2), whereas T790M- was 4.1 (range, 0-89.8) (p=0.0801). Prevalence of PD-L1+ >/=1%, >/=5%, and >/=10% in T790M+ vs. T790M- samples were 77% vs. 83% (p=0.5476), 31% vs. 49% (p=0.1419), and 12% vs. 27% (p=0.1213), respectively. In 9 of 11 patients receiving multiple rebiopsies, T790M and/or PD-L1 expression revealed temporal dynamism. Survival curves according to PD-L1 expression/T790M status suggested better prognosis in PD-L1-/T790M+ population. CONCLUSIONS: T790M+ status was correlated to lower PD-L1 expression. PD-L1 expression might have a prognostic value and interaction with T790M mutation in EGFR-mutant NSCLC.

Hatae, R. and K. Chamoto (2016). "Immune checkpoint inhibitors targeting programmed cell death-1 (PD-1) in cancer therapy." Rinsho Ketsueki **57**(10): 2224-2231.

Immune checkpoint inhibitors, especially anti-programmed cell death-1 (PD-1) antibodies, have revolutionized cancer therapy. A PD-1 antibody, nivolumab, was the first of these agents to be approved by the Pharmaceuticals and Medical Devices Agency (PMDA) of Japan, as a new cancer drug for melanoma, in July 2014. While PD-1 mAb therapy has so far been approved only for untreated malignant melanomas and non-small cell lung cancer, many clinical studies on various types of cancer have been conducted worldwide. Immune checkpoint inhibitors target lymphocytes rather than cancer cells, and evoke an anti-tumor immune reaction. Since the activated lymphocytes recognize various tumor-associated antigens including a mutated antigen, immune checkpoint inhibitors exhibit continuous long-term effectiveness, despite the generation of genetic mutations in cancer cells. As compared with previous cancer treatments, immune checkpoint inhibitors show superior efficacy against tumors with fewer side effects. Therefore, these novel immune checkpoint inhibitor agents are anticipated to become a 4(th) cancer treatment option following surgery, chemotherapy, and radiation therapy. Herein, we review the main clinical results of PD-1 mAb cancer immunotherapy obtained to date and discuss issues relevant to administering this form of treatment.

Hess, D., et al. (2010). "Inhibition of stearoylCoA desaturase activity blocks cell cycle progression and induces programmed cell death in lung cancer cells." PLoS One **5**(6): e11394.

Lung cancer is the most frequent form of cancer. The survival rate for patients with metastatic lung cancer is approximately 5%, hence alternative therapeutic strategies to treat this disease are critically needed. Recent studies suggest that lipid biosynthetic pathways, particularly fatty acid synthesis and desaturation, are promising molecular targets for cancer therapy. We have previously reported that inhibition of stearoylCoA desaturase-1 (SCD1), the enzyme that produces monounsaturated fatty acids (MUFA), impairs lung cancer cell proliferation, survival and invasiveness, and dramatically reduces tumor formation in mice. In this report, we show that inhibition of SCD activity in human lung cancer cells with the small molecule SCD inhibitor CVT-11127

reduced lipid synthesis and impaired proliferation by blocking the progression of cell cycle through the G (1)/S boundary and by triggering programmed cell death. These alterations resulting from SCD blockade were fully reversed by either oleic (18:1n-9), palmitoleic acid (16:1n-7) or cis-vaccenic acid (18:1n-7) demonstrating that cis-MUFA are key molecules for cancer cell proliferation. Additionally, co-treatment of cells with CVT-11127 and CP-640186, a specific acetylCoA carboxylase (ACC) inhibitor, did not potentiate the growth inhibitory effect of these compounds, suggesting that inhibition of ACC or SCD1 affects a similar target critical for cell proliferation, likely MUFA, the common fatty acid product in the pathway. This hypothesis was further reinforced by the observation that exogenous oleic acid reverses the anti-growth effect of SCD and ACC inhibitors. Finally, exogenous oleic acid restored the globally decreased levels of cell lipids in cells undergoing a blockade of SCD activity, indicating that active lipid synthesis is required for the fatty acid-mediated restoration of proliferation in SCD1-inhibited cells. Altogether, these observations suggest that SCD1 controls cell cycle progression and apoptosis and, consequently, the overall rate of proliferation in cancer cells through MUFA-mediated activation of lipid synthesis.

Iafolla, M. A. J. and R. A. Juergens (2017). "Update on Programmed Death-1 and Programmed Death-Ligand 1 Inhibition in the Treatment of Advanced or Metastatic Non-Small Cell Lung Cancer." Front Oncol **7**: 67.

PURPOSE: Non-small-cell lung cancer (NSCLC) has a large worldwide prevalence with a high mortality rate. Chemotherapy has offered modest improvements in survival over the past two decades. Immune checkpoint modulation with programmed death-1 (PD-1) or programmed death-ligand 1 (PD-L1) inhibition has shown the promise of changing the future landscape of cancer therapy. This update reviews recent advances in the treatment of NSCLC with immune checkpoint modulation. METHODS: Publications and proceedings were identified from searching PubMed and proceedings from the annual meetings of the American Society of Clinical Oncology, European Society for Medical Oncology, and European Lung Cancer Conference. RESULTS: Atezolizumab, nivolumab, and pembrolizumab increase overall survival in second-line treatment of Stage III/IV squamous and non-squamous NSCLC when compared to docetaxel. Pembrolizumab increases progression-free survival in the first-line treatment of Stage IV NSCLC with 50% PD-L1 expression when compared to platinum-based chemotherapy. Combination therapy with chemotherapy and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors has shown promise in early trials. CONCLUSION: Immune checkpoint modulation produces durable responses and overall survival benefits with less toxicity compared to conventional chemotherapy. Future investigations are combining PD-1/L1 inhibition with chemotherapy, targeted therapy, or other immuno-oncology agents in an effort to further improve efficacy.

Igal, R. A. (2010). "Stearoyl-CoA desaturase-1: a novel key player in the mechanisms of cell proliferation, programmed cell death and transformation to cancer." Carcinogenesis **31**(9): 1509-1515.

As part of a shift toward macromolecule production to support continuous cell proliferation, cancer cells coordinate the activation of lipid biosynthesis and the signaling networks that stimulate this process. A ubiquitous metabolic event in cancer is the constitutive activation of the fatty acid biosynthetic pathway, which produces saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) to sustain the increasing demand of new membrane phospholipids with appropriate acyl composition. In cancer cells, the tandem activation of the fatty acid biosynthetic enzymes adenosine triphosphate citrate lyase, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) leads to increased synthesis of SFA and their further conversion into MUFA by stearoyl-CoA desaturase (SCD) 1. The roles of adenosine triphosphate citrate lyase, ACC and FAS in the pathogenesis of cancer have been a subject of extensive investigation. However, despite early experimental and epidemiological observations reporting elevated levels of MUFA in cancer cells and tissues, the involvement of SCD1 in the mechanisms of carcinogenesis remains surprisingly understudied. Over the past few years, a more detailed picture of the functional relevance of SCD1 in cell proliferation, survival and transformation to cancer has begun to emerge. The present review addresses the mounting evidence that argues for a key role of SCD1 in the coordination of the intertwined pathways of lipid biosynthesis, energy sensing and the transduction signals that influence mitogenesis and tumorigenesis, as well as the potential value of this enzyme as a target for novel pharmacological approaches in cancer interventions.

Ilie, M., et al. (2018). "Use of the 22C3 anti-programmed death-ligand 1 antibody to determine programmed death-ligand 1 expression in cytology samples obtained from non-small cell lung cancer patients." Cancer Cytopathol **126**(4): 264-274.

BACKGROUND: Pembrolizumab monotherapy is a standard-of-care treatment for the first- and second-line treatment of advanced non-small cell lung cancer with programmed death-ligand 1 (PD-L1) tumor proportion score (TPS) values >/= 50% and >/= 1%, respectively. PD-L1 testing with the PD-L1 immunohistochemistry (IHC) 22C3 pharmDx companion assay has been validated on tumor tissue with the Dako Autostainer Link 48 (ASL48). 22C3 anti-PD-L1 antibody-based laboratory-developed tests (LDTs) compatible with other autostainers and cytology samples are essential to support pembrolizumab treatment decisions across institutions globally. METHODS: ASL48 and BenchMark Ultra LDTs were optimized for the evaluation of cytology samples through comparisons with cell lines with known PD-L1 expression levels (strong, moderate, and negative). The PD-L1 TPS was then evaluated for 70 paired biopsy and cytology samples (bronchial washes, n = 40; pleural effusions, n = 30) with these LDTs. Biopsy and cytology LDT TPS values were also compared with a subset of biopsy samples (n = 37) evaluated with the PD-L1 IHC 22C3 pharmDx assay on the ASL48. RESULTS: Intraclass correlation coefficients of 0.884 to 0.898 were observed for biopsy samples versus cytology samples with the ASL48 and BenchMark Ultra LDTs. Concordance was high, regardless of the TPS cut point (<1% vs >/= 1% and <50% vs >/= 50%), sample type (pleural effusion vs bronchial wash), or tumor histology (adenocarcinoma vs squamous cell carcinoma). Concordance was high for each LDT versus the PD-L1 IHC 22C3 pharmDx assay. CONCLUSIONS: ASL48 and BenchMark Ultra 22C3 antibody concentrate-based LDTs have been validated for PD-L1 testing in cytology samples, and they will support reliable, high-quality PD-L1 testing across regions globally. Cancer Cytopathol 2018;126:264-74. (c) 2018 American Cancer Society.

Imai, D., et al. (2017). "The prognostic impact of programmed cell death ligand 1 and human leukocyte antigen class I in pancreatic cancer." Cancer Med **6**(7): 1614-1626.

Pancreatic ductal adenocarcinoma (PDA) is associated with an immunosuppressive tumor-microenvironment (TME) that supports the growth of tumors and mediates tumors enabling evasion of the immune system. Expression of programmed cell death ligand 1 (PD-L1) and loss of human leukocyte antigen (HLA) class I on tumor cells are methods by which tumors escape immunosurveillance. We examined immune cell infiltration, the expression of PD-L1 and HLA class I by PDA cells, and the correlation between these immunological factors and clinical prognosis. PDA samples from 36 patients were analyzed for HLA class I, HLA-DR, PD-L1, PD-1, CD4, CD8, CD56, CD68, and FoxP3 expression by immunohistochemistry. The correlations between the expression of HLA class I, HLA-DR, PD-L1 or PD-1 and the pattern of tumor infiltrating immune cells or the patients' prognosis were assessed. PD-L1 expression correlated with tumor infiltration by CD68(+) and FoxP3(+) cells. Low HLA class I expression was an only risk factor for poor survival. PD-L1 negative and HLA class I high-expressing PDA was significantly associated with higher numbers of infiltrating CD8(+) T cells in the TME, and a better prognosis. Evaluation of both PD-L1 and HLA class I expression by PDA may be a good predictor of prognosis for patients. HLA class I expression by tumor cells should be evaluated when selecting PDA patients who may be eligible for treatment with PD-1/PD-L1 immune checkpoint blockade therapies.

Isaacs, J. T. (1994). "Advances and controversies in the study of programmed cell death/apoptosis in the development of and therapy for cancer." Curr Opin Oncol **6**(1): 82-89.

Whether normal or malignant, cells possess within their repertoire of epigenetic programs the ability to undergo a process of cellular suicide, termed programmed cell death. This programmed cell death process involves an epigenetic reprogramming of the cell that results in an energy-dependent cascade of biochemical and morphologic changes within the cell (also termed apoptosis), resulting in its death and elimination. Activation of programmed cell death is controlled by a series of endogenous cell-type-specific signals. In addition, various exogenous cell-damaging treatments (eg, radiation, chemicals, and viruses) can activate this pathway if sufficient injury to the cell occurs.

Ishii, H., et al. (2017). "Programmed cell death-ligand 1 expression and immunoscore in stage II and III non-small cell lung cancer patients receiving adjuvant chemotherapy." Oncotarget **8**(37): 61618-61625.

Programmed cell death 1 (PD-1) receptor-ligand interaction is a major pathway that is often hijacked by tumors to suppress immune control. Immunoscore (IS), a combinational index of CD3 and CD8 tumor-infiltrating lymphocyte (TIL) density in the tumor's center and invasive margin, is a new prognostic tool suggested to be superior to conventional tumor-staging methods in various tumors. This retrospective study aimed to investigate the prevalence and prognostic roles of PD-ligand 1 (PD-L1) expression and IS in non-small cell lung cancer (NSCLC) patients receiving adjuvant chemotherapy. PD-L1 expression and TIL density were evaluated by immunohistochemical

analysis in 36 patients with stage II and III NSCLC. Tumors with staining in over 1% of their cells were scored as positive for PD-L1 expression, and we determined the median number of CD3- and CD8-positive TILs as the cutoff point for TIL density. To determine IS, each patient was given a binary score (0 for low and 1 for high) for CD3 and CD8 density in both the tumor center and invasive margin region. PD-L1 expression in tumor cells was observed in 61.1% (22/36) of patients. PD-L1 expression was significantly associated with high IS, and highest IS tended to have a favorable disease-free survival.

Ishii, H., et al. (2015). "Significance of programmed cell death-ligand 1 expression and its association with survival in patients with small cell lung cancer." J Thorac Oncol **10**(3): 426-430.

BACKGROUND: Programmed cell death 1 receptor-ligand interaction is a major pathway often hijacked by tumors to suppress immune control. The aim of this retrospective study was to investigate the prevalence and prognostic roles of programmed cell death -ligand 1 (PD-L1) expression in small cell lung cancer (SCLC). METHODS: The expression of PD-L1 was evaluated by immunohistochemical analysis in 102 specimens of SCLC. Tumors with staining in over 5% of tumor cells were scored as positive for PD-L1 expression. Survival analysis was performed using the Kaplan-Meier method. RESULTS: Expression of PD-L1 in tumor cells was observed in 71.6% (73 of 102) of SCLCs, and was significantly correlated with a limited disease (LD) stage. SCLC patients with PD-L1-positive tumors showed significantly longer overall survival (OS) than those with PD-L1-negative (median OS, 16.3 versus 7.3 months; $p < 0.001$, respectively). Multivariate analyses demonstrated that a good performance status, LD stage, and expression of PD-L1 were significantly predictive of better OS, independently of other factors. We found no relevance between PD-L1 expression and progression-free survival for first-line treatment in LD- and extensive disease-SCLC patients. CONCLUSIONS: In patients with SCLC, expression of PD-L1 is positively correlated with a LD stage, and is independently predictive of a favorable outcome.

Ishizaki, Y., et al. (1995). "Programmed cell death by default in embryonic cells, fibroblasts, and cancer cells." Mol Biol Cell **6**(11): 1443-1458.

We recently proposed that most mammalian cells constitutively express all of the proteins required to undergo programmed cell death (PCD) and undergo PCD unless continuously signaled by other cells not to. Although some cells have been shown to work this way, the vast majority of cell types remain to be tested. Here we tested purified fibroblasts isolated from developing or adult rat sciatic nerve, a mixture of cell types isolated from normal or p53-null mouse embryos, an immortalized rat fibroblast cell line, and a number of cancer cell lines. We found the following: 1) All of these cells undergo PCD when cultured at low cell density in the absence of serum and exogenous signaling molecules but can be rescued by serum or specific growth factors, suggesting that they need extracellular signals to avoid PCD. (2) The mixed cell types dissociated from normal mouse embryos can only support one another's survival in culture if they are in aggregates, suggesting that cell survival in embryos may depend on short-range signals. (3) Some cancer cells secrete factors that support their own survival. (4) The survival requirements of a human leukemia cell line change when the cells differentiate. (5) All of the cells studied can undergo PCD in the presence of cycloheximide, suggesting that they constitutively express all of the protein components required to execute the death program.

Jarry, A., et al. (2004). "Position in cell cycle controls the sensitivity of colon cancer cells to nitric oxide-dependent programmed cell death." Cancer Res **64**(12): 4227-4234.

Mounting evidence suggests that the position in the cell cycle of cells exposed to an oxidative stress could determine their survival or apoptotic cell death. This study aimed at determining whether nitric oxide (NO)-induced cell death in colon cancer cells might depend on their position in the cell cycle, based on a clone of the cancer cell line HT29 exposed to an NO donor, in combination with the manipulation of the cell entry into the cell cycle. We show that PAPA NONOate (pNO), from 10(-4) m to 10(-3) m, exerted early and reversible cytostatic effects through ribonucleotide reductase inhibition, followed by late resumption of cell growth at 5 x 10(-4) m pNO. In contrast, 10(-3) m pNO led to late programmed cell death that was accounted for by the progression of cells into the cell cycle as shown by (a) the accumulation of apoptotic cells in the G (2)-M phase at 10(-3) m pNO treatment; and (b) the prevention of cell death by inhibiting the entry of cells into the cell cycle. The entry of pNO-treated cells into the G (2)-M phase was associated with actin depolymerization and its S-glutathionylation in the same way as in control cells. However, the pNO treatment interfered with the build-up of a high reducing power, associated in control cells with a dramatic increase in reduced glutathione biosynthesis in the G (2)-M phase. This oxidative stress prevented the exit from the G (2)-M phase, which requires a high reducing power for actin deglutathionylation and its repolymerization. Finally, our demonstration that programmed cell death occurred through a caspase-independent pathway is in

line with the context of a nitrosative/oxidative stress. In conclusion, this work, which deciphers the connection between the position of colonic cancer cells in the cell cycle and their sensitivity to NO-induced stress and their programmed cell death, could help optimize anticancer protocols based on NO-donating compounds.

Jiang, X. M., et al. (2017). "Osimertinib (AZD9291) decreases programmed death ligand-1 in EGFR-mutated non-small cell lung cancer cells." Acta Pharmacol Sin **38**(11): 1512-1520.

Osimertinib (AZD9291) is a third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) that has been approved for the treatment of EGFR-mutated non-small cell lung cancer (NSCLC). In NSCLC patients, an EGFR mutation is likely to be correlated with high levels of expression of programmed death ligand-1 (PD-L1). Here, we showed that osimertinib decreased PD-L1 expression in human EGFR mutant NSCLC cells in vitro. Osimertinib (125 nmol/L) markedly suppressed PD-L1 mRNA expression in both NCI-H1975 and HCC827 cells. Pretreatment with the N-linked glycosylation inhibitor tunicamycin, osimertinib clearly decreased the production of new PD-L1 protein probably due to a reduction in mRNA. After blocking transcription and translation processes with actinomycin D and cycloheximide, respectively, osimertinib continued to reduce the expression of PD-L1, demonstrating that osimertinib might degrade PD-L1 at the post-translational level, which was confirmed by a cycloheximide chase assay, revealing that osimertinib (125 nmol/L) decreased the half-life of PD-L1 from approximately 17.8 h and 13.8 h to 8.6 h and 4.6 h, respectively, in NCI-H1975 and HCC827 cells. Pretreatment with the proteasome inhibitors (MG-132 or bortezomib) blocked the osimertinib-induced degradation of PD-L1, but an inhibitor of autophagy (chloroquine) did not. In addition, inhibition of GSK3beta by LiCl prevented osimertinib-induced PD-L1 degradation. The results demonstrate that osimertinib reduces PD-L1 mRNA expression and induces its protein degradation, suggesting that osimertinib may reactivate the immune activity of T cells in the tumor microenvironment in EGFR-mutated NSCLC patients.

Jin, J., et al. (2018). "Elevated serum soluble programmed cell death ligand 1 concentration as a potential marker for poor prognosis in small cell lung cancer patients with chemotherapy." Respir Res **19**(1): 197.

BACKGROUND: Potential relationship between serum soluble programmed cell death ligand 1 and prognosis of small cell lung cancer is not well explored. The aim of the study was to reveal the prognostic significance of serum soluble programmed cell death ligand 1 in patients with small cell lung cancer. METHODS: A total of 250 small cell lung cancer patients and 250 controls were included. Research information was obtained from their medical records. Blood samples were collected on admission. Serum concentration of programmed cell death ligand 1 was measured using Enzyme-Linked Immunosorbent Assay. The patients underwent cisplatin-etoposide chemotherapy with a maximum of six cycles. Subsequently, they were followed-up for 12 months, and therapeutic response and cancer death were recorded. RESULTS: Serum concentration of programmed cell death ligand 1 was higher in the patients than in the controls on admission ($P < 0.001$). After chemotherapy, 112 patients had no response to this therapy. In the 12-month follow up period, 118 patients died due to this cancer. Multivariate Cox regression model revealed that the higher serum concentration of programmed cell death ligand 1 on admission was associated with the higher risk of no response to chemotherapy or cancer caused death (HR: 1.40, 95% CI: 1.05 ~ 1.87; HR: 1.43, 95% CI: 1.08 ~ 1.87). CONCLUSION: Elevated serum concentration of soluble programmed cell death ligand 1 might be an independent risk factor for non-response to chemotherapy and cancer caused death in small cell lung cancer patients.

Johar, D., et al. (2004). "Inflammatory response, reactive oxygen species, programmed (necrotic-like and apoptotic) cell death and cancer." Rocz Akad Med Bialymst **49**: 31-39.

In this short review we attempt to establish and/or strengthen connections between clinical, inflammatory manifestation of cancer, inflammatory processes driven by lipoxy-metabolites and their contribution to immortalized phenotype and apoptosis inhibition. Particularly the resemblance between symptoms of inflammation and signs associated with cancer chemotherapy and/or cytokine therapy is illustrated. In this context the role of apoptosis and necrosis in inflammation as well as the role of RedOx processes and lipid-oxidizing enzymes particularly cyclooxygenase-2 (COX-2) and also to lesser extend the 5-lipooxygenase (5-LOX) is highlighted. The multitude of biological effects of reactive oxygen species is shortly summarized and some aspects of it are being discussed in greater detail. Apoptotic cell death is discussed in the context of the "resolve-phase" of an inflammatory response. The disturbance of apoptosis is mainly deliberated in the framework of insufficient removal of immuno-effector cells that may cause autoimmunity. The role of COX-2 in apoptosis resistance is being highlighted mainly in the context of

malignant transformation. The mechanism of cell death (apoptotic or necrotic) and its influence on the immune system and potential benefits of necrotic cell death induction during cancer chemotherapy is indicated.

Khan, I., et al. (2018). "Andrographolide Exhibits Anticancer Potential Against Human Colon Cancer Cells by Inducing Cell Cycle Arrest and Programmed Cell Death via Augmentation of Intracellular Reactive Oxygen Species Level." Nutr Cancer **70**(5): 787-803.

Andrographolide, a diterpenoid lactone and a major constituent of Andrographis paniculata Nees, exhibits remarkable anticancer activity. However, the effect of andrographolide on colon cancer has not been completely elucidated yet. Thus, we investigated the chemopreventive potential of andrographolide in colon cancer HT-29 cells. The cytotoxic potential of andrographolide on HT-29 cells was determined by MTT assay, trypan blue exclusion assay, colony formation assay, and morphological analysis; and apoptotic property by DAPI and Hoechst staining, FITC-Annexin V assay, DNA fragmentation assay and caspase-3 activity assay. To elucidate andrographolide action, intracellular reactive oxygen species (ROS) level was determined by DCFDA dye; change in mitochondrial potential by Rhodamine123 and Mito Tracker Red CMXRos dye; and cell cycle modulatory property by flow cytometric analysis. Results of the study have shown that andrographolide decreased cell viability of HT-29 cells in a dose- and time-dependent manner. Furthermore, andrographolide induced apoptosis in HT-29 cells which seemed to be linked with augmented intracellular ROS level and disruption of mitochondrial membrane potential. Interestingly, andrographolide caused significant cell cycle arrest in G2/M phase at lower doses, but, in G0/G1 phase at higher doses. In summary, our results indicated that andrographolide exhibited antiproliferative and apoptotic properties against colon cancer HT-29 cells.

Khunger, M., et al. (2017). "Incidence of Pneumonitis With Use of Programmed Death 1 and Programmed Death-Ligand 1 Inhibitors in Non-Small Cell Lung Cancer: A Systematic Review and Meta-Analysis of Trials." Chest **152**(2): 271-281.

BACKGROUND: Programmed death 1 (PD-1) programmed death-ligand 1 (PD-L1) inhibitors show significant clinical activity in non-small cell lung carcinoma (NSCLC). However, they are often associated with potentially fatal immune-mediated pneumonitis. Preliminary reports of trials suggest a difference in the rate of pneumonitis with PD-1 and PD-L1 inhibitors. We sought to determine the overall incidence of pneumonitis and differences according to type of inhibitors and prior chemotherapy use. METHODS: MEDLINE, Embase, and Scopus databases were searched up to November 2016. Rates of pneumonitis of any grade and grade >/= 3 from all clinical trials investigating nivolumab, pembrolizumab, atezolizumab, durvalumab, and avelumab as single agents in NSCLC were collected. The incidence of pneumonitis across trials was calculated using DerSimonian-Laird random effects models. We compared incidences between PD-1 and PD-L1 inhibitors and between treatment naive and previously treated patients. RESULTS: Nineteen trials (12 with PD-1 inhibitors [n = 3,232] and 7 with PD-L1 inhibitors [n = 1,806]) were identified. PD-1 inhibitors were found to have statistically significant higher incidence of any grade pneumonitis compared with PD-L1 inhibitors (3.6%; 95% CI, 2.4%-4.9% vs 1.3%; 95% CI, 0.8%-1.9%, respectively; P =.001). PD-1 inhibitors were also associated with higher incidence of grade 3 or 4 pneumonitis (1.1%; 95% CI, 0.6%-1.7% vs 0.4%; 95% CI, 0%-0.8%; P =.02). Treatment naive patients had higher incidence of grade 1 through 4 pneumonitis compared with previously treated patients (4.3%; 95% CI, 2.4%-6.3% vs 2.8%; 95% CI, 1.7%-4%; P =.03). CONCLUSIONS: There was a higher incidence of pneumonitis with use of PD-1 inhibitors compared with PD-L1 inhibitors. Higher rate of pneumonitis was more common in treatment naive patients.

Kim, A., et al. (2017). "Programmed death-ligand 1 (PD-L1) expression in tumour cell and tumour infiltrating lymphocytes of HER2-positive breast cancer and its prognostic value." Sci Rep **7**(1): 11671.

Immunotherapy targeting PD-1/PD-L1 axis showed benefits in cancer. Prognostic significance of tumour infiltrating lymphocytes (TILs) has been determined. We evaluated PD-L1 protein expression in tumour cells and TILs, PD-L1 mRNA level and various histopathologic factors including TILs using 167 formalin-fixed paraffin embedded tissues and 39 fresh tissue of HER2-positive breast cancer. TILs level and PD-L1 expression in tumour cells and TILs were significantly correlated one another. PD-L1 positivity in tumour cells was associated with high histologic grade and high TILs level ($p < 0.001$, both). High PD-L1 immunoscore in TILs and high total immunoscore (in tumour cells and TILs) of PD-L1 were correlated with high histologic grade ($p = 0.001$ and $p < 0.001$, respectively), absence of lymphovascular invasion ($p = 0.012$ and $p = 0.007$, respectively), negative hormone receptor expression ($p = 0.044$ and $p = 0.001$, respectively) and high TILs level ($p < 0.001$, both). High PD-L1 mRNA expression was associated with high TILs level ($p < 0.001$, both). PD-L1 positivity in tumour cells was associated with better disease-free survival in HR-/HER2+ breast cancer ($p = 0.039$). PD-

L1 expression in tumour cells and TILs are significantly associated with TILs level in HER2-positive breast cancer. PD-L1 expression in tumour cells might be positive prognostic factor in HR-/HER2+ breast cancers.

Kim, H., et al. (2018). "Clinicopathological analysis and prognostic significance of programmed cell death-ligand 1 protein and mRNA expression in non-small cell lung cancer." PLoS One **13**(6): e0198634.

In this study, we present the clinicopathological features associated with PD-L1 protein and mRNA expression in a large Asian cohort of patients with non-small cell lung cancer (NSCLC) and assessed the prognostic implications of PD-L1 expression, particularly in early stage NSCLC. We retrospectively analyzed 687 NSCLC specimens (476 adenocarcinoma and 211 squamous cell carcinoma) using tissue microarray. PD-L1 immunohistochemistry (IHC) was performed using Dako 22C3 pharmDx assay and PDL1 mRNA was measured using RNA in situ hybridization (RISH). The overall prevalence of PD-L1 protein expression was 25.2% in tumor cells and PDL1 mRNA expression was 11.9%. There was a strong positive correlation between PD-L1 IHC and RISH results (Spearman's rho = 0.6, $p<0.001$). In adenocarcinoma, PD-L1 protein and mRNA expressions significantly correlated with poorly differentiated histologic subtype ($p<0.001$ and $p = 0.002$, respectively). PD-L1 expression was also associated with genetic alteration in adenocarcinoma. High PD-L1 expression level was associated with EGFR-naive and KRAS-mutant subgroup ($p = 0.001$ and $p = 0.017$, respectively). With a 1% cut-off value, PD-L1 protein expression showed a short overall survival duration in early stage adenocarcinoma with marginal significance ($p = 0.05$, Hazard ratio = 1.947). Our study revealed that PD-L1 expression varied with histologic subtype and genomic alteration status in lung adenocarcinoma, and activation of the PD-L1 pathway may be a poor prognostic factor especially in early stage lung adenocarcinoma. In addition, PDL1 RISH showed promising results in predicting PD-L1 protein expression in NSCLC.

Kim, H. R., et al. (2017). "Concordance of programmed death-ligand 1 expression between primary and metastatic non-small cell lung cancer by immunohistochemistry and RNA in situ hybridization." Oncotarget **8**(50): 87234-87243.

We investigated the concordance of programmed death-ligand 1 (PD-L1) expression between primary cancer at initial diagnosis and metastasis at recurrence in resected non-small cell lung cancer (NSCLC). PD-L1 expression was evaluated using the SP142 assay in 37 NSCLC patients with paired primary lung cancer and surgically resected metastases at recurrence. PD-L1 positivity was defined as immunohistochemistry (IHC) and also evaluated by RNA in situ hybridization (RISH). The concordance rate of PD-L1 between primaries and metastases and correlation with clinicopathological factors were analyzed. PD-L1 expression was higher in squamous cell carcinoma, wild-type EGFR, and smokers than in non-squamous carcinoma, mutant EGFR, and never smokers, respectively. PD-L1 positivity was observed in 18.9% of primaries and 21.6% of metastases. IHC demonstrated 78.4% concordance of PD-L1 positivity between primary and metastatic cancers. In 10.8% of cases, PD-L1 positivity was higher in primaries than in metastases, and vice versa in the remaining 10.8%. By PD-L1 RISH, 35.1% of primaries and 27.0% of metastases demonstrated PD-L1 positivity. There was 62.2% concordance in PD-L1 by RISH between the primaries and metastases. Our results thus highlight the clinical importance of replacing metastases with primary archival tissue, particularly when re-biopsy is difficult at recurrence.

Kim, H. S., et al. (2018). "Expression of programmed cell death ligand 1 and immune checkpoint markers in residual tumors after neoadjuvant chemotherapy for advanced high-grade serous ovarian cancer." Gynecol Oncol.

OBJECTIVE: To investigate the prognostic value of the expressions of programmed cell death ligand 1 (PD-L1) and immune checkpoint markers in residual tumors after neoadjuvant chemotherapy (NAC) for advanced high-grade serous ovarian cancer (HGSOC). METHODS: We collected pre- and post-NAC tumor samples from patients with advanced HGSOC between 2006 and 2017. Post-NAC tumor tissue samples were available for immunostaining from 131 patients. The expressions of PD-L1 and immune checkpoint markers were assessed by immunohistochemical staining and the status of tumor-infiltrating lymphocytes (TILs) was also evaluated. We examined whether there are significant associations between protein expression status and patient outcomes and whether significant changes in protein expression levels occurred in response to NAC. RESULTS: PD-L1 expression in the tumor cells was evaluated in 113 patients, 12 (10.6%) of whom had high PD-L1 expression (>/=25%) in post-NAC tissues. However, these high levels were not associated with progression-free survival (PFS; P=0.348) or overall survival (OS; P=0.699). Similarly, high stromal TILs [>/=50%; n=16 (15.0%)] among the 107 patients evaluated did not show any significant impact on PFS (P=0.250) or OS (P=0.800). Moreover, an abundance of TILs (intraepithelial, CD8+, and Foxp3+) and the expression of immune checkpoint

markers (PD-1, ICOS, and LAG-3) in residual tumors did not confer any significant survival benefit. The impact of NAC on PD-L1 expression and stromal TILs varied considerably among individual patients. CONCLUSION: Although the expression of PD-L1 and immune checkpoint markers in residual tumors after NAC had no prognostic impact on survival in patients with HGSOC, post-NAC evaluation of these proteins in chemoresistant tumors may help select patients for immunotherapy trials.

Kim, J., et al. (2018). "Prognostic implication of programmed cell death 1 protein and its ligand expressions in endometrial cancer." Gynecol Oncol **149**(2): 381-387.

OBJECTIVE: Monoclonal antibodies targeting programmed cell death-1 (PD-1)/programmed death ligand 1 (PD-L1) demonstrated promising clinical response. The predictive/prognostic value of PD-1/PD-L1 immunohistochemistry (IHC) has been evaluated in many cancer types. However, the prognostic value of PD-1/PD-L1 IHC has not been evaluated in endometrial cancer. METHODS: We conducted a retrospective study to quantify the IHC CD8, PD-1, and PD-L1 expressions in immune cells at center of tumor (CT), invasive margin (IM), and/or tumor cell in 183 primary endometrial cancer samples from a single cohort, followed by their reciprocal combinations, including compartmental differences, and correlated them with overall survival (OS) and progression-free survival (PFS). RESULTS: In repeated Cox multivariable models adjusted by clinicoimmunopathologic factors, high CT-PD-L1 was an independent adverse prognostic factor for PFS in all patients and in the microsatellite-stable subgroup. Immune marker ratios revealed independently shorter PFS for high CT-PD-L1/CT-CD8 and CT-PD-L1/CT-PD-1 ratios. Classification of endometrial cancer into four groups based on CT-CD8 and CT-PD-L1 revealed significantly different survival among groups. CONCLUSIONS: The high PD-L1/CD8 ratio and the high expression of PD-L1 on immune cells were independent poor prognostic factors for PFS in endometrial cancer, providing insights into the tumor microenvironment.

Kim, J. H., et al. (2012). "Suppression of tumor growth in H-ras12V liver cancer mice by delivery of programmed cell death protein 4 using galactosylated poly (ethylene glycol)-chitosan-graft-spermine." Biomaterials **33**(6): 1894-1902.

Non-viral gene delivery systems based on polyethyleneimine (PEI) are efficient due to their proton-sponge effect within endosomes, but they have poor physical characteristics such as slow dissociation, cytotoxicity, and non targeted gene delivery. To overcome many of the problems associated with PEI, we synthesized a galactosylated poly (ethylene glycol)-chitosan-graft-spermine (GPCS) copolymer with low cytotoxicity and optimal gene delivery to hepatocytes using an amide bond between galactosylated poly (ethylene glycol) and chitosan-graft-spermine. The GPCS copolymer formed complexes with plasmid DNA, and the GPCS/DNA complexes had well-formed spherical shapes. The GPCS/DNA complexes were nanoscale size with homogenous size distribution and a positive zeta potential by dynamic light scattering (DLS). The GPCS copolymer had lower cytotoxicity than that of PEI 25K in HepG2, HeLa, and A549 cell lines at various concentrations and showed good hepatocyte-targeting ability. Furthermore, GPCS/DNA complexes showed higher levels of GFP expression in the liver in model mice after intravenous injection than naked DNA and metoxy-poly (ethylene glycol)-chitosan-graft-spermine as controls without remarkable fibrosis, inflammation, lipidosis, or necrosis. In a tumor suppression study, an intravenous injection of the GPCS/Pdcd4 complexes significantly suppressed tumor growth, activated apoptosis, and suppressed proliferation and angiogenesis in liver tumor-bearing H-ras12V mice. Our results indicate that the GPCS copolymer has potential as a hepatocyte-targeting gene carrier.

Kim, T. H., et al. (2017). "Effects of 1alpha, 25-dihydroxyvitamin D3 on programmed cell death of Ishikawa endometrial cancer cells through ezrin phosphorylation." J Obstet Gynaecol **37**(4): 503-509.

This study investigated the effects of 1alpha, 25-dihydroxyvitamin D3-induced cell death and its underlying molecular mechanisms in Ishikawa endometrial carcinoma cells. The effects of 1alpha, 25-dihydroxyvitamin D3 on Ishikawa cells were examined by 3-[4,5-dimethylthiazol-2-yl]-2.5-diphenyl-tetrazolium bromide, thiazolyl blue (MTT) assay. 1alpha, 25-dihydroxyvitamin D3 was shown to induce programmed cell death in Ishikawa endometrial carcinoma cells by activation of caspase-3 and caspase-9, along with elevation of Bcl-2 and Bcl-xL. Cell viability was reduced by 1alpha, 25-dihydroxyvitamin D3 in a concentration-dependent manner up to 2.5 muM. In addition, ezrin phosphorylation increased with the 1alpha, 25-dihydroxyvitamin D3 concentration (0-0.5 muM). The protein level of caspase-9 was increased by 1alpha, 25-dihydroxyvitamin D3 up to 0.5 muM. This is the first report regarding the efficacy and molecular mechanisms underlying the effects of 1alpha, 25-dihydroxyvitamin D3 in endometrial cancer cells. Our findings indicate that 1alpha, 25-dihydroxyvitamin D3 induces endometrial cancer cell death in a

concentration-dependent manner. Impact statement Up to date, there is no report about the efficacy and molecular underlying mechanisms on the effect of vitamin D3 in endometrial cancer cells. Our findings indicate that 1alpha, 25-dihydroxyvitamin D3. which is an active metabolite of vitamin D3, induces Ishikawa endometrial cancer cell death in a concentration-dependent manner by activation of caspase-3 and -9, along with elevation of Bcl-2 and Bcl-xL. In addition, the same concentration of 1alpha, 25-dihydroxyvitamin D3 that provoked apoptotic signals caused phosphorylation of ezrin at threonine 567 in a VDR-dependent manner. This study suggests that 1alpha, 25-dihydroxyvitamin D3 within the optimal range (0.5 uM) would induce apoptosis through Fas-ezrin-caspase-3, -8, -9 signalling axis which may be a critical cell death regulator in Ishikawa endometrial cancer cell. Further study will be more interesting to address molecular connections or prove this critical optimal concentration range of vitamin D.

Kim, Y. K., et al. (2014). "Aerosol delivery of programmed cell death protein 4 using polysorbitol-based gene delivery system for lung cancer therapy." J Drug Target **22**(9): 829-838.

The development of a safe and effective gene delivery system is the most challenging obstacle to the broad application of gene therapy in the clinic. In this study, we report the development of a polysorbitol-based gene delivery system as an alternative gene carrier for lung cancer therapy. The copolymer was prepared by a Michael addition reaction between sorbitol diacrylate (SD) and spermine (SPE); the SD-SPE copolymer effectively condenses with DNA on the nanoscale and protects it from nucleases. SD-SPE/DNA complexes showed excellent transfection with low toxicity both in vitro and in vivo, and aerosol delivery of SD-SPE complexes with programmed cell death protein 4 DNA significantly suppressed lung tumorigenesis in K-ras (LA1) lung cancer model mice. These results demonstrate that SD-SPE has great potential as a gene delivery system based on its excellent biocompatibility and high gene delivery efficiency for lung cancer gene therapy.

Kitazono, S., et al. (2015). "Reliability of Small Biopsy Samples Compared With Resected Specimens for the Determination of Programmed Death-Ligand 1 Expression in Non--Small-Cell Lung Cancer." Clin Lung Cancer **16**(5): 385-390.

BACKGROUND: Several studies have assessed the expression of programmed death-ligand 1 (PD-L1) in resected surgical specimens of non-small-cell lung cancer (NSCLC). However, the expression of PD-L1 in smaller biopsy samples of advanced NSCLC has not been reported. PATIENTS AND METHODS: A total of 79 patients with NSCLC at our institution with available biopsy samples and resected specimens were retrospectively enrolled in the present study. PD-L1 expression was assessed by immunohistochemistry and scored using the hybrid scoring method. The concordance rates for the expression of PD-L1 between the 2 samples were analyzed. RESULTS: The pathologic stage of the patients (51 men, 28 women; median age, 68 years) was stage I in 37, stage II in 18, and stage III in 24. The diagnostic procedures included transbronchial biopsy in 59, transbronchial needle aspiration biopsy in 14, and computed tomography (CT)-guided needle biopsy in 6. The positivity rate of PD-L1 in these samples was 38.0% (27 transbronchial biopsies, 6 transbronchial needle aspiration biopsies, 3 CT-guided needle biopsies) versus 35.4% in the resected specimens. The median hybrid score was 0 (range, 0-170), and the mean score was 28.7 +/- 43.4. Comparing the biopsy samples and resected specimens with a score of >/= 1 as positive for PD-L1 staining, 6 tumors were discordant for PD-L1 expression and 73 were concordant, for a concordance rate of 92.4% and kappa value of 0.8366. CONCLUSION: PD-L1 status showed good concordance between the biopsy samples and resected specimens. These small samples, even those derived from transbronchial needle aspiration biopsies, appear adequate for the assessment of PD-L1 expression.

Kolacinska, A., et al. (2015). "Immune checkpoints: Cytotoxic T-lymphocyte antigen 4 and programmed cell death protein 1 in breast cancer surgery." Oncol Lett **10**(2): 1079-1086.

Immune checkpoints refer to a plethora of inhibitory pathways built into the immune system, and recent studies have emphasized the role of these checkpoints in carcinogenesis. The aim of the present study was to evaluate two major immune checkpoints, the cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1), in the serum of 35 patients with stage I and II breast cancer. Serum concentrations of CTLA-4 and PD-1 were measured at three time points: i) Preoperatively; ii) during anesthesia following the harvesting of sentinel nodes (SNs); and iii) 24 h postoperatively. Control samples were obtained from 25 healthy, age-matched females. Assessment of CTLA-4 and PD-1 expression levels was conducted using flow cytometry. A statistically significant difference in PD-1 expression was identified between breast cancer patients preoperatively and healthy controls (26.31+/-11.87 vs. 12.72+/-8.15; P<0.0001). In addition, a statistically significant association was found between CTLA-4 and PD-1 levels prior to surgery (P=0.0084). In addition, CTLA-4 expression was associated with age

(P=0.0453), with elevated levels of CTLA-4 detected in older breast cancer patients. Higher PD-1 expression levels were observed in T2 tumors compared with T1 tumors prior to surgery and intraoperatively; however, the differences were not statistically significant. Furthermore, a decrease in PD-1 levels was observed subsequent to harvesting SNs with metastasis, but not in SN-negative patients (P=0.05). A negative correlation was also observed between PD-1 expression and progesterone receptor (PR) status following surgery (P=0.024). These results provided a basis for further investigation of immune checkpoints in breast cancer. Breast cancer patients exhibit an altered profile of immune checkpoint markers, with higher concentrations of PD-1 observed in larger, PR-negative tumors.

Koty, P. P., et al. (1999). "Antisense bcl-2 treatment increases programmed cell death in non-small cell lung cancer cell lines." Lung Cancer **23**(2): 115-127.

Programmed cell death (PCD) is a genetically regulated pathway that is altered in many cancers. This process is, in part, regulated by the ratio of PCD inducers (Bax) or inhibitors (Bcl-2). An abnormally high ratio of Bcl-2 to Bax prevents PCD, thus contributing to resistance to chemotherapeutic agents, many of which are capable of inducing PCD. Non-small cell lung cancer (NSCLC) cells demonstrate resistance to these PCD-inducing agents. If Bcl-2 prevents NSCLC cells from entering the PCD pathway, then reducing the amount of endogenous Bcl-2 product may allow these cells to spontaneously enter the PCD pathway. Our purpose was to determine the effects of bcl-2 antisense treatment on the levels of programmed cell death in NSCLC cells. First, we determined whether bcl-2 and bax mRNA were expressed in three morphologically distinct NSCLC cell lines: NCI-H226 (squamous), NCI-H358 (adenocarcinoma), and NCI-H596 (adenosquamous). Cells were then exposed to synthetic antisense bcl-2 oligonucleotide treatment, after which programmed cell death was determined, as evidenced by DNA fragmentation. Bcl-2 protein expression was detected immunohistochemically. All three NSCLC cell lines expressed both bcl-2 and bax mRNA and had functional PCD pathways. Synthetic antisense bcl-2 oligonucleotide treatment resulted in decreased Bcl-2 levels, reduced cell proliferation, decreased cell viability, and increased levels of spontaneous PCD. This represents the first evidence that decreasing Bcl-2 in three morphologically distinct NSCLC cell lines allows the cells to spontaneously enter a PCD pathway. It also indicates the potential therapeutic use of antisense bcl-2 in the treatment of NSCLC.

Kurozumi, S., et al. (2017). "Significance of evaluating tumor-infiltrating lymphocytes (TILs) and programmed cell death-ligand 1 (PD-L1) expression in breast cancer." Med Mol Morphol **50**(4): 185-194.

The immune system affects all phases of tumor growth from initiation to progression and dissemination. Tumor-infiltrating lymphocytes (TILs) are mononuclear immune cells that infiltrate tumor tissue. Several retrospective studies have suggested the potential of TILs as a prognostic as well as predictive factor of chemotherapy in some breast cancers. On the other hand, programmed cell death protein-1 (PD-1) and programmed cell death-ligand 1 (PD-L1) eliminate T cell activation in various types of cancers. Prospective trials to evaluate the efficacy of antibody agents to PD-1 and PD-L1 are ongoing in patients with breast cancer. The findings of these studies appear to support the potential of immune checkpoint inhibitors targeting the PD-1/PD-L1 axis in triple negative breast cancer. Further studies are needed in order to confirm previous findings on TILs and promote the development of new immune therapy approaches for breast cancer patients. Furthermore, the search for TILs will soon be introduced into actual clinical practice, for which the standardization of evaluation methods and establishment of a simple evaluation method are expected.

Kyprianou, N., et al. (1991). "Programmed cell death during regression of the MCF-7 human breast cancer following estrogen ablation." Cancer Res **51**(1): 162-166.

To study the mechanism of regression of human mammary cancer following estrogen ablation, estrogen-responsive MCF-7 human mammary adenocarcinoma cells were inoculated into ovariectomized female nude mice supplemented with exogenous 17 beta-estradiol (E2) via an E2 implant. Implants were then removed when MCF-7 tumors were 400 mm3 in size. Removal of the E2 implants resulted in a 50% tumor regression by 2 weeks following E2 ablation. Associated with this regression is a rapid (i.e., within 1 day following E2 ablation) enhanced expression of the transforming growth factor beta 1 and TRPM-2-genes, two genes the expression of which has been previously demonstrated to be enhanced in a variety of cell types induced to undergo programmed cell death (i.e., apoptosis). The enhanced expression of transforming growth factor beta 1 and TRPM-2 is not a nonspecific response since the expression of other genes, like c-fos, c-H-ras, and pS2, decrease following E2 ablation. Fragmentation of tumor DNA into nucleosomal oligomers and histological appearance of apoptotic bodies are characteristic early events that precede the dramatic reduction in tumor volume following E2 ablation.

These results demonstrate that the regression of MCF-7 human mammary cancers in nude mice following estrogen ablation is due to a sequence of biochemical and morphological changes that result in both the cessation of cell proliferation and activation of programmed death or apoptosis of these MCF-7 cancer cells. Clarification of the biochemical pathway involved in the activation of this programmed cell death should identify new targets of therapy for even estrogen-independent human mammary cancer cells.

Kyprianou, N., et al. (1990). "Programmed cell death during regression of PC-82 human prostate cancer following androgen ablation." Cancer Res **50**(12): 3748-3753.

To study the mechanism of regression of human prostatic cancer following androgen ablation, the androgen-responsive PC-82 human prostatic adenocarcinoma xenograft was used as a model system. Castration of male nude mice bearing PC-82 xenografts results in a 50% tumor regression by 2 wk following androgen ablation. This regression is due to a sequence of biochemical and morphological events that results in both the cessation of cell proliferation and activation of programmed death or apoptosis of the androgen-dependent prostatic cancer cells. Associated with this response are an enhanced expression of the transforming growth factor beta 1 gene, a potent inhibitor of cell proliferation, and testosterone-repressed prostatic message 2 (designated TRPM-2), a programmed cell death-associated gene. Fragmentation of tumor DNA into nucleosomal oligomers and histological appearance of apoptotic bodies are characteristic early events that preceded the dramatic reduction in tumor volume following androgen ablation. These results suggest that androgen-dependent human prostatic cancer cells, like normal prostatic cells, retain the ability to inhibit proliferation and to activate programmed cell death in response to androgen ablation. Clarification of the biochemical pathway involved in the activation of this programmed cell death should identify new targets of therapy for even androgen-independent human prostatic cancer.

Kyprianou, N., et al. (1991). "Programmed cell death as a new target for prostatic cancer therapy." Cancer Surv **11**: 265-277.

To increase survival of men with metastatic prostatic cancer, a modality that can effectively eliminate androgen independent cancer cells is desperately needed. By combining such an effective modality with androgen ablation, all of the heterogeneous populations of tumour cells within a prostatic cancer patient can be affected, thus optimizing the chances of cure. Unfortunately, such effective therapy for the androgen independent prostatic cancer cell is not yet available. This therapy will probably require two types of agents, one having antiproliferative activity affecting the small number of dividing androgen independent cells, and the other able to increase the low rate of cell death among the majority of non-proliferating (ie interphase) androgen independent prostatic cancer cells present. Androgen dependent prostatic epithelial cells can be made to undergo programmed death by means of androgen ablation, even if the cells are not in the proliferative cell cycle. Androgen independent prostatic cancer cells retain the major portion of this programmed cell death pathway, only there is a defect in the pathway such that it is no longer activated by androgen ablation. If the intracellular free Ca2+ is sustained at an elevated level for a sufficient time, androgen independent cells can be induced to undergo programmed death. The long term goal is therefore to develop some type of non-androgen ablative method that can be used in vivo to induce a sustained elevation in Ca2+ in androgen independent prostatic cancer cells. To accomplish this task, a more complete understanding of the biochemical pathways involved in programmed cell death is urgently needed. At present, studies are focusing on the mechanism involved in the Ca2+ elevation in the normal and malignant androgen dependent cell induced following androgen ablation and the role of the TRPM-2 protein in this process.

Lokshin, A., et al. (1995). "Mechanism of interferon beta-induced squamous differentiation and programmed cell death in human non-small-cell lung cancer cell lines." J Natl Cancer Inst **87**(3): 206-212.

BACKGROUND: Non-small-cell lung cancer (NSCLC) is one of the leading causes of cancer-related mortality due largely to the failure of systemic chemotherapy. Thus, new therapeutic paradigms involving the manipulation of normal physiologic growth-regulatory mechanisms, such as terminal cellular differentiation or programmed cell death, are being explored. Interferons may function as antineoplastic agents, in part because of their effects on cell proliferation and differentiation. We have previously demonstrated the antiproliferative and differentiating effects of interferon beta (IFN beta). PURPOSE: The present investigation was designed to study the mechanism of IFN beta on squamous differentiation and/or programmed cell death in cultured NSCLC cells. METHODS: Cross-linked envelope competence and transglutaminase expression and activity were measured in three NSCLC cell lines (NCI-H226, NCI-H358, and NCI-H596) as common markers for squamous differentiation and programmed cell death. DNA fragmentation, as determined by gel electrophoretic analysis, served as a marker for

programmed cell death. In addition, the expression of several regulatory and differentiation-related genes (measured by Northern blot analysis of messenger RNA levels) as well as protein kinase C activity was measured to begin to explore possible mechanisms of IFN beta activity. RESULTS: IFN beta-induced cross-linked envelope competence occurred in cell lines with squamous features (NCI-H226 and NCI-H596); conversely, DNA fragmentation occurred in cell lines with glandular features (NCI-H358 and NCI-H596). Stimulation of cross-linked envelope competence by IFN beta was associated with the induction of tissue transglutaminase activity. Both of these parameters were protein-synthesis independent. As previously observed for NCI-H596, IFN beta suppressed the growth of the other two cell lines. Total protein kinase C activity was not altered. Expression of a variety of possibly relevant oncogenes and other genes was variably altered by IFN beta. CONCLUSIONS: IFN beta induces programmed cell death in NSCLC cell lines in a phenotype-specific manner. The programmed cell death pathway represented by cross-linked envelope competence is dependent on the expression of the squamous phenotype and is protein-synthesis independent, suggesting post-translational mechanisms. In addition, squamous differentiation itself may be induced. Changes in gene expression, while not necessary for induction of cross-linked envelope competence, may be involved in other aspects of cellular homeostasis, such as growth suppression. IMPLICATIONS: By inducing terminal cellular differentiation or programmed cell death, IFN beta may be therapeutically useful in NSCLC. The post-translational nature of IFN beta-induced effects suggests that it will be best used in combination with other agents that can regulate these cellular pathways at the pretranslational level, increasing the proportion of cells capable of being driven to a terminal state by this biotherapeutic agent.

Ma, G., et al. (2018). "The prognostic role of programmed cell death-ligand 1 expression in non-small cell lung cancer patients: An updated meta-analysis." Clin Chim Acta **482**: 101-107.

BACKGROUND: Programmed cell death-ligand 1 (PD-L1) seemed to be associated with the outcomes of non-small cell lung cancer. However the prognostic role of PD-L1 expression among NSCLC remained unclear and inconsistent. The aim of the study set out to evaluate the correlation between PD-L1 expression and the prognosis of patients that developed NSCLC. METHODS: Identified literatures were extracted of various electronic databases and a meta-analysis was performed to evaluate the prognostic role of PD-L1 among NSCLC patients. RESULTS: Totally 25 studies from 11 countries containing 5861 patients were included in the meta-analysis. The pooled hazard ratios (HRs) for overall survival (OS) and progression-free survival (PFS) were 1.176 (95% CI: 1.016-1.361, P=0.029) and 1.170 (95% CI: 0.984-1.392, P=0.076), respectively. High PD-L1 expression on NSCLC tissue was also related with worse OS among Asian patients (HR=1.381, 95% CI: 1.127-1.629, P=0.002), adenocarcinomas (HR=1.899, 95% CI: 1.306-2.762, P=0.001) and poor PFS in non-Asian patients (HR=1.695, 95% CI: 1.158-2.480, P=0.002). Sensitivity analysis indicated that removal of any particular included literature won't affect the pooled results. Publication bias among the studies was not significant neither. CONCLUSIONS: PD-L1 expression is a prognostic factor related with poor survival among patients that developed NSCLC.

Ma, G., et al. (2005). "[Expression of programmed cell death 4 and its clinicopathological significance in human pancreatic cancer]." Zhongguo Yi Xue Ke Xue Yuan Xue Bao **27**(5): 597-600.

OBJECTIVE: To investigate the expression of programmed cell death 4 (PDCD4) protein and its clinicopathological significance in human pancreatic cancer. METHODS: Immunohistochemistry was used to examine the expression of PDCD4 protein in 69 specimens of pancreatic cancer and Western blot in 8 fresh specimens. RESULTS: The expression of PDCD4 protein was significantly lower in all 8 fresh pancreatic cancer tissues than that in non-cancerous tissues detected by Western blot. Compared with non-cancerous pancreatic tissue (> 80% of positive cells), low PDCD4 expression was shown in 69 pancreatic cancer tissues (< 30% of positive cells in 36 cases and 30%-80% of positive expression cells in 33 cases). In the 33 cases with 30% and 80% of positive expression cells, the expression rates of PDCD4 protein were 57.6%, 24.2%, and 18.2% in well, moderately, and poorly differentiated cancers, respectively. In the 36 cases less than 30% of positive expression cells, however, the expression rate of PDCD4 protein in well, moderately, and poorly differentiated cases were 19.4%, 41.7%, and 38.9%, respectively. 67.4% (15/23) of the moderately differentiated cases and 70% (14/20) of the poorly differentiated cases showed < 30% of positive expression cells. Only 26.9% (7/26) of the well differentiated cases, however, showed < 30% of positive expression cells, indicating that low PDCD4 expression was associated with histological grade ($P < 0.01$). There was no relationship between PDCD4 expression and other clinicopathological parameters including patients' sex, age, and TNM stage. CONCLUSIONS: Expression of PDCD4 protein is low in human pancreatic cancer and is correlated with the differentiation levels of human pancreatic cancer.

PDCD4 may play an important role in the occurrence and development of pancreatic carcinomas.

Ma, Y., et al. (2016). "Induction of Patient-Derived Xenograft Formation and Clinical Significance of Programmed Cell Death Ligand 1 (PD-L1) in Lung Cancer Patients." Med Sci Monit **22**: 4017-4025.

BACKGROUND The immune checkpoint of programmed cell death ligand 1 (PD-L1) commonly expressed in solid cancers, and the blockade of this molecule show promising results in advanced cancers, including lung cancer. The relevance of PD-L1 to patient-derived xenograft (PDX) formation and clinicopathological characteristics in early stage lung cancer have not been fully elucidated. MATERIAL AND METHODS Cell counting kit-8 and flow cytometry were carried out to examine proliferation and apoptosis in PC9 and H520 cells transfected with siRNAs. Nod-scid mice were used to establish PDX. Immunohistochemistry was done to investigate PD-L1 expression in tumor tissues. RESULTS PD-L1 was detected in lung cancer cell lines and 45.45% of primary tumor tissues from a cohort of 209 lung cancer patients. Cell growth was restrained and apoptosis was induced when PD-L1 was inhibited in PC9 and H520 cells. In addition, we successfully established 16 PDX models from tissues from 43 cases of primary lung cancer. Higher PD-L1 expression rates (75%) was observed in primary tumors with PDX formation compared to protein expression rate (44.44%) in tumors without PDX formation. Consistently, a 1.9-fold increase of PDX formation frequency was identified in the PD-L1 positive tumors than in the PD-L1 negative tumors. Moreover, PD-L1 was found to be related to smoking, histological type, and pathological stage. Importantly, PD-L1 overexpression was associated with shorter overall survival (OS) of lung cancer patients. CONCLUSIONS This study suggests that overexpression of PD-L1 could induce PDX formation and is related to poor outcome for the lung cancer patients.

Maccarrone, M., et al. (1997). "Involvement of 5-lipoxygenase in programmed cell death of cancer cells." Cell Death Differ **4**(5): 396-402.

We investigated the involvement of 5-lipoxygenase activity in the early phases of programmed cell death (PCD) induced by H2O2 or retinoids in different human tumour cells (erythroleukaemia, neuroblastoma, melanoma). Apoptotic cells showed enhanced 5-lipoxygenase activity which was paralleled by decreased superoxide dismutase activity and increased light emission. Ultraweak luminescence, mainly due to membrane lipid peroxidation by lipoxygenase activation, increased in all cell lines tested within 10-15 min after induction of PCD, in a concentration and time-dependent manner. At the same time, we observed a significant increase in the intracellular steady state level of the 5-lipoxygenase metabolite leukotriene B4. Furthermore, 5-lipoxygenase metabolite 5-hydroxyeicosatetraenoic acid was able to induce PCD in all cell lines tested. Conversely, the general lipoxygenase inhibitor nordihydroguaiaretic acid and the selective 5-lipoxygenase inhibitor caffeic acid protected the different tumour cells from H2O2-induced PCD to a similar extent. These results show the activation of the 5-lipoxygenase pathway in PCD of three different cancer cell lines.

Mahmoud, E. H., et al. (2018). "Serum MicroRNA-21 Negatively Relates to Expression of Programmed Cell Death-4 in Patients with Epithelial Ovarian Cancer." Asian Pac J Cancer Prev **19**(1): 33-38.

Background: Ovarian cancer is the third most common cancer of the female genital tract and the leading cause of cancer death associated with gynecologic tumors. MicroRNAs regulate at least 60% of human genes, including tumor suppressor genes and oncogenes and, thereby, can affect cancer risk. Aim of the work: We aimed to assess any diagnostic role for serum miR-21 as a biomarker in human ovarian cancer and to study relations with programmed cell death-4 (PDCD4), one of its target proteins, hoping to help explain heterogeneity of this cancer type and facilitate stratification of regimens for therapy. Subjects and Methods: A total of 60 newly diagnosed ovarian cancer cases and 30 apparently healthy females were recruited. Serum microRNA-21 levels were measured by TaqMan- Real time PCR assay and PDCD4 by ELISA. Results: Significant over-expression of serum miR-21 and lower serum PDCD4 levels were observed in ovarian cancer patients as compared to the control group. A statistically significant inverse correlation was also evident between miR-21 and PDCD4. However, no significant links were noted observed between miR-21 and tumor grade, stage or histopathological type. Conclusion: The present work showed significantly up-regulation of serum miR21 in the recruited group of patients and a significant inverse relation association between miR-21and PDCD4. These findings suggest that miR-21 may be used as a diagnostic biomarker for human ovarian cancer.

Mahmud, H., et al. (2009). "Induction of programmed cell death in ErbB2/HER2-expressing cancer cells by targeted delivery of apoptosis-inducing factor." Mol Cancer Ther **8**(6): 1526-1535.

Apoptosis-inducing factor (AIF) is a mitochondrial flavoprotein with NADH oxidase activity that has a vital function in healthy cells but is also an important mediator of caspase-independent programmed cell death in stressed and damaged cells. Here, we generated a truncated AIF derivative (AIF (Delta100)) that lacks the mitochondrial import signal of the protein. Bacterially expressed AIF (Delta100) was functionally active and induced cell death on microinjection into Vero cells accompanied by clear signs of apoptosis. For specific targeting to tumor cells, AIF (Delta100) was genetically fused to the scFv (FRP5) antibody fragment that recognizes the ErbB2 (HER2) receptor tyrosine kinase frequently overexpressed in many human cancers. Recombinant scFv (FRP5)-AIF (Delta100) (5-AIF (Delta100)) protein and a similar scFv (FRP5)-ETA (252-366)-AIF (Delta100) (5-E-AIF (Delta100)) molecule harboring in addition the nontoxic translocation domain of Pseudomonas exotoxin A as an endosome escape function displayed binding to ErbB2-expressing cells followed by protein internalization and accumulation in intracellular vesicles. In the presence of the endosomolytic reagent chloroquine 5-E-AIF (Delta100) but not the similar 5-AIF (Delta100) protein displayed potent cell killing activity, which was strictly dependent on the expression of ErbB2 on the target cell surface. Our results show that recombinant AIF specifically targeted to human cancer cells and delivered into the cytosol has potent cell killing activity, suggesting this molecule as an effector function suitable for the development of humanized immunotoxin-like molecules.

Mansfield, A. S., et al. (2016). "Temporal and spatial discordance of programmed cell death-ligand 1 expression and lymphocyte tumor infiltration between paired primary lesions and brain metastases in lung cancer." Ann Oncol **27**(10): 1953-1958.

BACKGROUND: The dynamics of PD-L1 expression may limit its use as a tissue-based predictive biomarker. We sought to expand our understanding of the dynamics of PD-L1 expression and tumor-infiltrating lymphocytes (TILs) in patients with lung cancer-related brain metastases. EXPERIMENTAL DESIGN: Paired primary lung cancers and brain metastases were identified and assessed for PD-L1 and CD3 expression by immunohistochemistry. Lesions with 5% or greater PD-L1 expression were considered positive. Agreement statistics and the chi (2) or Fisher's exact test were used for analysis. RESULTS: We analyzed 146 paired lesions from 73 cases. There was disagreement of tumor cell PD-L1 expression in 10 cases (14%, kappa = 0.71), and disagreement of TIL PD-L1 expression in 19 cases (26%, kappa = 0.38). Most paired lesions with discordant tumor cell expression of PD-L1 were obtained 6 or more months apart. When specimens were categorized using a proposed tumor microenvironment categorization scheme based on PD-L1 expression and TILs, there were significant changes in the classifications because many of the brain metastases lacked either PD-L1 expression, tumor lymphocyte infiltration or both even when they were present in the primary lung cancer specimens (P = 0.009). CONCLUSIONS: We identified that there are significant differences between the tumor microenvironment of paired primary lung cancers and brain metastases. When physicians decide to treat patients with lung cancer with a PD-1 or PD-L1 inhibitor, they must do so in the context of the spatial and temporal heterogeneity of the tumor microenvironment.

Mansfield, A. S., et al. (2016). "Heterogeneity of Programmed Cell Death Ligand 1 Expression in Multifocal Lung Cancer." Clin Cancer Res **22**(9): 2177-2182.

PURPOSE: The expression of programmed cell death ligand 1 (PD-L1) provides limited predictive value in identifying patients most likely to respond to immunotherapy. As the heterogeneity of PD-L1 expression may lead to sampling error and the misclassification of PD-L1 status, we assessed the distribution of PD-L1 expression in paired, resected multifocal lung cancers. EXPERIMENTAL DESIGN: PD-L1 was assessed by IHC. Paired lesions were defined as independent primaries or related lesions using mate pair next-generation sequencing. Agreement statistics were used for analysis. RESULTS: Sixty-seven multifocal lung cancers from 32 patients were sequenced and stained for PD-L1. There was agreement of PD-L1 expression by the tumor cells in paired lesions of 20 patients and disagreement of PD-L1 expression by the tumor cells in paired lesions of 12 patients (kappa = 0.01). Sequencing identified that 23 patients had independent primary lung cancers and that 9 patients had related cancers. In paired lesions of patients with independent cancers, there was agreement of PD-L1 expression by the tumor cells in 12 patients and disagreement in 11 patients (kappa = 0.31). In paired lesions of patients with related lung cancers, there was agreement of PD-L1 expression by the tumor cells in 8 patients and disagreement in 1 patient (kappa = 0.73). CONCLUSIONS: The expression of PD-L1 is heterogeneous among paired independent lung cancers, but there are high levels of agreement in intrapulmonary metastasis. Clin Cancer Res; 22(9); 2177-82. (c)2015 AACR.

Marks, P. A. and X. Jiang (2005). "Histone deacetylase inhibitors in programmed cell death and cancer therapy." Cell Cycle **4**(4): 549-551.

Histone deacetylase (HDAC) inhibitors, such as suberoylanilide hydroxamic acid (SAHA), are targeted anticancer agents that have significant anticancer activity at doses well tolerated by patients. Recently, we found that HDAC inhibitors can trigger both mitochondria-mediated apoptosis and caspase-independent autophagic cell death, indicating potential benefit of HDAC inhibitors in treating cancers with apoptotic defects. We also found that thioredoxin (TRX) might play a significant role in HDAC inhibitor-induced cell death, and HDAC inhibitors increase TRX levels in normal cells but not transformed cells, which is likely to be one of the reasons why HDAC inhibitors preferentially kill cancer cells. In this review, we discuss the study of HDAC inhibitors in cell death and cancer research, the implications of our recent findings, and some outstanding questions that need to be addressed.

Massard, C., et al. (2016). "Safety and Efficacy of Durvalumab (MEDI4736), an Anti-Programmed Cell Death Ligand-1 Immune Checkpoint Inhibitor, in Patients With Advanced Urothelial Bladder Cancer." J Clin Oncol **34**(26): 3119-3125.

PURPOSE: To investigate the safety and efficacy of durvalumab, a human monoclonal antibody that binds programmed cell death ligand-1 (PD-L1), and the role of PD-L1 expression on clinical response in patients with advanced urothelial bladder cancer (UBC). METHODS: A phase 1/2 multicenter, open-label study is being conducted in patients with inoperable or metastatic solid tumors. We report here the results from the UBC expansion cohort. Durvalumab (MEDI4736, 10 mg/kg every 2 weeks) was administered intravenously for up to 12 months. The primary end point was safety, and objective response rate (ORR, confirmed) was a key secondary end point. An exploratory analysis of pretreatment tumor biopsies led to defining PD-L1-positive as >/= 25% of tumor cells or tumor-infiltrating immune cells expressing membrane PD-L1. RESULTS: A total of 61 patients (40 PD-L1-positive, 21 PD-L1-negative), 93.4% of whom received one or more prior therapies for advanced disease, were treated (median duration of follow-up, 4.3 months). The most common treatment-related adverse events (AEs) of any grade were fatigue (13.1%), diarrhea (9.8%), and decreased appetite (8.2%). Grade 3 treatment-related AEs occurred in three patients (4.9%); there were no treatment-related grade 4 or 5 AEs. One treatment-related AE (acute kidney injury) resulted in treatment discontinuation. The ORR was 31.0% (95% CI, 17.6 to 47.1) in 42 response-evaluable patients, 46.4% (95% CI, 27.5 to 66.1) in the PD-L1-positive subgroup, and 0% (95% CI, 0.0 to 23.2) in the PD-L1-negative subgroup. Responses are ongoing in 12 of 13 responding patients, with median duration of response not yet reached (range, 4.1+ to 49.3+ weeks). CONCLUSION: Durvalumab demonstrated a manageable safety profile and evidence of meaningful clinical activity in PD-L1-positive patients with UBC, many of whom were heavily pretreated.

Mayer, V. and P. Ebbesen (1997). "Programmed cell death: will it become a factor in cancer prevention?" Eur J Cancer Prev **6**(4): 323-329.

Among the factors triggering programmed cell death (PCD) are a number of known carcinogens, and several consequences of DNA abnormalities characteristic of cancer have been shown capable of eliciting the PCD response. So although elimination of a potentially malignant cell is likely to be a rare consequence of PCD it could turn out to be important for cancer development. A brief survey is given of the most well-known triggering factors, the molecular mechanisms of the pathways involved and the emerging experimental and clinical data relating capacity of PCD to cancer initiation and progression. It is suggested that future cancer prevention will have to consider also those factors which may abrogate normal PCD.

McCloskey, D. E., et al. (1996). "Programmed cell death in human breast cancer cells." Recent Prog Horm Res **51**: 493-508.

The need for improved systemic therapy for breast cancer is great. Cancer growth represents an imbalance between cell proliferation and cell death: thus, effective anti-cancer therapies may act to decrease cell proliferation or increase cell death, or both. This chapter delineates the role of the programmed cell death process in maintaining homeostasis in normal mammary tissues. The preservation of such death pathways in malignant mammary cells and the ability of chemotherapeutic agents to initiate the programmed cell death process in these cells is reviewed. Finally, ongoing research exploring new ways to take advantage of these death pathways in the clinical setting is examined.

McCloskey, D. E., et al. (1995). "Induction of programmed cell death in human breast cancer cells by an unsymmetrically alkylated polyamine analogue." Cancer Res **55**(15): 3233-3236.

The need for antineoplastic compounds with novel mechanisms of action is great. One such agent is the recently synthesized polyamine analogue N1-ethyl-N11-((cyclopropyl)methyl)-4,8-diazaundecane (CPENSpm). Exposure of hormone-dependent and -

independent human breast cancer cells to 0.1-10 microM CPENSpm led to both growth inhibition and induction of programmed cell death. Fragmentation of DNA to high molecular weight fragments and oligonucleosomal-sized fragments, both characteristic of programmed cell death, was determined to be time and concentration dependent. Depletion of natural polyamine pools and accumulation of the analogue was also demonstrated. These data provide the first evidence that a polyamine analogue induces programmed cell death.

McCloskey, D. E., et al. (1996). "Paclitaxel induces programmed cell death in MDA-MB-468 human breast cancer cells." Clin Cancer Res **2**(5): 847-854.

The ability of paclitaxel, one of the most active chemotherapeutic agents against breast cancer, to induce programmed cell death in hormone-independent MDA-MB-468 human breast cancer cells was assessed. Treatment of MDA-MB-468 cells led to growth inhibition, high-molecular-weight and oligonucleosomal DNA fragmentation, and apoptosis-associated morphological changes after either 3- or 24-h exposure to paclitaxel concentrations >/=10 nM. Additionally, cleavage products of poly (ADP-ribose) polymerase and lamin B1, two proteins that are cleaved early in the execution phase of programmed cell death, were detected. Quantitative studies indicated that exposure to paclitaxel for 24 h resulted in more DNA fragmentation than did 3-h exposure. Rapid induction of the early-response gene c-jun but not c-myc was associated with paclitaxel treatment. The ability of paclitaxel to induce high-molecular-weight DNA fragmentation and apoptosis-associated morphological changes in three other breast cancer cell lines was also established. These data suggest that paclitaxel, an agent known to stabilize microtubules and prevent cell division but not to act directly on DNA, induces programmed cell death in breast cancer cells.

Meng, H., et al. (2015). "MicroRNA-330-3p functions as an oncogene in human esophageal cancer by targeting programmed cell death 4." Am J Cancer Res **5**(3): 1062-1075.

MicroRNAs comprise a family of small non-coding RNA molecules that have emerged as key post-transcriptional regulators of gene expression. Aberrant miRNA expression has been linked to various human tumors. This study was aimed to identify novel miRNAs involved in the carcinogenesis of esophageal squamous cell carcinoma (ESCC) and their potential functions. We performed miRNA microarray and found that miR-330-3p was highly expressed in ESCC tumor tissues. qRT-PCR further confirmed the result in other 35 pairs of ESCC tumor tissues and ESCC cell lines. Ectopic expression of miR-330-3p significantly promoted ESCC cell proliferation, survival, migration, invasion in vitro and stimulated tumor formation in nude mice. Knockdown of miR-330-3p leaded to the opposite effects. The luciferase assay confirmed that miR-330-3p directly interacted with the PDCD4 mRNA 3' un-translated region (UTR). Moreover, expression of PDCD4 was inversely associated with miR-330-3p in ESCC tissues. Silencing of PDCD4 significantly promoted cell growth, cell migration, invasion and inhibited cisplatin-induced apoptosis in ESCC cells. This study suggested that miR-330-3p might play an oncogenic role in the development of ESCC partially via suppression of PDCD4 expression.

Merhi, M., et al. (2018). "Squamous Cell Carcinomas of the Head and Neck Cancer Response to Programmed Cell Death Protein-1 Targeting and Differential Expression of Immunological Markers: A Case Report." Front Immunol **9**: 1769.

Targeting the programmed cell death protein-1 (PD-1)/PD-1 ligand (PD-L1) pathway has been shown to enhance T cell-mediated antitumor immunity. Clinical responses are limited to subgroups of patients. The search for biomarkers of response is a strategy to predict response and outcome of PD-1/PD-L1 checkpoint intervention. The NY-ESO-1 cancer testis antigen has been considered as a biomarker in head and neck squamous cell carcinoma (HNSCC) patients and can induce both specific NY-ESO-1 antibody and T cells responses. Here, we correlated clinical responsiveness to anti-PD-1 (nivolumab) treatment with immunity to NY-ESO-1 in a patient with recurrent HNSCC. The patient was treated with second-line treatment of nivolumab and had a stable disease for over 7 months. His NY-ESO-1 antibody was found to be lower after the third (****$p < 0.0001$) and the fifth (****$p < 0.0001$) cycles of treatment compared to base line, and this was in line with the stability of the disease. The NY-ESO-1-specific T cells response of the patient was found to be increased after the third and the fifth (**$p = 0.002$) cycles of treatment but had a significant decline after progression (**$p = 0.0028$). The PD-1 expression by the patient's T cells was reduced 15-folds after nivolumab treatment and was uniquely restricted to the CD8(+) T cells population. Several cytokines/chemokines involved in immune activation were upregulated after nivolumab treatment; two biomarkers were reduced at progression [interleukin (IL)-10: ****$p < 0.0001$ and CX3CL1: ****$p < 0.0001$]. On the other hand, some cytokines/chemokines contributing to immune inhibition were downregulated after nivolumab treatment; two biomarkers were increased at

progression (IL-6: ****p < 0.0001 and IL-8: ****p < 0.0001). This data support the notion that the presence of anti-NY-ESO-1 integrated immunity and some cytokines/chemokines profile may potentially identify a response to PD-1 blockade in HNSCC patients.

Minami, T., et al. (2015). "Identification of Programmed Death Ligand 1-derived Peptides Capable of Inducing Cancer-reactive Cytotoxic T Lymphocytes From HLA-A24+ Patients With Renal Cell Carcinoma." J Immunother **38**(7): 285-291.

Molecular therapy targeting tumor angiogenesis has been the standard treatment for metastatic renal cell carcinoma (mRCC). However, despite their significant antitumor effects, most of patients with mRCC have not been cured. Under such circumstances, anticancer immunotherapy has been considered a promising treatment modality for mRCC, and cancer-reactive cytotoxic T lymphocytes (CTLs) are the most powerful effectors among several immune cells. However, anticancer CTLs can be inhibited by several immune inhibitory mechanisms, including the interaction between programmed death 1 (PD-1) and its ligand PD-L1, on T cells and cancer cells, respectively. Alternatively, this also means that PD-L1 could be a promising target for anticancer immunotherapy. Therefore, we searched for PD-L1-derived peptides that are applicable for anticancer vaccine for HLA-A24(+) RCC patients. Among 5 peptides derived from PD-L1, which were prepared based on the binding motif to the HLA-A24(+) allele, both PD-L1(11-19) and PD-L1(41-50) peptides induced peptide-specific CTLs from peripheral blood mononuclear cells of HLA-A24(+) RCC patients. Such PD-L1 peptide-stimulated CD8 T cells showed cytotoxicity against HLA-A24(+) and PD-L1-expressing RCC cells. Although IFN-gamma treatment increased PD-L1 expression on PD-L1(low) RCC cells, their sensitivity to cytotoxicity of PD-L1 peptide-stimulated CD8(+) T cells varied between patients. Altogether, these results indicate that both PD-L1(11-19) and PD-L1(41-50) peptides could be candidates for peptide-based anticancer vaccines for HLA-A24(+) mRCC patients.

Mino-Kenudson, M. (2016). "Programmed cell death ligand-1 (PD-L1) expression by immunohistochemistry: could it be predictive and/or prognostic in non-small cell lung cancer?" Cancer Biol Med **13**(2): 157-170.

Blockade of immune checkpoints has recently emerged as a novel therapeutic strategy in various tumors. In particular, monoclonal antibodies targeting programmed cell death 1 (PD-1) or its ligand (PD-L1) have been most studied in lung cancer, and PD-1 inhibitors are now established agents in the management of non-small cell lung cancer (NSCLC). The reports on high-profile clinical trials have shown the association of PD-L1 expression by immunohistochemistry (IHC) with higher overall response rates to the PD-1/PD-L1 axis blockade suggesting that PD-L1 expression may serve as a predictive marker. Unfortunately, however, each PD-1 or PD-L1 inhibitor is coupled with a specific PD-L1 antibody, IHC protocol and scoring system for the biomarker assessment, making the head-to-head comparison of the studies difficult. Similarly, multiple clinical series that correlated PD-L1 expression with clinicopathologic and/or molecular variables and/or survival have reported conflicting results. The discrepancy could be explained by the differences in ethnicity and/or histologic types included in the studies, but it appears to be attributed in part to the differences in PD-L1 IHC methods. Thus, orchestrated efforts to standardize the PD-L1 IHC are warranted to establish the IHC as a predictive and/or prognostic biomarker in NSCLC.

Mudduluru, G., et al. (2007). "Loss of programmed cell death 4 expression marks adenoma-carcinoma transition, correlates inversely with phosphorylated protein kinase B, and is an independent prognostic factor in resected colorectal cancer." Cancer **110**(8): 1697-1707.

BACKGROUND: Programmed cell death 4 (Pdcd4) inhibits malignant transformation, and initial studies of Pdcd4 suggested the regulation of Pdcd4 localization by protein kinase B (Akt). However, supporting patient tissue data are missing, and the diagnostic/prognostic potential of Pdcd4 rarely has been studied. The objectives of the current were 1) to determine Pdcd4 as a diagnostic marker in the adenoma-carcinoma sequence, 2) to support phosphorylated Akt (pAkt)-mediated Pdcd4 regulation in vivo, and 3) to obtain the first prognostic evidence of Pdcd4 in colorectal cancer. METHODS: Tumor samples and normal tissues from 71 patients with colorectal cancer who were followed prospectively (median follow-up, 36 months) and 42 adenomas were analyzed for Pdcd4, Akt, and pAkt in immunohistochemical and Western blot analyses. RESULTS: A significant reduction in Pdcd4 was observed between normal mucosa and adenomas and between adenomas and tumor samples (P <.01 and P <.01, respectively). Normal mucosa demonstrated strong nuclear Pdcd4, which was reduced significantly in adenomas (P <.01) and almost was lost in tumors (P <.01). pAkt was correlated inversely with Pdcd4 and with the transition of Pdcd4 from nucleus to cytoplasm (P <.01). Kaplan-Meier analysis (using the Mantel-Cox log-rank test) indicated a significant correlation between the loss of total and nuclear Pdcd4 in tumors

and overall survival (P <.05 and P <.02, respectively) and disease-specific survival (P <.01 and P <.01, respectively). In multivariate analysis, loss of total or nuclear Pdcd4 was an independent predictor of disease-specific or overall survival. CONCLUSIONS: To the authors' knowledge, this is the first study to demonstrate an independent prognostic impact of Pdcd4 and its expression pattern in colorectal cancer. Data from this study support the regulation of Pdcd4 localization by pAkt in vivo. Pdcd4 immunohistochemistry may be useful as a supportive diagnostic tool for the transition between normal, adenoma, and tumor tissues.

Murthy, K. N., et al. (2015). "Cytotoxicity of obacunone and obacunone glucoside in human prostate cancer cells involves Akt-mediated programmed cell death." Toxicology **329**: 88-97.

Obacunone and obacunone glucoside (OG) are naturally occurring triterpenoids commonly found in citrus and other plants of the Rutaceae family. The current study reports the mechanism of cytotoxicity of citrus-derived obacunone and OG on human androgen-dependent prostate cancer LNCaP cells. Both limonoids exhibited time- and dose-dependent inhibition of cell proliferation, with more than 60% inhibition of cell viability at 100 muM, after 24 and 48 h. Analysis of fragmentation of DNA, activity of caspase-3, and cytosolic cytochrome-c in the cells treated with limonoids provided evidence for activation of programmed cell death by limonoids. Treatment of LNCaP cells with obacunone and OG resulted in dose-dependent changes in expression of proteins responsible for the induction of programmed cell death through the intrinsic pathway and down-regulation of Akt, a key molecule in cell signaling pathways. In addition, obacunone and OG also negatively regulated an inflammation-associated transcription factor, androgen receptor, and prostate-specific antigen, and activated proteins related to the cell cycle, confirming the ability of limonoids to induce cytotoxicity through multiple pathways. The results of this study provided, for the first time, an evidence of the cytotoxicity of obacunone and OG in androgen-dependent human prostate cancer cells.

Naha, N., et al. (2008). "Rare sugar D-allose induces programmed cell death in hormone refractory prostate cancer cells." Apoptosis **13**(9): 1121-1134.

Development of effective agents for treatment of hormone-refractory prostate cancer (HRPC) has become a national medical priority. D-Allose is a monosaccharide (C-3 epimer of glucose) distributed rarely in nature; because of its scarcity and cost, the biological effect has hardly been studied. In the present study, we demonstrated the inhibitory action of D-allose on proliferation of human HRPC cell lines, DU145 and PC-3 in a dose- and time-dependent manner, while human normal prostate epithelial (NPE) cell line, PrEC showed no remarkable effect. In vitro treatment of D-allose resulted in the alteration of Bcl-2/Bax ratio in favor of apoptosis (programmed cell death, PCD) in both the HRPC cell lines, which was associated with the lowering of mitochondrial transmembrane potential (Deltapsi (m)) and the release of cytochrome C (cyt C), the cleavage of caspase 3 and poly (ADP-ribose) polymerase (PARP), and the elevation of calcium concentration in cytosol ([Ca (2+)] (c)). D-Allose also induced G1 phase arrest of the cell cycle in DU145 cell line. This study for the first time suggested the antiproliferative effect of D-allose through induction of PCD in HRPC cell lines, which could be due to the modulation of mitochondria mediated intrinsic apoptotic pathway.

Nakamura, S., et al. (2017). "Intratumoral heterogeneity of programmed cell death ligand-1 expression is common in lung cancer." PLoS One **12**(10): e0186192.

Programmed cell death ligand-1 (PD-L1) expression may predict the response to both programmed cell death-1 and PD-L1 inhibitors in lung cancer. However, the extent of intratumoral heterogeneity of PD-L1 expression, which may cause false negative results, is largely unexplored. We aimed to assess the intratumoral heterogeneity of PD-L1 expression in surgically resected lung cancer specimens by applying a novel method of tissue microarray, namely Spiral Arrays, which enables us to observe the heterogeneity in spiral-shaped tissue cores. Adenocarcinoma and squamous cell carcinoma specimens were obtained from consecutive patients with lung cancer who had undergone surgical resection at Nagasaki University Hospital (Nagasaki, Japan) since 2009. Small cell lung cancer and large cell carcinoma specimens were selected from patients in the same archive who had undergone resection since 1998. Spiral Arrays were constructed of spiral-shaped cores, prepared from representative blocks of each case, which were subjected to immunohistochemistry using an anti-PD-L1 antibody. Each core was divided into 8 segments and each segment was classified as either PD-L1-positive or PD-L1-negative using thresholds of 1.0%, 5.0%, 10.0%, and 50.0%, respectively. In total, 138 specimens were selected, including 60 adenocarcinomas, 59 squamous cell carcinomas, 12 small cell lung cancers, and 7 large cell carcinomas. The majority of specimens with PD-L1-positive segments exhibited heterogeneous expression (i.e., had a mixture of PD-L1-positive and PD-L1-negative segments within a core) irrespective of the threshold (1.0%, 66.7%; 5.0%, 74.4%; 10.0%, 75.8%;

and 50.0%, 85.7%]. Large variations in the ratios of PD-L1-positive segments were observed. At least 50.0% of the segments within a core were negative in no fewer than 50.0% (range, 50.0-76.0%) of cases with heterogeneous PD-L1 expression. In conclusion, intratumoral heterogeneity of PD-L1 expression was frequently observed in cases of lung cancer. Thus, multiple tumor biopsy specimens may be needed to accurately determine the PD-L1 expression status.

Nedaeinia, R., et al. (2017). "Inhibition of microRNA-21 via locked nucleic acid-anti-miR suppressed metastatic features of colorectal cancer cells through modulation of programmed cell death 4." Tumour Biol **39**(3): 1010428317692261.

Colorectal cancer is among the most lethal of malignancies, due to its propensity to metastatic spread and multifactorial-chemoresistance. The latter property supports the need to identify novel therapeutic approaches for the treatment of colorectal cancer. MicroRNAs are endogenous non-coding small RNA molecules that function as post-transcriptional regulators of gene expression. Recently, programmed cell death 4 has been identified as a protein that increases during apoptosis. This gene is among the potential targets of miR-21 (OncomiR). Locked nucleic acid-modified oligonucleotides have recently emerged as a potential therapeutic option for targeting microRNAs. The aim of this study was to explore the functional role of locked nucleic acid-anti-miR-21 in the LS174T cell line in vitro and in vivo models. LS174T cells were treated with locked nucleic acid-anti-miR-21 for 24, 48, and 72 h in vitro. The expression of miR-21 and PDCD4 at messenger RNA (mRNA) level was evaluated by quantitative real-time polymerase chain reaction, while the protein level of PDCD4 was determined by Western blotting. Cell migratory behavior and the cluster-forming ability of cells were assessed before and after therapy. The disseminated tumor cells were assessed in the chick chorioallantoic membrane model by Alu quantitative polymerase chain reaction. Locked nucleic acid-anti-miR-21 was transfected successfully into the LS174T cells and inhibited the expression of miR-21. Locked nucleic acid-anti-miR-21 inhibited the migration and the number of cells forming clusters. Moreover, we found that locked nucleic acid-anti-miR-21 transfection was associated with a significant reduction in metastatic properties as assessed by the in ovo model. Our findings demonstrated the novel therapeutic potential of locked nucleic acid-anti-miR-21 in colon adenocarcinoma with high miR-21 expression.

Ness, N., et al. (2017). "The prognostic role of immune checkpoint markers programmed cell death protein 1 (PD-1) and programmed death ligand 1 (PD-L1) in a large, multicenter prostate cancer cohort." Oncotarget **8**(16): 26789-26801.

Programmed cell death protein 1 (PD-1) and its ligand Programmed death ligand 1 (PD-L1) have gained massive attention in cancer research due to recent availability and their targeted antitumor effects. Their role in prostate cancer is still undetermined. We constructed tissue microarrays from prostatectomy specimens from 535 prostate cancer patients. Following validation of antibodies, immunohistochemistry was used to evaluate the expression of PD-1 in lymphocytes and PD-L1 in epithelial and stromal cells of primary tumors. PD-L1 expression was commonly seen in tumor epithelial cells (92% of cases). Univariate survival analysis revealed a positive association between a high density of PD-1+ lymphocytes and worse clinical failure-free survival, limited to a trend ($p = 0.084$). In subgroups known to indicate unfavorable prostate cancer prognosis (Gleason grade 9, age < 65, preoperative PSA > 10, pT3) patients with high density of PD-1+ lymphocytes had a significantly higher risk of clinical failure ($p = < 0.001$, $p = 0.025$, $p = 0.039$ and $p = 0.011$, respectively). In the multivariate analysis, high density of PD-1+ lymphocytes was a significant negative independent prognostic factor for clinical failure-free survival (HR = 2.48, CI 95% 1.12-5.48, $p = 0.025$).

Nieves-Alicea, R., et al. (2009). "Programmed cell death 4 inhibits breast cancer cell invasion by increasing tissue inhibitor of metalloproteinases-2 expression." Breast Cancer Res Treat **114**(2): 203-209.

High levels of the cyclooxygenase-2 (COX-2) protein have been associated with invasion and metastasis of breast tumors. Both prostaglandin E (2) (PGE (2)) and interleukin-8 (IL-8) have been shown to mediate the invasive activity of COX-2 in breast cancer cells. Here we expand these studies to determine how COX-2 uses PGE (2) and IL-8 to induce breast cancer cell invasion. We demonstrated that PGE (2) and IL-8 decreased the expression of the tumor suppressor protein Programmed Cell Death 4 (PDCD4). We hypothesized that suppression of PDCD4 expression is vital to the invasive activity of PGE (2) and IL-8. In MCF-7 cells overexpressing PDCD4 (MCF-7/PDCD4), PGE (2) and IL-8 failed to induce invasion, in contrast to the parental MCF-7 cells, thus indicating that PDCD4 blocks breast cancer cell invasion. MCF-7/PDCD4 cells produced higher levels of the Tissue Inhibitor of Metalloproteinases-2 (TIMP-2) than the parental cells. Silencing TIMP-2 mRNA in MCF-7/PDCD4 cells reversed the anti-invasive effects of PDCD4, allowing PGE (2) and IL-8 to induce the invasion of these cells. Here we report

the novel findings that suppression of PDCD4 expression is vital for the invasive activity of COX-2 mediated by PGE (2) and IL-8, and that PDCD4 increases TIMP-2 expression to inhibit breast cancer cell invasion.

Nishino, M., et al. (2016). "Incidence of Programmed Cell Death 1 Inhibitor-Related Pneumonitis in Patients With Advanced Cancer: A Systematic Review and Meta-analysis." JAMA Oncol **2**(12): 1607-1616.

Importance: Programmed cell death 1 (PD-1) inhibitor-related pneumonitis is a rare but clinically serious and potentially life-threatening adverse event. Little is known about its incidence across different tumor types and treatment regimens. Objective: To compare the incidence of PD-1 inhibitor-related pneumonitis among different tumor types and therapeutic regimens. Data Sources: A PubMed search through November 10, 2015, and a review of references from relevant articles. For the PubMed search, the following keywords or corresponding Medical Subject Heading terms were used: nivolumab, pembrolizumab, and PD-1 inhibitor. Study Selection: Twenty-six original articles of PD-1 inhibitor trial results were identified. Among them, 20 studies of melanoma, non-small cell lung cancer (NSCLC), or renal cell carcinoma (RCC) were eligible for a meta-analysis. Data Extraction and Synthesis: The data were extracted by 1 primary reviewer and then independently reviewed by 2 secondary reviewers following Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Comparisons of the incidence were based on marginal, exact generalized linear models with generalized estimating equations. Main Outcomes and Measures: Incidence of all-grade and grade 3 or higher pneumonitis and pneumonitis-related deaths. Results: Twenty studies of single-tumor-type trials of PD-1 inhibitor (12 melanoma studies, 5 NSCLC studies, and 3 RCC studies) (a total of 4496 unique patients) were included in the meta-analysis. The overall incidence of pneumonitis during PD-1 inhibitor monotherapy was 2.7% (95% CI, 1.9%-3.6%) for all-grade and 0.8% (95% CI, 0.4%-1.2%) for grade 3 or higher pneumonitis. The incidence was higher in NSCLC for all-grade (4.1% vs 1.6%; P =.002) and grade 3 or higher pneumonitis (1.8% vs 0.2%; P <.001) compared with melanoma. The incidence in RCC was higher than in melanoma for all-grade pneumonitis (4.1% vs 1.6%; P <.001) but not for grade 3 or higher pneumonitis. Four pneumonitis-related deaths were observed in patients with NSCLC in the monotherapy group. Pneumonitis was more frequent during combination therapy than monotherapy for all-grade (6.6% vs 1.6%; P <.001) and grade 3 or higher pneumonitis (1.5% vs 0.2%; P =.001) in melanoma, with 1 pneumonitis-related death during combination therapy. Multivariable analyses demonstrated higher odds of pneumonitis in NSCLC for all-grade (odds ratio [OR], 1.43; 95% CI, 1.08-1.89; P =.005) and grade 3 or higher pneumonitis (OR, 2.85; 95% CI, 1.60-5.08; P <.001) and in RCC for all-grade pneumonitis (OR, 1.59; 95% CI, 1.32-1.92; P <.001) compared with melanoma. The combination therapy had significantly higher odds than monotherapy for all-grade (OR, 2.04; 95% CI, 1.69-2.50; P <.001) and grade 3 or higher pneumonitis (OR, 2.86; 95% CI, 1.79- 4.35; P <.001). Conclusions and Relevance: The incidence of PD-1 inhibitor-related pneumonitis was higher in NSCLC and RCC and during combination therapy. These findings contribute to enhance awareness among clinicians and support further investigations to meet the clinical needs.

Ogura, A., et al. (2018). "Pattern of programmed cell death-ligand 1 expression and CD8-positive T-cell infiltration before and after chemoradiotherapy in rectal cancer." Eur J Cancer **91**: 11-20.

BACKGROUND: The synergistic effect of combining immune checkpoint inhibitors with radiotherapy was reported recently, but there are few studies on programmed cell death-ligand 1 (PD-L1) expression in rectal cancer treated by preoperative chemoradiotherapy (CRT). The aim of the present study was to investigate the PD-L1 expression status before and after CRT and its association with clinicopathological characteristics and recurrence in rectal cancer. METHODS: Immunostainings of PD-L1 and CD8 were performed in 287 patients with rectal cancer treated by CRT. PD-L1 expression on the tumour cells (tPD-L1) and on the stromal immune cells (iPD-L1) was evaluated before and after CRT. CD8+ cell density in tumour area (tCD8+) before CRT and in the stromal area (sCD8+) before and after CRT was also evaluated. RESULTS: High tPD-L1 expression was observed in only three patients (1.0%). High iPD-L1 expression significantly increased from 31.7% before CRT to 49.2% after CRT (P < 0.0001). The increase in high iPD-L1 expression after CRT was only observed in patients with tumour regression grades 1 and 2. High iPD-L1 expression was associated with high tCD8+ cell density before CRT (P < 0.0001) and sCD8+ cell density after CRT (P < 0.0001). High tCD8+ cell density before CRT was associated with better disease-free survival (DFS) (P = 0.0331), but its improved effect on DFS could be observed in patients with high iPD-L1 expression (P = 0.0081), not in patients with low iPD-L1 expression (P = 0.516). CONCLUSION: The present study demonstrated the significant correlations between iPD-L1 expression and CD8+ cell density both before and after CRT.

O'Kane, G. M., et al. (2017). "Monitoring and Management of Immune-Related Adverse Events Associated With Programmed Cell Death Protein-1 Axis Inhibitors in Lung Cancer." Oncologist **22**(1): 70-80.

Monoclonal antibodies targeting programmed cell death protein-1 (PD-1) represent a new treatment paradigm in non-small cell lung cancer. Three phase III trials have demonstrated a survival benefit and improved tolerability of nivolumab and pembrolizumab when compared with standard second-line chemotherapy. Nevertheless, the adverse events associated with PD-1 inhibitors are unique; early recognition and treatment are essential. This review summarizes the required monitoring and appropriate management of immune-related adverse events in lung cancer patients receiving these agents. THE ONCOLOGIST: 2017;22:70-80 IMPLICATIONS FOR PRACTICE:: The potential adverse events of immune checkpoint inhibitors differ from conventional chemotherapy and can require a multidisciplinary approach. Continued education is important for all physicians to ensure optimal care for patients.

Okuma, Y., et al. (2017). "High plasma levels of soluble programmed cell death ligand 1 are prognostic for reduced survival in advanced lung cancer." Lung Cancer **104**: 1-6.

OBJECTIVES: Programmed cell death-ligand 1 (PD-L1) expressed in tumor tissues is a key molecule for immune suppression, given its role in immune checkpoints. The significance and implication of soluble PD-L1 (sPD-L1) in the blood of lung cancer patients remain unknown. PATIENTS AND METHODS: Blood samples were prospectively collected from patients with advanced lung cancer, and the plasma sPD-L1 concentrations were measured by enzyme-linked immunosorbent assay. The correlations of the plasma sPD-L1 levels with clinico-pathological status, laboratory data, and survival of the patients were analyzed. RESULTS: Ninety-six patients with advanced lung cancer were analyzed, including 73 with adenocarcinoma, 12 with squamous cell carcinoma, and seven with small-cell lung cancer. Sixty-five were naive to chemotherapy, and 20 had received two or more lines of chemotherapy. The mean plasma sPD-L1 concentration of all the patients was 6.95+/-2.90ng/ml (range 2.30-20.0ng/ml), and this value is significantly increased compared with that previously reported for normal subjects. No correlation of the plasma sPD-L1 level with histological subtypes, adenocarcinoma genetic status, smoking history, clinical stage or laboratory data was found. However, overall survival was significantly reduced in patients with high (>/=7.32ng/ml) compared with low (<7.32ng/ml) plasma sPD-L1 levels (13.0 vs. 20.4 months, p=0.037). Multivariate analysis revealed that high sPD-L1 levels were significantly related to poor prognosis (hazard ratio 1.99, p=0.041). CONCLUSION: High plasma sPD-L1 levels were associated with poor prognosis in patients with advanced lung cancer, possibly associated with suppression of anti-tumor immunity. Clinical trial register and their clinical registration number: UMIN%000014760.

Okuma, Y., et al. (2018). "Soluble Programmed Cell Death Ligand 1 as a Novel Biomarker for Nivolumab Therapy for Non-Small-cell Lung Cancer." Clin Lung Cancer **19**(5): 410-417 e411.

BACKGROUND: Biomarkers for predicting the effect of anti-programmed cell death 1 (PD-1) monoclonal antibody against non-small-cell lung cancer (NSCLC) are urgently required. Although it is known that the blood levels of soluble programmed cell death ligand 1 (sPD-L1) are elevated in various malignancies, the nature of sPD-L1 has not been thoroughly elucidated. We investigated the significance of plasma sPD-L1 levels as a biomarker for anti-PD-1 monoclonal antibody, nivolumab therapy. PATIENTS AND METHODS: The present prospective study included 39 NSCLC patients. The patients were treated with nivolumab at the dose of 3 mg/kg every 2 weeks, and the effects of nivolumab on NSCLC were assessed according to the change in tumor size, time to treatment failure (TTF), and overall survival (OS). The baseline plasma sPD-L1 concentration was determined using an enzyme-linked immunosorbent assay. RESULTS: The area under the curve of the receiver operating characteristic curve was 0.761. The calculated optimal cutoff point for sPD-L1 in the plasma samples was 3.357 ng/mL. Of the 39 patients, 59% with low plasma sPD-L1 levels achieved a complete response or partial response and 25% of those with high plasma sPD-L1 levels did so. In addition, 22% of the patients with low plasma sPD-L1 levels developed progressive disease compared with 75% of those with high plasma sPD-L1 levels. The TTF and OS were significantly longer for those patients with low plasma sPD-L1 levels compared with the TTF and OS for those with high plasma sPD-L1 levels. CONCLUSION: The clinical benefit from nivolumab therapy was significantly associated with the baseline plasma sPD-L1 levels. Plasma sPD-L1 levels might represent a novel biomarker for the prediction of the efficacy of nivolumab therapy against NSCLC.

Omori, S., et al. (2018). "Changes in programmed death ligand 1 expression in non-small

cell lung cancer patients who received anticancer treatments." Int J Clin Oncol.

BACKGROUND: The expression of programmed death ligand 1 (PD-L1) is considered a predictive biomarker of anti-programmed death 1 (PD-1)/PD-L1 cancer therapies. However, changes in PD-L1 expression of tumor cells during clinical courses have not been fully evaluated. We evaluated changes in PD-L1 expression for non-small cell lung cancer (NSCLC) patients who received anticancer treatments during clinical courses. METHODS: In 76 NSCLC patients, PD-L1 expression was evaluated before and after anticancer treatment by immunohistochemical (IHC) analysis using an anti-PD-L1 antibody. We defined two cut-off points of PD-L1 expression (1 and 50%) and three corresponding IHC groups (A: 0%, B: 1-49%, and C: >/=50%). IHC group B and C were considered to be positive expression, and we defined the difference of IHC group between pre- and post-treatment as 'major change' in PD-L1 expression. RESULTS: Before anticancer treatment, PD-L1 expression was observed in 38/76 (50%) patients, and was significantly less common in patients harboring mutations in the epidermal growth factor receptor gene (EGFR) than in those without (P = 0.039). After anticancer treatment, PD-L1 expression was observed in 36/76 (47%) patients. Major increases in PD-L1 expression were seen in 11 (14%), and major decreases in 18 (24%) patients. Among 13 patients harboring EGFR mutations treated with EGFR tyrosine-kinase inhibitor (EGFR-TKI), five (38%) showed major increases. CONCLUSION: Major changes of PD-L1 expression in tumor cells were observed in 38% of NSCLC patients who received anticancer treatments. And, treatments with EGFR-TKI may increase PD-L1 expression in NSCLC patients harboring EGFR mutations.

Ondrouskova, E. and B. Vojtesek (2014). "[Programmed cell death in cancer cells]." Klin Onkol **27 Suppl 1**: S7-14.

Resistance to programmed cell death is one of the hallmarks of cancer cells that affects the process of malignant transformation as well as response to cancer therapy. The goal of this review is to summarize recent information about programmed cell death (PCD) in healthy and cancer cells, as well as new perspectives for anticancer treatments targeting these signaling pathways. Three main types of PCD are described in detail: apoptosis, necrosis/ necroptosis and cell death associated with autophagy. Among them, apoptosis plays the key role in both malignant transformation and response to therapy. In this review, we describe main signaling pathways and molecules participating in apoptosis regulation in healthy cells. In most cancer cells, mutations or aberrant expression of proteins directly or indirectly involved in induction and execution of cell death can be detected - p53, Bcl 2 family proteins, inhibitors of apoptosis, death receptors/ ligands and other proteins. Mutations or changes in expression of these proteins and their relation to certain types of tumors are described. Finally, we provide a review of recently developed treatments that target and reactivate the machinery of programmed cell death and are currently tested in clinical trials.

Owa, C., et al. (2013). "Triptolide induces lysosomal-mediated programmed cell death in MCF-7 breast cancer cells." Int J Womens Health **5**: 557-569.

BACKGROUND: Breast cancer is a major cause of death; in fact, it is the most common type, in order of the number of global deaths, of cancer in women worldwide. This research seeks to investigate how triptolide, an extract from the Chinese herb Tripterygium wilfordii Hook F, induces apoptosis in MCF-7 human breast cancer cells. Accumulating evidence suggests a role for lysosomal proteases in the activation of apoptosis. However, there is also some controversy regarding the direct participation of lysosomal proteases in activation of key apoptosis-related caspases and release of mitochondrial cytochrome c. In the present study, we demonstrate that triptolide induces an atypical, lysosomal-mediated apoptotic cell death in MCF-7 cells because they lack caspase-3. METHODS: MCF-7 cell death was characterized via cellular morphology, chromatin condensation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric cell growth inhibition assay and the expression levels of proapoptotic proteins. Acridine orange and LysoTracker (R) staining were performed to visualize lysosomes. Lysosomal enzymatic activity was monitored using an acid phosphatase assay and western blotting of cathepsin B protein levels in the cytosolic fraction, which showed increased enzymatic activity in drug-treated cells. RESULTS: These experiments suggest that triptolide-treated MCF-7 cells undergo atypical apoptosis and that, during the early stages, lysosomal enzymes leak into the cytosol, indicating lysosomal membrane permeability. CONCLUSION: Our results suggest that further studies are warranted to investigate triptolide's potential as an anticancer therapeutic agent.

Peters, S., et al. (2017). "Phase II Trial of Atezolizumab As First-Line or Subsequent Therapy for Patients With Programmed Death-Ligand 1-Selected Advanced Non-Small-Cell Lung Cancer (BIRCH)." J Clin Oncol **35**(24): 2781-2789.

Purpose BIRCH was designed to examine the efficacy of atezolizumab, a humanized anti-

programmed death-ligand 1 (PD-L1) monoclonal antibody, in advanced non-small-cell lung cancer (NSCLC) across lines of therapy. Patients were selected on the basis of PD-L1 expression on tumor cells (TC) or tumor-infiltrating immune cells (IC). Patients and Methods Eligible patients had advanced-stage NSCLC, no CNS metastases, and zero to two or more lines of prior chemotherapy. Patients whose tumors expressed PD-L1 using the SP142 immunohistochemistry assay on >/= 5% of TC or IC (TC2/3 or IC2/3 [TC or IC >/= 5% PD-L1-expressing cells, respectively]) were enrolled. Atezolizumab 1,200 mg was administered intravenously every 3 weeks. Efficacy-evaluable patients (N = 659) comprised three cohorts: first line (cohort 1; n = 139); second line (cohort 2; n = 268); and third line or higher (cohort 3; n = 252). The primary end point was independent review facility-assessed objective response rate (ORR; Response Evaluation Criteria in Solid Tumors [RECIST] version 1.1). Secondary end points included median duration of response, progression-free survival, and overall survival (OS). Results BIRCH met its primary objective of demonstrating a significant ORR versus historical controls. With a minimum of 12 months of follow-up, the independent review facility-assessed ORR was 18% to 22% for the three cohorts, and 26% to 31% for the TC3 or IC3 subgroup; most responses are ongoing. Responses occurred regardless of EGFR or KRAS mutation status. The median OS from an updated survival analysis (minimum of 20 month follow up) for cohort 1 was 23.5 months (26.9 months for TC3 or IC3 patients); the median OS in cohorts 2 and 3 was 15.5 and 13.2 months, respectively. The safety profile was similar across cohorts and consistent with previous atezolizumab monotherapy trials. Conclusion BIRCH demonstrated responses with atezolizumab monotherapy in patients with PD-L1-selected advanced NSCLC, with good tolerability. PD-L1 status may serve as a predictive biomarker for identifying patients most likely to benefit from atezolizumab.

Petrou, P. (2018). "A systematic review of economic evaluations of tyrosine kinase inhibitors of vascular endothelial growth factor receptors, mammalian target of rapamycin inhibitors and programmed death-1 inhibitors in metastatic renal cell cancer." Expert Rev Pharmacoecon Outcomes Res **18**(3): 255-265.

INTRODUCTION: The therapeutic categories of tyrosine kinase inhibitors of vascular endothelial growth factor receptors, mammalian target of rapamycin inhibitors and programmed death-1 inhibitors have transformed the treatment of metastatic renal cell cancer. Nevertheless, this comes at an increased cost, in tandem with similar fiscal pressures in the broader oncology sector, which may jeopardize the sustainability of health systems. Areas covered: To this direction, the economic evaluation of these agents is essential for rational and efficient decision-making and resource allocation process. The aim of this study is to glean, assess and present an outline of the available cost-effectiveness studies of these agents in the management of metastatic renal cell cancer. Expert Commentary: We concluded that the results of the economic evaluations are pertinent, apart from the product under evaluation, to the country setting as well.

Piacentini, M., et al. (1991). "The expression of "tissue" transglutaminase in two human cancer cell lines is related with the programmed cell death (apoptosis)." Eur J Cell Biol **54**(2): 246-254.

The expression of "tissue" transglutaminase (tTG) in two human tumor cell lines (the cervix adenocarcinoma line HeLa-TV and the neuroblastoma cells SK-N-BE-2) was found to be in correlation with the rate of physiological cell death (apoptosis) in culture. We investigated the effect of retinoic acid (RA) and alpha-difluoromethylornithine (DFMO) in order to elucidate the relationship between tTG expression and apoptosis. RA led to a 6-fold increase of tTG activity in HeLa-TV cells and to a 12-fold increase in SK-N-BE (2) cells, which was paralleled in both cell lines by a proportional increase in the number of apoptotic bodies recovered from the cultures. On the contrary, DFMO determined a dramatic reduction of tTG expression and of the apoptotic index. Immunohistochemical analysis using an anti-tTG antibody showed that the enzyme was accumulated in both cell lines within typical apoptotic bodies. Immunocytochemistry and cell cloning of SK-N-BE (2) line demonstrated that tTG was absent in cells showing neurite outgrowth, indicating that the enzyme expression is not associated with neural differentiation, even though both phenomena are elicited by retinoic acid. On the whole, these data indicate that also in tumors tTG activation takes place in cells undergoing apoptosis. The enzyme is activated in apoptotic cells to form cross-linked protein envelopes which are insoluble in detergents and chaotropic agents. The number of insoluble protein envelopes as well as the N,N-bis (gamma-glutamyl)polyamine cross-links is related with both tTG expression and apoptotic index, strongly suggesting the participation of the enzyme in the apoptotic program. (ABSTRACT TRUNCATED AT 250 WORDS)

Pietruszewska, W., et al. (2000). "[Programmed cell death research in laryngeal cancer]." Otolaryngol Pol **54 Suppl 31**: 212-215.

Apoptosis--the programmed sell death is the process of characteristic events on morphological, biochemical and molecular level which lead consequently to cell death. This process require activation of some genes i.e. p-53, mdm2 and inhibiting others i.e. bcl-2. Sixty patients with laryngeal cancer treated in ENT Department of Medical Academy of Lodz were analysed. Expression of the p-53 and bcl-2 genes' products was examined by means immunohistochemical techniques carried out on laryngeal cancer paraffin samples. Above-mentioned markers were correlated with: stage of cancer progression, recurrences and metastasis of laryngeal cancer and follow-up of the patients. Initial results indicate the possible utilisation of apoptosis as prognostic factors for the patients with laryngeal cancer.

Pignatelli, M., et al. (2005). "15-deoxy-Delta-12,14-prostaglandin J2 induces programmed cell death of breast cancer cells by a pleiotropic mechanism." Carcinogenesis **26**(1): 81-92.

Activation of peroxisome proliferator-activated receptor gamma (PPARgamma) has been found to induce cell death in a variety of cells. In this regard, we reported recently that 15-deoxy-Delta-(12,14)-prostaglandin J2 (15dPG-J2), a specific ligand of the nuclear receptor PPARgamma, inhibits proliferation and induces cellular differentiation and apoptosis in the breast cancer cell line MCF-7. In addition to PPARgamma activation other proteins, such as NF-kappaB and AP1, have been shown to be targets of 15dPG-J2. However, the mechanism by which 15dPG-J2 triggers cell death is still elusive. Our results demonstrate that 15dPG-J2 initiates breast cancer cell death via a very rapid and severe impairment of mitochondrial function, as revealed by a drop in mitochondrial membrane potential (DeltaPsi (m)), generation of reactive oxygen species (ROS) and a decrease in oxygen consumption. In addition, 15dPG-J2 can also activate an intrinsic apoptotic pathway involving phosphatidyl serine externalization, caspase activation and cytochrome c release. Bcl-2 over-expression and zVADfmk, albeit preventing caspase activation, have no effect on 15dPG-J2-mediated mytochondrial dysfunction and loss of cell viability. In contrast, the addition of radical scavengers or rotenone, which prevent 15dPG-J2-induced ROS production, block the loss of cell viability induced by this prostaglandin. Finally, 15dPG-J2-induced cell death appears to involve disruption of the microtubule cytoskeletal network. Together, these results suggest that PG-J2-induced mitochondrial dysfunction and ROS production inevitably leads to death, with or without caspases.

Pizer, E. S., et al. (1996). "Inhibition of fatty acid synthesis induces programmed cell death in human breast cancer cells." Cancer Res **56**(12): 2745-2747.

One of the key limiting factors in the treatment of advanced stage human epithelial malignancies is the lack of new, selective molecular targets for antineoplastic therapy. A substantial subset of human breast, ovarian, endometrial, colorectal, and prostatic cancers express elevated levels of fatty acid synthase, the major enzyme required for endogenous fatty acid biosynthesis, and carcinoma lines are growth inhibited by cerulenin, a noncompetitive inhibitor of fatty acid synthase. We have shown previously that the difference in fatty acid biosynthesis between cancer and normal cells is an exploitable target for metabolic inhibitors in the in vitro setting and in vivo in a human ovarian carcinoma xenograft in nude mice. Here, we report that cerulenin treatment of human breast cancer cells inhibits fatty acid synthesis within 6 h after exposure, that loss of clonogenic capacity occurs within the same interval, and that DNA fragmentation and morphological changes characteristic of apoptosis ensue.

Polonia, A., et al. (2017). "Prognostic value of stromal tumour infiltrating lymphocytes and programmed cell death-ligand 1 expression in breast cancer." J Clin Pathol **70**(10): 860-867.

AIM: The present work aims to evaluate the presence of stromal tumour-infiltrating lymphocytes (TILs) and programmed cell death-ligand 1 (PDL1) expression in breast carcinomas and their correlation with available clinicopathological features. METHODS: Two independent series of invasive breast cancer (IBC), one including ductal carcinoma in situ (DCIS) pair-matched cases, were selected, and quantification of TILs was accomplished in each case. Immunohistochemistry was also performed to evaluate the expression of PDL1. RESULTS: In both cohorts evaluated, increased stromal TILs and PDL1 expression were present in about 10% of IBCs, being significantly associated with each other and both with grade 3 and triple-negative subtype. We observed a similar distribution of stromal TILs and PDL1 expression between DCIS and IBC. Finally, we observed that increased stromal TILs and PDL1 expression were significantly associated with cancer stem cell (CSC) markers, basal cell markers and vimentin expression. Interestingly, in IBC cases with vimentin expression, increased stromal TILs, as well as decreased PDL1 expression, disclosed a better clinical outcome, independently of the main classical BC prognostic factors. CONCLUSIONS: We have confirmed the association of stromal TILs and PDL1 expression with aggressive forms of BC and that both are already found in in situ stages. We also showed

that stromal TILs and PDL1 expression are associated with clinical outcome in cases enriched for a mesenchymal immunophenotype. We describe for the first time a close relationship between CSC markers and PDL1 expression.

Rashed, H. E., et al. (2017). "Prognostic Significance of Programmed Cell Death Ligand 1 (PD-L1), CD8+ Tumor-Infiltrating Lymphocytes and p53 in Non-Small Cell Lung Cancer: An Immunohistochemical Study." Turk Patoloji Derg **1**(1): 211-222.

OBJECTIVE: Programmed cell death ligand-1 interacts with the immune receptors on the surface of CD8+ tumor infiltrating lymphocytes and PD-1, thereby blocking its anti-tumor activity. Therapeutics suppression of this interaction will show a promise in the treatment of non-small cell lung cancer by restoring the functional anti-tumor T-cell activity. We aimed to evaluate the association between the immunohistochemical expression of PD-L1, stromal CD8+ tumor infiltrating lymphocytes and p53 with the clinicopathological characteristics, response to chemotherapy, progression-free-survival, and overall survival. MATERIAL AND METHOD: We examined the immunohistochemical expression of PD-L1, stromal CD8+ TILs, and p53 expression in 50 patients with advanced stage (III & IV) non-small cell lung cancer. RESULTS: PD-L1 was expressed in 56% of the studied cases. PD-L1 expression was related to unfavorable response to the therapy without significant difference. PD-L1 expression was significantly associated with disease progression, poor progression-free-survival & overall survival. CD8+ TILs were high in 32% of the cases. Tumors with high CD8+ TILs showed a partial response to therapy and had a better progression-free-survival and overall survival. p53 expressed in 82% of the studied cases. There was a significant negative association between PD-L1 and CD8+ TILs (p=0.009), while a non-significant association was found between p53 and PD-L1 (p=0.183). CONCLUSION: PD-L1 overexpression is an unfavorable prognostic marker, while the high CD8 + TILs is a good prognostic marker in non-small cell lung cancer. PD-L1 immunohistochemical assessment may be used for the selection of patients legible for treatment with anti-PD-L1 therapy.

Ratcliffe, M. J., et al. (2017). "Agreement between Programmed Cell Death Ligand-1 Diagnostic Assays across Multiple Protein Expression Cutoffs in Non-Small Cell Lung Cancer." Clin Cancer Res **23**(14): 3585-3591.

Purpose: Immunotherapies targeting programmed cell death-1 (PD-1) and programmed cell death ligand-1 (PD-L1) demonstrate encouraging antitumor activity and manageable tolerability in non-small cell lung cancer (NSCLC), especially in patients with high tumor PD-L1 expression, as detected by companion or complementary diagnostic assays developed for individual agents. A laboratory is unlikely to use multiple assay platforms. Furthermore, commercially available diagnostic assays are not standardized, and different assay methods could lead to inappropriate treatment selection. This study establishes the extent of concordance between three validated, commercially available PD-L1 IHC diagnostic assays for NSCLC patients [Ventana SP263 (durvalumab), Dako 22C3 (pembrolizumab), and Dako 28-8 (nivolumab)].Experimental Design: Five hundred formalin-fixed, paraffin-embedded archival NSCLC samples were obtained from commercial sources. Stained slides were read in batches on an assay-by-assay basis by a single pathologist trained in all methods, in a Clinical Laboratory Improvements Amendments program-certified laboratory. An additional pathologist performed an independent review of 200 stained samples for each assay.Results: PD-L1 expression was evaluable with all assays in 493 samples. The three assays showed similar patterns of tumor membrane staining, with high correlation between percent PD-L1 staining. An overall percentage agreement of >90% was achieved between assays at multiple expression cutoffs, including 1%, 10%, 25%, and 50% tumor membrane staining.Conclusions: This study builds optimism that harmonization between assays may be possible, and that the three assays studied could potentially be used interchangeably to identify patients most likely to respond to anti-PD-1/PD-L1 immunotherapies, provided the appropriate clinically defined algorithm and agent are always linked. Clin Cancer Res; 23(14); 3585-91. (c)2017 AACR.

Ravi, D., et al. (1999). "De novo programmed cell death in oral cancer." Histopathology **34**(3): 241-249.

AIM: The importance of programmed cell death or apoptosis in the maintenance of tissue homoeostasis and the pathogenesis of oral cancer was analysed in relation to apoptosis regulatory proteins, tissue proliferation and tumour histology. METHODS AND RESULTS: The extent of apoptosis was defined by morphological criteria and the TUNEL (terminal deoxy nucleotidyl transferase-mediated dUTP biotin nick end labelling) assay. p53, bax, bcl-2 and cyclin D1 expression was evaluated by immunocytochemistry. The presence of mutant p53 was analysed using a mutant p53-specific ELISA. An inverse correlation was observed between TUNEL reactivity and histology of the lesion (r = -0.555, P = 0.0001). There was also correlation between TUNEL

reactivity and immunoreactivity of apoptosis regulatory proteins. p53 (r = 0.641, P = 0.00023), bcl-2 (r = -0.642, P = 0.00014) and bax (r = 0.651, P = 0.00002). The presence of mutant p53 protein showed an inverse correlation to the extent of apoptosis (r = -0.301, P = 0.00063). Significant correlation was evident between the bax/bcl-2 ratio and TUNEL (r = 0.652, P = 0.00001) as well as between cyclin D1 and TUNEL reactivity (r = 0.577, P = 0.00001). CONCLUSIONS: Results from this study suggest that apoptosis decreases as histological abnormality increases. Apoptotic regulatory proteins are also altered in a histologically dependent manner. Deregulated proliferation occurs simultaneously with decreased apoptosis during tumour progression in the oral mucosa.

Rebelatto, M. C., et al. (2016). "Development of a programmed cell death ligand-1 immunohistochemical assay validated for analysis of non-small cell lung cancer and head and neck squamous cell carcinoma." Diagn Pathol **11**(1): 95.

BACKGROUND: A high-quality programmed cell-death ligand 1 (PD-L1) diagnostic assay may help predict which patients are more likely to respond to anti-programmed cell death-1 (PD-1)/PD-L1 antibody-based cancer therapy. Here we describe a PD-L1 immunohistochemical (IHC) staining protocol developed by Ventana Medical Systems Inc. and key analytical parameters of its use in formalin-fixed, paraffin-embedded (FFPE) samples of non-small cell lung cancer (NSCLC) and head and neck squamous cell carcinoma (HNSCC). METHODS: An anti-human PD-L1 rabbit monoclonal antibody (SP263) was optimized for use with the VENTANA OptiView DAB IHC Detection Kit on the automated VENTANA BenchMark ULTRA platform. The VENTANA PD-L1 (SP263) Assay was validated for use with FFPE NSCLC and HNSCC tissue samples in a series of studies addressing sensitivity, specificity, robustness, and precision. Samples from a subset of 181 patients from a Phase 1/2 study of durvalumab (NCT01693562) were analyzed to determine the optimal PD-L1 staining cut-off for enriching the probability of responses to treatment. The scoring algorithm was defined using statistical analysis of clinical response data from this clinical trial and PD-L1 staining parameters in HNSCC and NSCLC tissue. Inter-reader agreement was established by three pathologists who evaluated 81 NSCLC and 100 HNSCC samples across the range of PD-L1 expression levels. RESULTS: The VENTANA PD-L1 (SP263) Assay met all pre-defined acceptance criteria. For both cancer types, a cut-off of 25 % of tumor cells with PD-L1 membrane staining of any intensity best discriminated responders from nonresponders. Samples with staining above this value were deemed to have high PD-L1 expression, and those with staining below it were deemed to have low or no PD-L1 expression. Inter-reader agreement on PD-L1 status was 97 and 92 % for NSCLC and HNSCC, respectively. CONCLUSIONS: These results highlight the robustness and reproducibility of the VENTANA PD-L1 (SP263) Assay and support its suitability for use in the evaluation of NSCLC and HNSCC FFPE tumor samples using the devised >/=25 % tumor cell staining cut-off in a clinical setting. The clinical utility of the PD-L1 diagnostic assay as a predictive biomarker will be further validated in ongoing durvalumab studies. TRIAL REGISTRATION: ClinicalTrials.gov: NCT01693562.

Reed, C. J. (2000). "Apoptosis and cancer: strategies for integrating programmed cell death." Semin Hematol **37**(4 Suppl 7): 9-16.

Virtually all human cells are endowed with the capacity to commit suicide using an evolutionarily conserved mechanism that involves activation of caspase-family cell death proteases. Caspase activation culminates in a cell death process known as "apoptosis." The activation of these intracellular proteases is carefully controlled through a delicate balance of anti- and pro-death proteins, serving to precisely regulate cell life span. Defects in the natural death pathway promote tumorigenesis by prolonging cell life span and hence cell accumulation. Low-grade B-cell malignancies, particularly follicular lymphoma and chronic lymphocytic leukemia (CLL) represent quintessential examples of human neoplasms characterized primarily by a problem with cell death rather than cell cycle. Because the cell suicide pathway is also required for tumor eradication by the immune system, anticancer drugs, and irradiation, cancer-associated defects in the cellular apoptosis machinery also play an important role in treatment failures. Monoclonal antibody-based therapies may provide opportunities to either bypass defects in apoptosis pathways or to activate latent apoptotic programs in cancer cells, particularly in lymphoid malignancies where tissue-specific antigens can be exploited for cell-selective activation of apoptosis. Recent knowledge about apoptosis pathways is reviewed, and some examples of opportunities for therapeutic intervention are discussed.

Rehman, J. A., et al. (2017). "Quantitative and pathologist-read comparison of the heterogeneity of programmed death-ligand 1 (PD-L1) expression in non-small cell lung cancer." Mod Pathol **30**(3): 340-349.

PD-L1 is expressed in a percentage of lung cancer patients and those patients show increased likelihood of response to PD-1 axis therapies.

However, the methods and assays for the assessment of PD-L1 using immunohistochemistry are variable and PD-L1 expression appears to be highly heterogeneous. Here, we examine assay heterogeneity parameters toward the goal of determining variability of sampling and the variability due to pathologist-based reading of the immunohistochemistry slide. SP142, a rabbit monoclonal antibody, was used to detect PD-L1 by both chromogenic immunohistochemistry and quantitative immunofluorescence using a laboratory-derived test. Five pathologists scored the percentage of PD-L1 positivity in tumor- and stromal-immune cells of 35 resected non-small cell lung cancer cases, each represented on three separate blocks. An intraclass correlation coefficient of 94% agreement was seen among the pathologists for the assessment of PD-L1 in tumor cells, but only 27% agreement was seen in stromal/immune cell PD-L1 expression. The block-to-block reproducibility of each pathologist's score was 94% for tumor cells and 75% among stromal/immune cells. Lin's concordance correlation coefficient between pathologists' readings and the mean immunofluorescence score among blocks was 94% in tumor and 68% in stroma. Pathologists were highly concordant for PD-L1 tumor scoring, but not for stromal/immune cell scoring. Pathologist scores and immunofluorescence scores were concordant for tumor tissue, but not for stromal/immune cells. PD-L1 expression was similar among all the three blocks from each tumor, indicating that staining of one block is enough to represent the entire tumor and that the spatial distribution of heterogeneity of expression of PD-L1 is within the area represented in a single block. Future studies are needed to determine the minimum representative tumor area for PD-L1 assessment for response to therapy.

Remon, J., et al. (2016). "Predictive biomarkers for programmed death-1/programmed death ligand immune checkpoint inhibitors in nonsmall cell lung cancer." Curr Opin Oncol **28**(2): 122-129.

PURPOSE OF REVIEW: Immune checkpoint inhibitors, antiprogrammed death receptor 1 (anti-PD-1)/antiprogrammed death-ligand 1 (anti-PD-L1), are new therapeutic regimens for managing advanced nonsmall cell lung cancer patients, giving an overall response rate of approximately 20% as monotherapy in second-line treatment. The use of predictive biomarkers for identifying patients suitable for these therapies is an important issue not only for making treatment decisions, but also from a medical economic point of view. RECENT FINDINGS: Among potential predictive biomarker candidates for anti-PD-1/PD-L1 treatments in nonsmall cell lung cancer, the expression of PD-L1 (as determined by immunohistochemistry) is currently the most studied. PD-L1 positivity has been associated with higher response rate to anti-PD-1/PD-L1 therapies. However, several observations suggest that the predictive value of PD-L1 expression is not clear-cut. We review other potential predictive biomarkers, including programmed death-ligand 2, IFN-gamma, and genetic signatures. SUMMARY: Standardized techniques and conditions for evaluating PD-L1 expression (tissue quality and age, percentage positivity threshold, managing heterogeneous and dynamic expression) are critical for establishing the use of this protein as a predictive marker. Care should be also taken when using anti-PD-1/PD-L1 therapies in combination with other therapies, which may impact the predictive value of PD-L1 expression.

Rivera, N., et al. (2017). "Hair Repigmentation During Immunotherapy Treatment With an Anti-Programmed Cell Death 1 and Anti-Programmed Cell Death Ligand 1 Agent for Lung Cancer." JAMA Dermatol **153**(11): 1162-1165.

Importance: New targeted therapies for cancer have been released in recent years, opening new horizons in the treatment of patients with cancer. However, their related adverse events (AE) are not fully characterized. Hair repigmentation (HR) is a nondescribed effect secondary to anti-programmed cell death 1 (anti-PD-1) and anti-programmed cell death ligand 1 (anti-PD-L1) therapy for treatment of lung cancer (LC), in opposition to the vitiligo reactions that develop during melanoma treatment. Objective: To describe a new adverse event occurring during anti-PD-1/anti-PD-L1 therapy for LC. Design, Setting, and Participants: A case series from a descriptive observation of 14 patients with HR after anti-PD-1/anti-PD-L1 treatment, recruited between September and December, 2016, who were followed up to detect whether they developed cutaneous AE at the time HR was detected. The patients had all been treated in the dermatology department at Hospital Universitari Germans Trias i Pujol, Badalona, Spain. Main Outcomes and Measures: Clinical observation of HR during anti-PD-1/anti-PD-L1 therapy for LC, proved by comparing old pictures provided by the patients and recent pictures taken during the follow-up. Results: Fourteen patients (13 men and 1 woman; mean age, 64.9 years) receiving anti-PD-1 or anti-PD-L1 therapy for non-small-cell lung cancer (NSCLC) presented hair repigmentation during follow-up. This hair repigmentation consisted in a diffuse darkening of the hair in 13 of 14 patients, or in black patches between white hairs in 1. Thirteen of 14 patients presented a good clinical response to the treatment, with at least stable disease, and only 1 had to stop the therapy after only 4 cycles of treatment owing to a life-threatening progression of the disease. Conclusions and Relevance:

We present to our knowledge the first report of hair repigmentation owing to anti-PD-1/anti-PD-L1 therapy for lung cancer in a series of 14 patients. Hair repigmentation may be a good response marker in patients receiving anti-PD1/anti-PD-L1 therapy for LC.

Rizvi, H., et al. (2018). "Molecular Determinants of Response to Anti-Programmed Cell Death (PD)-1 and Anti-Programmed Death-Ligand 1 (PD-L1) Blockade in Patients With Non-Small-Cell Lung Cancer Profiled With Targeted Next-Generation Sequencing." J Clin Oncol **36**(7): 633-641.

Purpose Treatment of advanced non-small-cell lung cancer with immune checkpoint inhibitors (ICIs) is characterized by durable responses and improved survival in a subset of patients. Clinically available tools to optimize use of ICIs and understand the molecular determinants of response are needed. Targeted next-generation sequencing (NGS) is increasingly routine, but its role in identifying predictors of response to ICIs is not known. Methods Detailed clinical annotation and response data were collected for patients with advanced non-small-cell lung cancer treated with anti-programmed death-1 or anti-programmed death-ligand 1 [anti-programmed cell death (PD)-1] therapy and profiled by targeted NGS (MSK-IMPACT; n = 240). Efficacy was assessed by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, and durable clinical benefit (DCB) was defined as partial response/stable disease that lasted > 6 months. Tumor mutation burden (TMB), fraction of copy number-altered genome, and gene alterations were compared among patients with DCB and no durable benefit (NDB). Whole-exome sequencing (WES) was performed for 49 patients to compare quantification of TMB by targeted NGS versus WES. Results Estimates of TMB by targeted NGS correlated well with WES (rho = 0.86; P <.001). TMB was greater in patients with DCB than with NDB (P =.006). DCB was more common, and progression-free survival was longer in patients at increasing thresholds above versus below the 50th percentile of TMB (38.6% v 25.1%; P <.001; hazard ratio, 1.38; P =.024). The fraction of copy number-altered genome was highest in those with NDB. Variants in EGFR and STK11 associated with a lack of benefit. TMB and PD-L1 expression were independent variables, and a composite of TMB plus PD-L1 further enriched for benefit to ICIs. Conclusion Targeted NGS accurately estimates TMB and elevated TMB further improved likelihood of benefit to ICIs. TMB did not correlate with PD-L1 expression; both variables had similar predictive capacity. The incorporation of both TMB and PD-L1 expression into multivariable predictive models should result in greater predictive power.

Rom-Jurek, E. M., et al. (2018). "Regulation of Programmed Death Ligand 1 (PD-L1) Expression in Breast Cancer Cell Lines In Vitro and in Immunodeficient and Humanized Tumor Mice." Int J Mol Sci **19**(2).

Programmed death ligand 1 (PD-L1) expression is an efficient strategy of tumor cells to escape immunological eradiation. However, only little is known about the factors that affect the cellular expression levels. Here we assessed the PD-L1 expression on different breast cancer cell lines under standard in vitro culture conditions and as a function of Epirubicin or Paclitaxel treatment. Moreover, we evaluated the expression in immunodeficient tumor mice as well as in humanized tumor mice (i.e., in the presence of a human immune system). We found highest PD-L1 levels in JIMT-1 and MDA-MB-231 cells. Epirubicin treatment caused a decrease and Paclitaxel treatment an increased PD-L1 expression in MDA-MB-231 cells. In addition, we identified nuclear PD-L1 in MDA-MB-231 cells. All in vivo transplanted breast cancer cell lines downregulated PD-L1 expression compared to their in vitro counterpart. Neither the gene copy number nor the presence of human immune system in humanized tumor mice had an effect on the PD-L1 content. We demonstrate that the degree of PD-L1 expression amongst breast cancer cell lines varies considerably. In addition, cytotoxic treatments and other extrinsic parameters differentially affect the expression. Hence, further investigations including in vivo evaluations are necessary to understand PD-L1 regulation for advanced breast cancer stratification.

Roy, A., et al. (2018). "Methylglyoxal at metronomic doses sensitizes breast cancer cells to doxorubicin and cisplatin causing synergistic induction of programmed cell death and inhibition of stemness." Biochem Pharmacol **156**: 322-339.

Potent anticancer activity coupled with absence of toxicity at therapeutic dose established the glycolytic metabolite, methylglyoxal, as a promising candidate against malignant neoplasia. In this preclinical study we illustrate the applicability of methylglyoxal in formulating an optimally designed combination regimen with chemotherapeutic drugs against breast cancer. Results demonstrated a synergistic augmentation in doxorubicin and cisplatin mediated cytotoxicity in human breast cancer cell lines MDA MB 231 & MCF 7 with methylglyoxal co-treatment at metronomic concentrations. The cell death due to combination treatment was significantly prevented by N-Acetylcysteine and the synergistic effects were attenuated in presence of inhibitors for apoptosis and necroptosis, in MDA MB 231 and MCF 7 cells, respectively. Additionally, acridine orange

staining and immunoblotting with LC3B antibody indicated the suppression of doxorubicin induced autophagy flux with methylglyoxal co-treatment. This report documents for the first time the preferential targeting of breast cancer stem cells by methylglyoxal. Combination treatment with doxorubicin or cisplatin hindered mammosphere forming efficiency and inclusively eliminated both cancer stem as well as non-stem cancer cells. The synergistic effect was validated in Ehrlich mammary carcinoma cell induced murine ascites model and the combination advantage in vivo was achieved without any additional deleterious effect to liver and kidney. Our present study evidences the implications of methylglyoxal inclusion in adjuvant multimodal chemotherapeutics against breast cancer and offers noteworthy insights into the possible outcome.

Scordino, A., et al. (2014). "Delayed luminescence to monitor programmed cell death induced by berberine on thyroid cancer cells." J Biomed Opt **19**(11): 117005.

Correlation between apoptosis and UVA-induced ultraweak photon emission delayed luminescence (DL) from tumor thyroid cell lines was investigated. In particular, the effects of berberine, an alkaloid that has been reported to have anticancer activities, on two cancer cell lines were studied. The FTC-133 and 8305C cell lines, as representative of follicular and anaplastic thyroid human cancer, respectively, were chosen. The results show that berberine is able to arrest cell cycle and activate apoptotic pathway as shown in both cell lines by deoxyribonucleic acid fragmentation, caspase-3 cleavage, p53 and p27 protein overexpression. In parallel, changes in DL spectral components after berberine treatment support the hypothesis that DL from human cells originates mainly from mitochondria, since berberine acts especially at the mitochondrial level. The decrease of DL blue component for both cell lines could be related to the decrease of intra-mitochondrial nicotinamide adenine dinucleotide and may be a hallmark of induced apoptosis. In contrast, the response in the red spectral range is different for the two cell lines and may be ascribed to a different iron homeostasis.

Shen, H., et al. (2018). "[Expression and distribution of programmed death receptor 1 and T cell immunoglobulin mucin 3 in breast cancer microenvironment and its relationship with clinicopathological features]." Zhonghua Yi Xue Za Zhi **98**(17): 1352-1357.

Objective: To explore the expression and distribution of programmed death receptor 1 (PD-1) and T-cell immunoglobulin mucin 3 (TIM-3) in breast cancer microenvironment and analyze the their correlation with the clinicopathological features. Methods: The specimens of tumor tissue and adjacent tissues from 30 patients with infiltrative breast cancer who were diagnosed as breast cancer from June 2016 to May 2017 in The First Hospital of Jiaxing were collected, and the specimen were divided into two parts along the center. After embedding and cryosectioning, the expression and distribution of PD-1 and TIM-3 protein in tumor tissues were observed by immunofluorescence staining. Another part of the specimen was cut and digested, and non-continuous density gradient centrifugation was used to extract tumor-infiltrating lymphocytes (TILs), real-time quantitative PCR (qRT-PCR) was used to detect the mRNA expression of PD-1 and TIM-3 in TILs. Meanwhile, the protein expression was determined by Western blotting. The relationship between the expression of PD-1 and TIM-3 and pathological parameters of breast cancer was analyzed with correlation analysis. Results: Immunofluorescence results showed that more PD-1 and TIM-3 positive cells were observed in the tumor tissues compared with the tumor-adjacent tissues. The qRT-PCR showed that the expression of PD-1 and TIM-3 mRNA in TILs were both significantly higher than those in paracancerous tissues (3.09+/-0.38 vs 1.26+/-0.23, 3.42+/-0.31 vs 1.57+/-0.29, t=4.16, 4.37, both P<0.05). At the protein level, the expression of PD-1 and TIM-3 in tumor tissue lymphocytes (0.66+/-0.08, 0.80+/-0.11) was significantly higher than those in cancerous tissues (0.10+/-0.01, 0.26+/-0.02) (t=6.79, 4.57, both P<0.05). There were significant differences in the expression of PD-1, TIM-3 mRNA in the TILs between the different tumor histological grades, tumor sizes, lymph node metastasis (t=2.22-2.99, all P<0.05). Correlation analysis showed that there was a significant positive correlation between the expression of PD-1 and TIM-3 in tumor tissues (r=0.616, P<0.01). Conclusions: In the breast cancer microenvironment, PD-1, TIM-3-mediated signaling pathway plays an important role in the occurrence and development of breast cancer, it provides a new basis for the combination therapy of breast cancer.

Shen, T., et al. (2017). "Prognostic value of programmed cell death protein 1 expression on CD8+ T lymphocytes in pancreatic cancer." Sci Rep **7**(1): 7848.

Pancreatic cancer is one of the most aggressive malignancies and has a highly immunosuppressive tumour microenvironment. Immune checkpoint blockade has led to remarkable and durable objective responses in a number of malignancies and antibody-based strategies targeting programmed cell death protein 1 (PD-1) are showing promise where traditional modalities of surgery, radiotherapy, and

chemotherapy have failed. In this study, we examined the clinical value of PD-1 protein expression by CD8+ peripheral T lymphocytes or tumour-infiltrating T lymphocytes (TILs) in pancreatic ductal adenocarcinoma (PDAC). Expression of PD-1 protein on CD8+ TILs correlated with overall survival and clinicopathological characteristics such as clinical stage, N classification, and M classification. Similar findings were observed for the expression of PD-1 protein on peripheral CD8+ T cells, whereas its expression on peripheral CD4+ T cells showed no significance. Comparison of the levels of PD-1 protein expressed by peripheral CD8+ T cells before and 4 weeks after surgery indicated that preoperative and postoperative status of peripheral PD-1 expression was unchanged. Our findings showed that PD-1 protein expressed by peripheral or tumour-infiltrated CD8+ T cells was a promising biomarker for diagnosis and prognosis in PDAC and might help guide future immunotherapies.

Shibutani, M., et al. (2017). "The Prognostic Significance of the Tumor-infiltrating Programmed Cell Death-1(+) to CD8(+) Lymphocyte Ratio in Patients with Colorectal Cancer." Anticancer Res **37**(8): 4165-4172.

BACKGROUND/AIM: Tumor-infiltrating lymphocytes (TILs) have been reported to reflect the antitumor immunity of the host and correlate with the therapeutic outcomes and survival. Nowadays TILs are attracting attention as new biomarkers of diseases such as colorectal cancer. TILs are classified into several subsets, among which CD8(+) T cells directly attack cancer cells and play a central role in antitumor immunity. A high density of CD8(+) TILs has been reported to correlate with a better clinical outcome. Programmed cell death-1 (PD-1) is recognized to be a surface marker for dysfunction of T lymphocytes. However, the prognostic significance of PD-1(+) TILs remains unclear. The aim of this study was to evaluate the prognostic significance of the number of PD-1(+) TILs and the tumor-infiltrating PD-1(+) to CD8(+) lymphocyte ratio (PD-1/CD8 ratio) in patients with colorectal cancer (CRC). PATIENTS AND METHODS: A total of 90 patients with stage II/III CRC who underwent curative surgery were enrolled in this study. Immunohistochemistry was used to assess the densities of PD-1(+) TILs and CD8(+) TILs. The PD-1/CD8 ratio was defined as the number of PD-1(+) TILs divided by the number of CD8(+) TILs. The optimum cut-off value for the number of PD-1(+) TILs and the PD-1/CD8 ratio was determined via a receiver operating characteristic analysis. We then assessed the prognostic significance of the number of PD-1(+) TILs and the PD-1/CD8 ratio. RESULTS: The relapse-free and overall survival rates were significantly worse in the high-PD-1/CD8 ratio group than in the low-PD-1/CD8 ratio group (relapse-free survival: p=0.0257, overall survival: p=0.0363), although the number of PD-1(+) TILs showed no prognostic significance. CONCLUSION: The PD-1/CD8 ratio may, therefore, be a useful prognostic marker for stage II/III CRC. What is important for predicting the prognosis may be the PD-1/CD8 ratio rather than the absolute number of PD-1(+) TILs.

Shimoji, M., et al. (2016). "Clinical and pathologic features of lung cancer expressing programmed cell death ligand 1 (PD-L1)." Lung Cancer **98**: 69-75.

BACKGROUND: Programmed cell death 1 (PD-1) negatively regulates antigen receptor signaling upon binding by either of its ligands, programmed cell death ligand 1 or 2 (PD-L1/2). Blockade of this interaction with either PD-1 or PD-L1 antibodies has been successful in the treatment of human cancer, especially melanoma and non-small cell lung cancer. PD-L1 expression has been proposed as a predictor of tumor response. However, the relationships between PD-L1 expression and various clinicopathological characteristics remain unclear. MATERIALS AND METHODS: PD-L1 expression was examined in 220 non-small cell lung cancer specimens that were consecutively resected at our hospital after validating the E1L3N antibody immunohistochemical assay by comparing IHC and RT-PCR data for lung cancer cell lines. We evaluated the relationships between PD-L1 positivity, several clinical factors and the immunohistochemical expression of epithelial-mesenchymal transition (EMT), cancer stem cell and proliferative markers. RESULTS: PD-L1 was expressed in 22% of lung adenocarcinomas and 60% of squamous cell lung cancers. There was no significant association between PD-L1 expression and clinicopathological features in squamous cell lung cancer. However, in patients with lung adenocarcinoma, PD-L1 expression was significantly correlated with solid subtype histology, vimentin expression, increased Ki-67 labeling index and poor prognosis by multivariate analysis. CONCLUSION: PD-L1 expression was associated with high proliferative activity and the EMT phenotype in adenocarcinoma but not in squamous cell carcinoma of the lung. PD-L1 expression was a significant poor prognostic factor in patients with lung adenocarcinoma.

Shiota, M., et al. (2009). "Programmed cell death protein 4 down-regulates Y-box binding protein-1 expression via a direct interaction with Twist1 to suppress cancer cell growth." Cancer Res **69**(7): 3148-3156.

Programmed cell death protein 4 (PDCD4) has recently been shown to be involved in both transcription and translation, and to regulate cell growth. However, the mechanisms underlying PDCD4 function are not well understood. In this study, we show that PDCD4 interacts directly with the transcription factor Twist1 and leads to reduced cell growth through the down-regulation of the Twist1 target gene Y-box binding protein-1 (YB-1). PDCD4 interacts with the DNA binding domain of Twist1, inhibiting its DNA binding ability and YB-1 expression. Immunohistochemical analysis showed that an inverse correlation between nuclear PDCD4 and YB-1 expression levels was observed in 37 clinical prostate cancer specimens. Growth suppression by PDCD4 expression was completely recovered by either Twist1 or YB-1 expression. Moreover, PDCD4-overexpressing cells are sensitive to cisplatin and paclitaxel but not to etoposide or 5-fluorouracil. In summary, PDCD4 negatively regulates YB-1 expression via its interaction with Twist1 and is involved in cancer cell growth and chemoresistance.

Shirali, A. C., et al. (2016). "Association of Acute Interstitial Nephritis With Programmed Cell Death 1 Inhibitor Therapy in Lung Cancer Patients." Am J Kidney Dis **68**(2): 287-291.

Immune checkpoint inhibitors that target the programmed death 1 (PD-1) signaling pathway have recently been approved for use in advanced pretreated non-small cell lung cancer and melanoma. Clinical trial data suggest that these drugs may have adverse effects on the kidney, but these effects have not been well described. We present 6 cases of acute kidney injury in patients with lung cancer who received anti-PD-1 antibodies, with each case displaying evidence of acute interstitial nephritis (AIN) on kidney biopsy. All patients were also treated with other drugs (proton pump inhibitors and nonsteroidal anti-inflammatory drugs) linked to AIN, but in most cases, use of these drugs long preceded PD-1 inhibitor therapy. The association of AIN with these drugs in our patients raises the possibility that PD-1 inhibitor therapy may release suppression of T-cell immunity that normally permits renal tolerance of drugs known to be associated with AIN.

Shosu, K., et al. (2016). "Programmed Cell Death Ligand 1 Expression in Canine Cancer." In Vivo **30**(3): 195-204.

BACKGROUND: Antibody therapy targeting programmed cell death-1 (PD-1) and programmed cell death-ligand 1 (PD-L1) is a promising therapy in human cancer, but only limited information on PD-L1 expression in canine tumors is available. MATERIALS AND METHODS: PD-L1 expression was examined in 31 canine tumor cell lines of various origins by flow cytometry and western blotting, and in canine tumor and normal tissue specimens by immunohistochemistry. RESULTS: PD-L1 was only expressed on the cell surface of a small number of cell lines but was found expressed within the cells of almost all cell lines. Immunohistochemistry revealed that PD-L1 is frequently expressed in malignant melanoma, mammary gland tumor, mast cell tumor and lymphoma, but less frequently in soft-tissue sarcoma and hemangiosarcoma. PD-L1 was also expressed in some of the cells of normal canine tissue specimens. CONCLUSION: Canine tumors with PD-L1 expression that were identified in this study are potential candidates for antiPD-1 and antiPD-L1 therapy.

Shrivastava, A., et al. (2011). "Cannabidiol induces programmed cell death in breast cancer cells by coordinating the cross-talk between apoptosis and autophagy." Mol Cancer Ther **10**(7): 1161-1172.

Cannabidiol (CBD), a major nonpsychoactive constituent of cannabis, is considered an antineoplastic agent on the basis of its in vitro and in vivo activity against tumor cells. However, the exact molecular mechanism through which CBD mediates this activity is yet to be elucidated. Here, we have shown CBD-induced cell death of breast cancer cells, independent of cannabinoid and vallinoid receptor activation. Electron microscopy revealed morphologies consistent with the coexistence of autophagy and apoptosis. Western blot analysis confirmed these findings. We showed that CBD induces endoplasmic reticulum stress and, subsequently, inhibits AKT and mTOR signaling as shown by decreased levels of phosphorylated mTOR and 4EBP1, and cyclin D1. Analyzing further the cross-talk between the autophagic and apoptotic signaling pathways, we found that beclin1 plays a central role in the induction of CBD-mediated apoptosis in MDA-MB-231 breast cancer cells. Although CBD enhances the interaction between beclin1 and Vps34, it inhibits the association between beclin1 and Bcl-2. In addition, we showed that CBD reduces mitochondrial membrane potential, triggers the translocation of BID to the mitochondria, the release of cytochrome c to the cytosol, and, ultimately, the activation of the intrinsic apoptotic pathway in breast cancer cells. CBD increased the generation of reactive oxygen species (ROS), and ROS inhibition blocked the induction of apoptosis and autophagy. Our study revealed an intricate interplay between apoptosis and autophagy in CBD-treated breast cancer cells and highlighted the value of continued investigation into the potential use of CBD as an antineoplastic agent.

Soo, R. A., et al. (2018). "Determinants of variability of five programmed death ligand-1 immunohistochemistry assays in non-small cell lung cancer samples." Oncotarget **9**(6): 6841-6851.

Programmed death ligand-1 (PD-L1) expression as determined by immunohistochemistry (IHC) is potentially predictive of clinical outcome. The aim of this study was to assess the concordance of reported PD-L1 IHC assays and investigate factors influencing variability. Consecutive sections from 20 non-small cell lung cancers (NSCLCs) comprising resection, core biopsy, cytology and pleural fluid samples underwent IHC with 5 different antibody/autostainer combinations: 22C3/Link48, 28-8/BOND-MAX, E1L3N/BOND-MAX, SP142/BenchMark and SP263/BenchMark. PD-L1 RNA levels were assessed using RNAscope. The frequency of positive cases using scoring thresholds from clinical trials was 72%, 33%, 61%, 56%, and 33% for the 5 IHC protocols respectively, and 33% for RNAscope. Pairwise agreement on the classification of cases as positive or negative for PD-L1 expression ranged from 61%-94%. On a continuous scale, the lowest correlation was between 28-8/BOND-MAX and SP142/BenchMark (R (2)=0.25) and highest was between 22C3/Link48 and E1L3N/BOND-MAX (R (2)=0.71). When cases were ordered according to tumor cell (TC)%, a similar ranking of cases across IHC protocols could be observed, albeit with different quanta and limits of detection. Single-slide OPAL 7-color fluorescence IHC analysis revealed a high degree of co-localization of staining from the 5 PD-L1 antibodies. Using SP142 antibody in a BOND-MAX protocol led to increased TC% quanta, while retaining a similar ranking of samples according to TC%. The results of this study highlight tumor PD-L1 status can vary significantly according to IHC protocol. Protocol-dependent staining intensities and nominated thresholds for positivity contribute to this variability, while the antibody used appears to be less of a factor.

Strasser, A., et al. (2011). "Deciphering the rules of programmed cell death to improve therapy of cancer and other diseases." EMBO J **30**(18): 3667-3683.

Apoptosis, the major form of programmed cell death in metazoan organisms, plays critical roles in normal development, tissue homeostasis and immunity, and its disturbed regulation contributes to many pathological states, including cancer, autoimmunity, infection and degenerative disorders. In vertebrates, it can be triggered either by engagement of 'death receptors' of the tumour necrosis factor receptor family on the cell surface or by diverse intracellular signals that act upon the Bcl-2 protein family, which controls the integrity of the mitochondrial outer membrane through the complex interactions of family members. Both pathways lead to cellular demolition by dedicated proteases termed caspases. This review discusses the groundbreaking experiments from many laboratories that have clarified cell death regulation and galvanised efforts to translate this knowledge into novel therapeutic strategies for the treatment of malignant and perhaps certain autoimmune and infectious diseases.

Su, Z., et al. (2001). "A combinatorial approach for selectively inducing programmed cell death in human pancreatic cancer cells." Proc Natl Acad Sci U S A **98**(18): 10332-10337.

Pancreatic cancer is an extremely aggressive neoplasm whose incidence equals its death rate. Despite intensive analysis, the genetic changes that mediate pancreatic cancer development and effective therapies for diminishing the morbidity associated with this disease remain unresolved. Through subtraction hybridization, we have identified a gene associated with induction of irreversible growth arrest, cancer reversion, and terminal differentiation in human melanoma cells, melanoma differentiation associated gene-7 (mda-7). Ectopic expression of mda-7 when using a recombinant adenovirus, Ad.mda-7, results in growth suppression and apoptosis in a broad spectrum of human cancers with diverse genetic defects, without exerting deleterious effects in normal human epithelial or fibroblast cells. Despite the apparently ubiquitous antitumor effects of mda-7, pancreatic carcinoma cells are remarkably refractory to Ad.mda-7 induced growth suppression and apoptosis. In contrast, the combination of Ad.mda-7 with antisense phosphorothioate oligonucleotides, which target the K-ras oncogene (a gene that is mutated in 85 to 95% of pancreatic carcinomas), induces a dramatic suppression in growth and a decrease in cell viability by induction of apoptosis. In mutant K-ras pancreatic carcinoma cells, programmed cell death correlates with expression and an increase, respectively, in MDA-7 and BAX proteins and increases in the ratio of BAX to BCL-2 proteins. Moreover, transfection of mutant K-ras pancreatic carcinoma cells with an antisense K-ras expression vector and infection with Ad.mda-7 inhibits colony formation in vitro and tumorigenesis in vivo in nude mice. These intriguing observations demonstrate that a combinatorial approach, consisting of a cancer-specific apoptosis-inducing gene and an oncogene inactivation strategy, may provide the foundation for developing an effective therapy for pancreatic cancer.

Suda, K., et al. (2017). "Increased EGFR Phosphorylation Correlates with Higher Programmed Death Ligand-1 Expression: Analysis of TKI-Resistant

Lung Cancer Cell Lines." Biomed Res Int **2017**: 7694202.

Despite the recent development of immunotherapies that target programmed death-1 (PD-1) or programmed death ligand-1 (PD-L1) in non-small cell lung cancer (NSCLC) treatment, these therapies are less effective in NSCLC patients with epidermal growth factor receptor (EGFR) mutations. However, the molecular mechanisms underlying this lower efficacy of immunotherapies in EGFR mutant lung cancers are still unclear. In this study, we analyzed PD-L1 protein expression in lung cancer cell lines with EGFR mutations prior to and after acquisition of resistance to EGFR tyrosine kinase inhibitors (TKIs). We found that parental lung cancer cell lines harboring EGFR mutations showed negative (PC9 and H3255 cells) and positive (HCC827 cells) staining for PD-L1 by immunohistochemistry. Comparing PD-L1 expression between EGFR-TKI resistant cell lines and their parental cells, we found that increased phosphorylation of EGFR was related to increased expression of PD-L1. Increased phosphorylation of EGFR was accompanied by the T790M secondary mutation. Acquired resistance cells with MET amplification or EGFR loss both showed decreased phosphorylation of EGFR and decreased PD-L1 expression. Our results indicate that lung cancer cell lines with EGFR mutations (parental cells) do not harbor high PD-L1 protein expression. In addition, EGFR phosphorylation affects PD-L1 expression after acquisition of resistance to EGFR-TKIs.

Sueoka, N., et al. (2000). "Insulin-like growth factor binding protein-6 activates programmed cell death in non-small cell lung cancer cells." Oncogene **19**(38): 4432-4436.

Insulin-like growth factor binding proteins (IGFBPs) are secreted into the extra-cellular matrix and inhibit cell growth through IGF-dependent and -independent mechanisms. In this study, we investigated the role of IGFBP-6, a relatively unexplored member of the IGFBP family, in the proliferation of non-small cell lung cancer (NSCLC) cells. Infection of NSCLC cell lines in vitro with an adenovirus expressing human IGFBP-6 under the control of a CMV promoter (Ad5CMV-BP6) reduced NSCLC cell number through activation of programmed cell death, as shown by cell staining with Hoechst 33342 or DNA end-labeling with bromodeoxyuridine triphosphate. The growth regulatory effect of IGFBP-6 was investigated in vivo by intratumoral injection of Ad5CMV-BP6 in NSCLC xenografts established in nu/nu mice. A single injection of Ad5CMV-BP6 reduced the size of NSCLC xenografts by 45%. These findings indicate that IGFBP-6 is a potent inducer of programmed cell death in cancer cells and support investigations into IGFBP-6 as a potential target in cancer therapeutics.

Sui, J. D., et al. (2018). "Risk of hematologic toxicities with programmed cell death-1 inhibitors in cancer patients: a meta-analysis of current studies." Drug Des Devel Ther **12**: 1645-1657.

Background: Programmed cell death-1 (PD-1) inhibitor-related hematologic toxicities are a category of rare but clinically serious and potentially life-threatening adverse events; however, little is known about their risks across different treatment regimens and tumor types. The objective of this study was to compare the incidences of PD-1 inhibitor-related hematologic toxicities among different therapeutic regimens and tumor types. Methods: Twenty-six original articles on PD-1 inhibitor trials were identified based on a PubMed search completed on September 26, 2017. The incidences of hematologic toxicities were collected. Results: A total of 26 studies containing 5,088 patients were included in the meta-analysis. PD-1 inhibitor monotherapy was associated with an increased risk of all-grade anemia in cancer patients (5%, 95% CI 4%-6%), particularly in patients with renal cell carcinoma (RCC) (8%, 95% CI 6%-12%), compared with all-grade thrombocytopenia (2%, 95% CI 1%-5%), leukopenia (2%, 95% CI 1%-3%), and neutropenia (1%, 95% CI 0-1%). However, low incidences of high-grade hematologic toxicities were observed in cancer patients treated with PD-1 inhibitor monotherapy. The use of PD-1 inhibitors in combination with ipilimumab, peptide vaccines, or chemotherapy had significantly higher risks than PD-1 inhibitor monotherapy for all-grade anemia (13%, 95% CI 5%-31%), thrombocytopenia (6%, 95% CI 2%-18%), leukopenia (5%, 95% CI 1%-35%), neutropenia (4%, 95% CI 1%-26%), and only high-grade thrombocytopenia (4%, 95% CI 1%-15%). In addition, all-grade and high-grade hematologic toxicities in chemotherapy and everolimus treatment arms were more frequent than in PD-1 inhibitor monotherapy arms. Conclusion: The risks of PD-1 inhibitor-related hematologic toxicities were higher in RCC than in other cancers, and during combination therapy. These results may contribute toward enhancing awareness among clinicians about frequent clinical monitoring when managing PD-1 inhibitors.

Sul, J., et al. (2016). "FDA Approval Summary: Pembrolizumab for the Treatment of Patients With Metastatic Non-Small Cell Lung Cancer Whose Tumors Express Programmed Death-Ligand 1." Oncologist **21**(5): 643-650.

UNLABELLED:: On October 2, 2015, the U.S. Food and Drug Administration (FDA) granted

accelerated approval for pembrolizumab, a breakthrough therapy-designated drug, for the treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors express programmed death-ligand 1 (PD-L1), as determined by an FDA-approved test, and who have disease progression on or after platinum-containing chemotherapy or targeted therapy against anaplastic lymphoma kinase or epidermal growth factor receptor, if appropriate. This indication was approved concurrently with the PD-L1 immunohistochemistry 22C3 pharmDx, a companion diagnostic test for patient selection based on PD-L1 tumor expression. The accelerated approval was granted based on durable objective response rate (ORR) and an acceptable toxicity profile demonstrated in a multicenter, open-label trial enrolling 550 patients with metastatic NSCLC. The efficacy population comprised 61 patients with tumors identified as strongly positive for PD-L1, and the confirmed ORR as determined by blinded independent central review was 41% (95% confidence interval: 28.6%, 54.3%); all were partial responses. At the time of the analysis, responses were ongoing in 21 of 25 patients (84%), with 11 patients (44%) having response duration of >/=6 months. The most commonly occurring (>/=20%) adverse reactions included fatigue, decreased appetite, dyspnea, and cough. The most frequent (>/=2%) serious adverse drug reactions were pleural effusion, pneumonia, dyspnea, pulmonary embolism, and pneumonitis. Immune-mediated adverse reactions occurred in 13% of patients and included pneumonitis, colitis, hypophysitis, and thyroid disorders. The accelerated approval regulations describe approval of drugs and biologic products for serious and life-threatening illnesses based on a surrogate endpoint likely to predict clinical benefit. Under these regulations, a confirmatory trial or trials is required to verify and describe the benefit of pembrolizumab for patients with metastatic NSCLC. IMPLICATIONS FOR PRACTICE: This report presents key information on the U.S. Food and Drug Administration (FDA) accelerated approval of pembrolizumab for the treatment of patients with metastatic non-small cell lung cancer whose tumors express programmed death-ligand 1, as determined by an FDA-approved test, and who have disease progression on or after platinum-containing chemotherapy or targeted therapy against anaplastic lymphoma kinase or epidermal growth factor receptor, if appropriate. The report discusses the data supporting the approval decision, specifically highlighting the incorporation of a companion diagnostic in the key study and the optimal dose of pembrolizumab.

Takahashi, N., et al. (2016). "Serum levels of soluble programmed cell death ligand 1 as a prognostic factor on the first-line treatment of metastatic or recurrent gastric cancer." J Cancer Res Clin Oncol **142**(8): 1727-1738.

PURPOSE: Immune checkpoint molecules are key targets for the treatment of various malignancies. Due to the heterogeneity of advanced gastric cancer (GC), the role of programmed cell death ligand 1 (PD-L1) expression as a tumor biomarker remains controversial. In this study, the prognostic value of soluble PD-L1 (sPD-L1) levels in serum samples was assessed in patients with metastatic GC. METHODS: All patients received first-line treatment with fluoropyrimidine and platinum chemotherapy, and trastuzumab was added for HER2-positive patients. Serum levels of sPD-L1 were measured by enzyme-linked immunosorbent assay. RESULTS: Among 75 metastatic GC patients, the median serum sPD-L1 level was 0.704 ng/ml (range <0.156-3.214). Serum sPD-L1 was significantly higher in patients with a high versus a low white blood cell count at baseline. When the cutoff value was set as the median, multivariate analyses showed that high sPD-L1 levels were associated with worse overall survival compared with low sPD-L1 levels (HR 2.218, 95 % CI 1.139-4.320, P = 0.019). Regardless of HER2 status, overall survival tended to be shorter in patients with high sPD-L1 compared with low sPD-L1. There was no significant association between sPD-L1 level and progression-free survival on the first-line treatment of metastatic GC. CONCLUSIONS: High serum levels of sPD-L1 correlated with worse overall survival on the first-line chemotherapy in metastatic GC patients.

Takamori, S., et al. (2017). "Discrepancy in Programmed Cell Death-Ligand 1 Between Primary and Metastatic Non-small Cell Lung Cancer." Anticancer Res **37**(8): 4223-4228.

AIM: To investigate the discordance in the programmed cell death-ligand 1 (PD-L1) expression between primary and metastatic tumors and analyze the association between the discordance and the clinical factors in non-small cell lung cancer (NSCLC) patients. PATIENTS AND METHODS: Twenty-one NSCLC patients who underwent surgery or biopsy for paired primary and metastatic lesions at our Institution from 2005 to 2016 were analyzed. Lesions with the PD-L1 expression being >/=5% were considered PD-L1-positive. RESULTS: The metastatic sites included the brain (n=16), adrenal gland (n=3), spleen (n=1) and jejunum (n=1). Negative conversion of the primary PD-L1-positive NSCLC and positive conversion of the primary PD-L1-negative NSCLC were observed in 3 (14%) and 2 (10%) cases, respectively. Radiotherapy for the metastatic brain

lesion before its resection showed a significant relationship with the positive conversion of the primary PD-L1-negative NSCLC (p=0.048). CONCLUSION: Radiotherapy-derived effects may contribute to the positive conversion of the primary PD-L1-negative NSCLC.

Tibaldi, C., et al. (2017). "Use of programmed cell death protein ligand 1 assay to predict the outcomes of non-small cell lung cancer patients treated with immune checkpoint inhibitors." World J Clin Oncol **8**(4): 320-328.

The recent discovery of immune checkpoints inhibitors, especially anti-programmed cell death protein 1 (PD-1) and anti-programmed cell death protein ligand 1 (PD-L1) monoclonal antibodies, has opened new scenarios in the management of non-small cell lung cancer (NSCLC) and this new class of drugs has achieved a rapid development in the treatment of this disease. However, considering the costs of these drugs and the fact that only a subset of patients experience long-term disease control, the identification of predictive biomarkers for the selection of candidates suitable for treatment has become a priority. The research focused mainly on the expression of the PD-L1 receptor on both tumor cells and/or immune infiltrates determined by immunohistochemistry (IHC). However, different checkpoint inhibitors were tested, different IHC assays were used, different targets were considered (tumor cells, immune infiltrates or both) and different expression thresholds were employed in clinical trials. In some trials the assay was used prospectively to select the patients, while in other trials it was evaluated retrospectively. Some confusion emerges, which makes it difficult to easily compare the literature data and to translate them in practice management. This mini-review shows the possibilities and pitfalls of the PD-L1 expression to predict the activity and efficacy of anti PD1/PD-L1 monoclonal antibodies in the treatment of NSCLC.

Tombal, B., et al. (1995). "[Role of intracellular calcium in the programmed cell death of prostatic cancer cells]." Acta Urol Belg **63**(1): 1-5.

The growth of any tissue depends on the quantitative relationship between the rate of cell proliferation and cell death. In normal adults tissues, this steady-state balance is regulated by a series of both systemic hormones and local growth factors. Programmed cell death, also called apoptosis, is one of the two pathways for cell death. In programmed cell death, specific intracellular signals induce the cell to undergo an active, energy-dependent process leading to a cascade of biochemical and morphologic events that result in the irreversible fragmentation of genomic DNA and then of the cell itself. Biochemical and morphological studies have demonstrated that the involution of the normal, hyperplastic and cancerous prostate after castration is the result of programmed cell death. Numerous group have studied the role of calcium in programmed cell death's triggering. In androgen-independent prostatic cell it had been proved that it was possible to trigger programmed cell death by inducing a sustained elevation of the intracellular calcium. Actually, some features tend to explain more precisely the exact role of calcium in apoptosis of prostatic cells.

Toyokawa, G., et al. (2016). "Favorable Disease-free Survival Associated with Programmed Death Ligand 1 Expression in Patients with Surgically Resected Small-cell Lung Cancer." Anticancer Res **36**(8): 4329-4336.

BACKGROUND: The prognostic significance of programmed death ligand 1 (PD-L1) has been reported in non-small cell lung cancer; however, the significance of PD-L1 expression in patients with resected small-cell lung cancer (SCLC) remains to be clarified. MATERIALS AND METHODS: Forty patients with SCLC whose resected specimens were available for immunohistochemistry for PD-L1 were evaluated to determine the association between its expression and the clinicopathological factors and prognosis. RESULTS: Among 40 patients, PD-L1 was expressed in tumor cells (TCs) of six (15%), tumor-infiltrating cells (ICs) of 16 (40%), and TCs and/or ICs cells of 18 (45%) patients. Patients with PD-L1-positve ICs and TCs and/or ICs exhibited significantly longer disease-free survival than those without PD-L1-expression (hazard ratio (HR)=0.268; 95% confidence interval (CI)=0.100-0.645; p=0.003 and HR=0.301; 95% CI=0.118-0.702; p=0.005, respectively). CONCLUSION: This study provides important evidence on the prognostic value of the PD-L1 expression in resected SCLC patients.

Tsai, E. B., et al. (2018). "Feasibility and Safety of Intrathoracic Biopsy and Repeat Biopsy for Evaluation of Programmed Cell Death Ligand-1 Expression for Immunotherapy in Non-Small Cell Lung Cancer." Radiology **287**(1): 326-332.

Purpose To determine feasibility and safety of biopsy and repeat biopsy for assessment of programmed cell death ligand-1 (PD-L1) status. Materials and Methods This retrospective analysis reviewed 101 patients who underwent transthoracic core needle biopsy for the KEYNOTE-001 (MK-3475) clinical trial of pembrolizumab, an antiprogrammed cell death-1 therapy for non-small cell lung cancer, from May 2012 to September 2014. Sixty-one male patients (mean age, 66.1 years; range 36-83 years) and 40 female patients (mean age, 66.8 years; age range,

36-90 years) were included. Data collected included population characteristics, treatment history, target location, size, and depth from pleura. Adequacy of the tissue sample for diagnostic testing and rates of biopsy-related complications were assessed. Statistical analysis was performed by using univariate and multivariate generalized linear models to determine significant risk factors for biopsy complications. Results A total of 110 intrathoracic biopsies were performed, and 101 (91.8%) were performed as repeat biopsies subsequent to a previous percutaneous or bronchoscopic biopsy or previous surgical biopsy or resection. More than 84.5% (93 of 110) of biopsies were performed in patients who had undergone previous local or systemic therapy. Specimens were adequate for evaluation of PD-L1 expression in 96.4% of biopsies. Procedure-related complications occurred in 28 biopsies (25.4%); pneumothorax was most common (22.7%). Overall mean number of core needle biopsy samples obtained was 7.9 samples. Conclusion Image-guided transthoracic core needle biopsy is an effective method for obtaining tissue for PD-L1 expression analysis. ((c)) RSNA, 2017.

van Dam, L. S., et al. (2015). "The role of programmed cell death-1 (PD-1) and its ligands in pediatric cancer." Pediatr Blood Cancer **62**(2): 190-197.

Programmed cell death-1 (PD-1) and its ligands, PD-L1 and PD-L2 maintain self-tolerance and modulate physiological immune responses. Recently, targeting the PD-1/PD-L1 pathway with blocking antibodies has emerged as a potentially promising approach to treat advanced cancers in adult patients. Since tumor PD-L1 expression is currently considered the most important predictive biomarker for successful checkpoint blockade, we summarize expression data for the most common tumors of childhood. Additionally, we give an introduction into PD-1 function in the immune system to then focus on PD-1 mediated tumor immune escape. Pediatr Blood Cancer 2015;62:190-197. (c) 2014 Wiley Periodicals, Inc.

Vecchiarelli, S., et al. (2018). "Circulating programmed death ligand-1 (cPD-L1) in non-small-cell lung cancer (NSCLC)." Oncotarget **9**(25): 17554-17563.

Background: This study aimed at investigating feasibility of programmed death ligand-1 (PD-L1) testing in plasma samples of advanced NSCLC patients receiving first-line treatment, assessing whether circulating (c)PD-L1 levels were modified by the therapy and whether baseline cPD-L1 levels were associated with patients' clinical responses and survival outcome. Methods: Peripheral blood samples were collected from 16 healthy volunteers and 56 newly diagnosed NSCLC patients before and at 12th week during the course of first-line therapy. The level of PD-L1 was measured in plasma samples using the human (PD-L1/CD274) ELISA kit (CUSABIO, MD, USA). The Mann Whitney test or Fisher's test were used for comparisons. Survival analysis was performed using Kaplan Meyer method, providing median and p-value. Results: Baseline median cPD-L1 was 42.21 pg/ml (range 12.00-143.49) in NSCLC patients and 37.81 pg/ml (range 9.73-90.21) in healthy control cohort ($p = 0.78$). Median cPD-L1 increased in patients treated with first-line chemotherapy (63.20 pg/ml vs 39.34 pg/ml; $p = 0.002$), with no changes in patients exposed to non-chemotherapy drugs (42.39 pg/ml vs 50.67 pg/ml; $p = 0.398$). Time to progression and overall survival were 4.4 vs 6.9 months ($p = 0.062$) and 8.8 vs 9.3 months ($p = 0.216$) in cPD-L1 positive vs cPD-L1 negative patients. Baseline cPD-L1 levels increased with the ascending number of metastatic sites, even if the association was not statistically significant ($p = 0.063$). Conclusions: This study showed that cPD-L1 testing is feasible, with chemotherapy influencing PD-L1 plasma levels. The possibility of using such test for predicting or monitoring the effect of immunotherapy or combination of chemotherapy and immunotherapy warrant further investigations.

Velcheti, V., et al. (2014). "Programmed death ligand-1 expression in non-small cell lung cancer." Lab Invest **94**(1): 107-116.

Recent strategies targeting the interaction of the programmed cell death ligand-1 (PD-L1, B7-H1, CD274) with its receptor, PD-1, resulted in promising activity in early phase clinical trials. In this study, we used various antibodies and in situ mRNA hybridization to measure PD-L1 in non-small cell lung cancer (NSCLC) using a quantitative fluorescence (QIF) approach to determine the frequency of expression and prognostic value in two independent populations. A control tissue microarray (TMA) was constructed using PD-L1-transfected cells, normal human placenta and known PD-L1-positive NSCLC cases. Only one of four antibodies against PD-L1 (5H1) validated for specificity on this TMA. In situ PD-L1 mRNA using the RNAscope method was similarly validated. Two cohorts of NSCLC cases in TMAs including 340 cases from hospitals in Greece and 204 cases from Yale University were assessed. Tumors showed PD-L1 protein expression in 36% (Greek) and 25% (Yale) of the cases. PD-L1 expression was significantly associated with tumor-infiltrating lymphocytes in both cohorts. Patients with PD-L1 (both protein and mRNA) expression above the detection threshold showed statistically significant better outcome in both series (log-rank P=0.036 and

P=0.027). Multivariate analysis showed that PD-L1 expression was significantly associated with better outcome independent of histology. Measurement of PD-L1 requires specific conditions and some commercial antibodies show lack of specificity. Expression of PD-L1 protein or mRNA is associated with better outcome. Further studies are required to determine the value of this marker in prognosis and prediction of response to treatments targeting this pathway.

Vitello, E. A., et al. (2016). "Cancer-secreted AGR2 induces programmed cell death in normal cells." Oncotarget **7**(31): 49425-49434.

Anterior Gradient 2 (AGR2) is a protein expressed in many solid tumor types including prostate, pancreatic, breast and lung. AGR2 functions as a protein disulfide isomerase in the endoplasmic reticulum. However, AGR2 is secreted by cancer cells that overexpress this molecule. Secretion of AGR2 was also found in salamander limb regeneration. Due to its ubiquity, tumor secretion of AGR2 must serve an important role in cancer, yet its molecular function is largely unknown. This study examined the effect of cancer-secreted AGR2 on normal cells. Prostate stromal cells were cultured, and tissue digestion media containing AGR2 prepared from prostate primary cancer 10-076 CP and adenocarcinoma LuCaP 70CR xenograft were added. The control were tissue digestion media containing no AGR2 prepared from benign prostate 10-076 NP and small cell carcinoma LuCaP 145.1 xenograft. In the presence of tumor-secreted AGR2, the stromal cells were found to undergo programmed cell death (PCD) characterized by formation of cellular blebs, cell shrinkage, and DNA fragmentation as seen when the stromal cells were UV irradiated or treated by a pro-apoptotic drug. PCD could be prevented with the addition of the monoclonal AGR2-neutralizing antibody P3A5. DNA microarray analysis of LuCaP 70CR media-treated vs. LuCaP 145.1 media-treated cells showed downregulation of the gene SAT1 as a major change in cells exposed to AGR2. RT-PCR analysis confirmed the array result. SAT1 encodes spermidine/spermine N1-acetyltransferase, which maintains intracellular polyamine levels. Abnormal polyamine metabolism as a result of altered SAT1 activity has an adverse effect on cells through the induction of PCD.

Wang, W., et al. (2016). "Roles of programmed cell death protein 5 in inflammation and cancer (Review)." Int J Oncol **49**(5): 1801-1806.

PDCD5 (programmed cell death 5) is an apoptosis related gene cloned in 1999 from a human leukemic cell line. PDCD5 protein containing 125 amino acid (aa) residues sharing significant homology to the corresponding proteins of species. Decreased expression of PDCD5 has been found in many human tumors, including breast, gastric cancer, astrocytic glioma, chronic myelogenous leukemia and hepatocellular carcinoma. In recent years, increased number of studies have shown the functions and mechanisms of PDCD5 protein in cancer cells, such as paraptosis, cell cycle and immunoregulation. In the present review, we provide a comprehensive review on the role of PDCD5 in cancer tissues and cells. This review summarizes the recent studies of the roles of PDCD5 in inflammation and cancer. We mainly focus on discoveries related to molecular mechanisms of PDCD5 protein. We also discuss some discrepancies between the current studies. Overall, the current available data will open new perspectives for a better understanding of PDCD5 in cancer.

Wang, W., et al. (2010). "Programmed cell death 4 (PDCD4) mediates the sensitivity of gastric cancer cells to TRAIL-induced apoptosis by down-regulation of FLIP expression." Exp Cell Res **316**(15): 2456-2464.

Tumor necrosis factor-related apoptosis induced ligand (TRAIL) is an important apoptosis inducer in a variety of tumor cells. In the present study, we determined the underlying molecular mechanisms by which certain gastric cancer cells are resistant to TRAIL. We first detected expression of programmed cell death 4 (PDCD4) in three gastric cancer cell lines and identified its association with the sensitivity of gastric cancer cells to TRAIL. We then stably transfected PDCD4 cDNA or shRNA into these gastric cell lines. Our data showed that restoration of PDCD4 expression induced TRAIL sensitivity, whereas knockdown of PDCD4 expression reduced the sensitivity of these tumor cells to TRAIL treatment. PDCD4 was able to suppress expression of FLICE-inhibiting protein (FLIP), a negative regulator of apoptosis. Knockdown of FLIP expression using FLIP shRNA had similar effects as those of restored PDCD4 expression. Furthermore, the proteasome inhibitor MG132 was able to inhibit expression of FLIP mRNA and protein and upregulate the sensitivity of these cells to TRAIL treatment. Taken together, the results from the current study demonstrated that PDCD4 plays an important role in mediating the sensitivity of gastric cancer cells to TRAIL-induced apoptosis through FLIP suppression. Therefore, the proteasome inhibitor MG132 should be further evaluated for combination therapy with TRAIL.

Wang, W. Q., et al. (2010). "Programmed cell death 4 (PDCD4) enhances the sensitivity of gastric cancer cells to TRAIL-induced apoptosis by inhibiting

the PI3K/Akt signaling pathway." Mol Diagn Ther **14**(3): 155-161.

OBJECTIVE: Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is thought to be a promising anti-neoplastic agent because of its ability to selectively induce apoptosis in cancer cells. However, some cancer cells are resistant to TRAIL. The mechanisms underlying this resistance are unclear. The aim of this study was to explore the role of programmed cell death 4 (PDCD4) in regulating TRAIL sensitivity in gastric cancer cells. METHODS: PDCD4 complementary DNA and PDCD4-specific short-hairpin RNA (shRNA) fragments were transfected into TRAIL-sensitive and -resistant gastric cancer cells. Expression of PDCD4 and Akt was detected via western blot. Cell survival and apoptosis were measured using 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT) and flow cytometry (FCM) assays. RESULTS: We found that upregulation of PDCD4 enhanced TRAIL sensitivity in gastric cancer cells. Downregulation of PDCD4 decreased TRAIL sensitivity. Inhibition of Akt by the phosphoinositide 3-kinase (PI3K) inhibitor LY294002 induced PDCD4 activity and enhanced TRAIL sensitivity in TRAIL-resistant gastric cancer cells. CONCLUSION: We demonstrated that PDCD4 regulates TRAIL sensitivity in gastric cancer cells by inhibiting the PI3K/Akt signaling pathway.

Wang, Z., et al. (2018). "Combination of Cytokine-Induced Killer Cells and Programmed Cell Death-1 Blockade Works Synergistically to Enhance Therapeutic Efficacy in Metastatic Renal Cell Carcinoma and Non-Small Cell Lung Cancer." Front Immunol **9**: 1513.

Introduction: Programmed cell death-1 (PD-1) inhibition therapy has changed the treatment paradigm of metastatic renal cell carcinoma (MRCC) and non-small cell lung cancer (NSCLC). However, attempts to use the drug as a single agent have achieved only limited clinical success. To further enhance the clinical benefits of monotherapy, combination therapies will likely be necessary. Cytokine-induced killer (CIK) cells are a heterogeneous subset of ex vivo expanded T lymphocytes that have been shown to prolong the survival of cancer patients. We are conducting a study to evaluate the efficacy of PD-1 inhibitor in combination with CIK cells in relapsed/refractory MRCC and NSCLC and to analyze potential biomarkers to predict which patients will benefit most from the combined therapy. Case presentation: The results of two patients treated in an ongoing clinical trial for MRCC and NSCLC are described here. The tumor biopsy from Patient 1 exhibited moderate CD3(+) T cell infiltration, but no PD-1 or PD-L1 expression. The tumor cells from Patient 2 strongly expressed PD-L1, and there was extensive tumor infiltration by CD3(+) T cells; however, no PD-1 staining was seen. Non-synonymous single nucleotide variant (nsSNVs), along with higher indel mutations, in Patient 1 and nsSNVs along with higher tumor mutation burden in Patient 2 correlate with tumor-infiltrating CD3(+) lymphocyte density. Patient 1 achieved a complete response, and Patient 2 achieved a near-complete response. Conclusion: A PD-1 inhibitor in combination with CIK cells led to potent antitumor activity in MRCC and NSCLC; CD3(+) T cell infiltration in baseline tumor biopsies is a potential predictive biomarker. This approach is being further investigated in an ongoing phase I trial.

Zerbini, L. F. and T. A. Libermann (2005). "Life and death in cancer. GADD45 alpha and gamma are critical regulators of NF-kappaB mediated escape from programmed cell death." Cell Cycle **4**(1): 18-20.

The NF-kappaB/IkappaB signaling pathway is a critical regulator of cell survival, and constitutive activation of NF-kappaB is a crucial step for many types of cancers to escape programmed cell death. Furthermore, chemotherapeutic agents activate NF-kappaB in cancer cells, and this may partially explain the resistance of cancer cells to chemotherapy. The precise mechanism of the anti-apoptotic action of NF-kappaB is not known, but involves the regulation of several cell cycle regulatory and anti-apoptotic genes. We recently demonstrated that NF-kappaB mediated cell survival is absolutely dependent on two GADD45 family members, GADD45alpha and gamma. In line with this, inhibition of NF-kappaB in cancer cells results in GADD45alpha and g dependent induction of apoptosis, JNK activation and inhibition of tumor growth. These findings establish an unambiguous role for the GADD45 family as an essential mediator of cell survival in cancer cells with implications for cancer chemotherapy and novel drug discovery.

Zschabitz, S., et al. (2017). "Response to anti-programmed cell death protein-1 antibodies in men treated for platinum refractory germ cell cancer relapsed after high-dose chemotherapy and stem cell transplantation." Eur J Cancer **76**: 1-7.

INTRODUCTION: Treatment options for patients with platinum refractory metastatic germ cell tumours (GCT) relapsing after high-dose chemotherapy and autologous stem cell transplantation are limited and survival is poor. Antibodies directed against programmed cell death protein-1 (PD-1) and programmed cell death ligand-1 (PD-L1) are currently assessed within clinical trials. We present updated data on our experience with checkpoint inhibitors as a compassionate use off-label treatment attempt for highly-pretreated patients with GCT and provide an

overview of the current literature on PD-L1 expression in this rare tumour entity. PATIENTS AND METHODS: We analysed all patients with platinum refractory GCT treated with checkpoint inhibitors at our institutions between 2015 and 2017. Data were retrieved retrospectively from the patient charts. RESULTS: Seven patients were treated with nivolumab or pembrolizumab. Four patients received single-dose treatment and died shortly afterwards due to tumour progression; the remaining three patients received treatment for at least 6 months. No significant treatment toxicity was observed. Long-term tumour response was achieved in two of the three patients, both of them highly positive for PD-L1 staining. INTERPRETATION: We consider checkpoint inhibition to be efficient in carefully selected patients with platinum refractory GCT. However, predictive markers associated with tumour response are not yet known and larger prospective clinical trials are warranted.

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