1735 Pten Deletion Synergizes with Tp53 Mutation to Drive Medulloblastoma and Highly Infiltrative Gliomas in Mouse Brain

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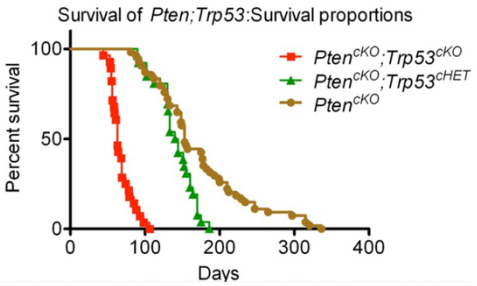
Background: *PTEN* and *TP53* are among the most frequently mutated tumor suppressor genes in human glioblastoma which is the most common and most aggressive primary brain tumor. Both pathways are concurrently dysregulated in greater than 90% of glioblastoma. How loss of PTEN and TP53 contribute to the gliomagenesis and the role of PTEN function in both developmental and oncogenic contexts need be further characterized.

Design: To investigate Pten function in early postnatal brain development and tumorigenesis, we conditionally inactivated *Pten* and *Tp53* in neural stem/progenitor cells in neonatal brain using *Nestin-CreER* transgenic mice. We generated *Nestin-CreER;Pten^{cKO}*, *Nestin-CreER;Pten^{cKO}; Tp53^{cKO}* and *Nestin-CreER;Pten^{cKO};Tp53^{cHET}* mice.

Results: *Pten ^{cKO}* mice had a median survival 153 days of age and succumb to macrocephaly and hydrocephaly due to developmental defects. *Pten ^{cKO};Tp53 ^{cKO}* mice had a median survival of 63 days of age and developed medulloblastoma with 100% penetrance. *Pten^{cKO};Tp53^{cHET}* mice had a median survival of 142 days of age and developed highly infiltrative gliomas throughout brain including brain stem, corpus callosum, neocortex, thalamus, cerebellum and subventricular zone, but not medulloblastoma.



Conclusions: Pten is required for normal development of early postnatal brain and loss of Pten in the neural stem/progenitor cells by itself is not sufficient for brain tumorigenesis. Both *Pten* and *Tp53* are critical tumor suppressor genes in preventing medulloblastoma or glioma formation from the neural stem/progenitor cells of different parts of brain. Homozygous *Pten* deletion synergizes with heterozygous *Tp53*deletion to drive gliomagenesis, and with homozygous *Tp53* deletionto drive medulloblastomagenesis, indicating different gene dosage effects of *Tp53* tumor suppressor function in glioma and medulloblastoma arising from early postnatal neural stem/progenitor cells.

1736 Histopathological Study of 167 Cases of Blind Painful Eyes: A Topographic Analysis

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Background: A blind painful eye (BPE) is the end stage clinical diagnosis of several ocular diseases, including inflammatory, vascular, and glaucomatous conditions. The morphological findings that can be encountered are broad and often related to the original condition. The aim of this study is to document the frequency of the most common morphological features in BPE by topographical location.

Design: In total, 167 cases clinically diagnosed with BPE over a 21-year period were reviewed. The cases were categorized as enucleations (54.5%) and eviscerations (44.5%). The morphological findings were subdivided accordingly to each topographic intraocular location.

Results: Mean age at diagnosis was 55.6 years (1–88) with equal sex distribution. In enucleation and evisceration specimens, the two most common findings in each topographic location were as follows (respectively): in the cornea, chronic keratitis with neovascularization (78%/85.3%) and subepithelial pannus (22%/30.7%); in the iris, anterior synechia (35.2%/17%) and rubeosis iridis (12,1/not applicable [NA]); in the trabecular meshwork, angle closure glaucoma (44%/NA); in the anterior chamber, epithelial ingrowth (3.3%/NA); in the lens, cataract (20.9%/24%); in the retina, retinal detachment (67%/NA) and retinal gliosis (64.8%/77.3%); in the choroid, non-granulomatous uveitis (44%/66.7%); and for optic nerve, optic nerve atrophy (45%/NA). Important diagnoses included two cases consistent with sympathetic ophthalmia, two Fuch's adenomas, and one toxoplasmosis case. Phthisical eyes were diagnosed by osseous metaplasia or shrunken eyes (<16 mm in diameter) and represented 23.9% of all enucleations.

Conclusions: To the best of our knowledge, this is the first topographic study of blind painful eyes describing the histopathological features. Pathological evaluation of blind painful eyes is mainly used to rule out symphathetic ophthalmia, neoplasias, and infections, but there are also other subtle findings that may be useful to confirm the underlying clinical condition.

1737 Malignant Apocrine Tumors of the Eyelid in a Review of 5,453 Specimens

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Background: Primary carcinomas with apocrine differentiation (CAD) are infrequent tumors with several subtypes, including apocrine adenocarcinomas (AAs), extramammary Paget's disease, adenoid cystic carcinoma, and mucinous carcinoma (MC) among others. The purpose of this study is to describe the clinical and pathological characteristics of CAD in order to assist with differential diagnoses.

Design: We reviewed 5,453 cases diagnosed at the Henry C. Witelson Ocular Pathology Laboratory, Montreal, Quebec over an 8-year period. Clinical and pathological data of all CAD diagnosed during this period were retrieved. Descriptive analysis, including frequency, size, location, gender, and age, was performed.

Results: Of the 5,453 specimens reviewed, 3,014 (55.3%) cases were eyelid lesions. Five CAD cases were identified. Two cases were AAs and three cases MC (0.07% and 0.17% of the eyelid lesions, respectively). The AAs were diagnosed in a 59-year-old male and an 81-year-old female in the lower and upper eyelid, respectively. Both were clinically diagnosed as chalazions. Average lesion size was 4 mm and both cases showed luminal border decapitation and eosinophilic cytoplasm. One carcinoma was well demarcated with high pleomorphism, while the other showed infiltrative borders and bland cytological features. After 2 and 3 years of follow-up, respectively, no recurrences were seen. The MCs were diagnosed in three males (61, 88, and 84 years) with an average size of 10 mm. Two of them occurred in the lower eyelid. They were all clinically diagnosed as cystic basal cell carcinoma. All of them were characterized by mucin pools separated by thin fibrous septa with nests of cribiform cells. In one case, an intraepithelial component was seen favoring the primary nature of the lesion. One patient experienced local recurrence after 6 years of follow-up.

Conclusions: To the best of our knowledge, this is the first comprehensive review of CAD of the eylid from a single institution. CAD are exceedingly rare low-grade malignant tumors with the potential for recurrence. The morphological spectrum of CAD is broad. Chalazion was the clinical misdiagnosis in all AAs, while MCs were misdiagnosed as cystic basal cell carcinomas. Identifying the intraepithelial portion of MCs may aid in the differential diagnosis of metastatic tumors. We encourage surgeons to submit all chalazions for pathological diagnosis due to the possibility that they may be malignant lesions.

Pancreas and Biliary Tree

1738 Epithelial Atypia in the Gallbladder: Diagnosis and Classification in an International Consensus Study

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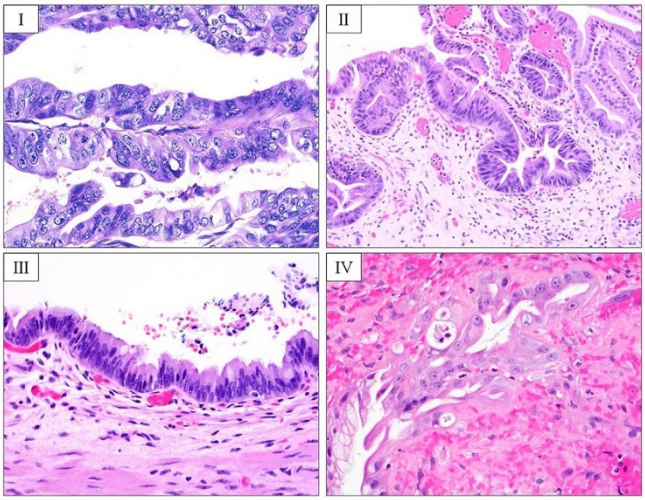
Background: Epithelial atypia (EA) in gallbladder (GB) is common and highly challenging.

Design: 12 international pathologists were asked to independently diagnose 77 GBs with EA into reactive, low-grade(LGD) and high-grade dysplastic/CIS(HGD). In subsequent discussions/reviews, 4 distinct patterns were recognized, and pairwise agreement within groups was analyzed.

Results: The patterns and the diagnoses for each were: **I. Extensive severe EA (HGD).** Hyperchromatic stratified columnar cells (pencillate/intestinal) or large cuboidal cells w/ prominent, cherry-red nucleoli (biliary), involving a significant proportion of the intact mucosa ("wild fire phenomenon"). In 87% of the 54 w/ this pattern, ≥9/12 experts rendered the diagnosis of HGD (p=0.0001). **II. Focal EA of healing erosion (Reactive/LGD).** Distinct basophilic foci in deep crevices w/ surface maturation, collagenization/hemorrhage of stroma, high mitosis, accentuated intercellular spaces, hypochromatic nuclei w/ small basophilic nucleoli. In 8/9 w/ this pattern, ≥9/12 experts agreed this was not HGD, and generally favored reactive over LGD (50 vs 28%). **III. FEA w/ surface columnar mucinous cells (LGD/Reactive).** Basophilic zones in the columnar surface mucosa w/ stratification, intestinal/goblet-cells. In 8/9 cases w/ this pattern, ≥9/12 experts agreed this was not HGD, and in 5, ≥9/12 gave LGD. **IV. Severe EA of ulceration (Reactive).** Fibrin/ulcer w/ cells showing significant acidophilic atypia, cytoplasmic vacuoles/globules, symplastic/hyperchromatic nuclei w/ nucleoli. For the 5 cases w/ this pattern, the most common diagnosis was reactive (63%), and in 3, ≥9/12 agreed on not HGD.



Conclusions: There is strong agreement among international experts as to what EA pattern qualifies as HGD (extensive severe EA,"wild-fire phenomenon"). For focal EA w/ basophilic patterns (healing erosion and surface intestinal types) the ddx is reactive vs LGD. A web-based illustration of these patterns is underway.

1739 Intra- and Interobserver Variability in the Assessment of Ampullary Dysplasia

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Background: Endoscopic mucosal biopsies of the Ampulla of Vater (amp bxs) are obtained to assess for neoplasia. A diagnosis of dysplasia or carcinoma may lead to interventions from surveillance to pancreaticoduodenectomy. A host of factors (stones, stents, sphincterotomy, etc.) can induce histologic changes that pose diagnostic challenges. We sought to investigate observer variability in the diagnosis of amp bxs and the impact clinical data may have on diagnosis.

Design: 31 cases from institutional archives were selected for inclusion based on diagnostic criteria developed for this study (>108 cases retrospectively reviewed). Cases included 12 reactive atypia (RA), 8 indefinite for dysplasia (ID) and 11 low-grade dysplasia (LGD). Slides were independently reviewed three times by 6 GI pathologists and categorized as RA, ID or LGD. 1st review: no clinical data; 2nd: ≥3 weeks later with clinical data; 3rd: >3 months after 2nd review with clinical data (pending). Kappa (κ) statistic was used to assess observer agreement.

Results: Interobserver variability:

1st review: κ for the overall study set between individual reviewers ranged from 0.07 (p=0.25) to 0.55 (p<0.001); κ for diagnostic categories were 0.42 for LGD (p<0.001), 0.07 for ID (p=0.08), and 0.27 for RA (p<0.001). Diagnostic agreement reached unanimity in 4 cases and majority in 16; there was no majority in 11 cases.

2nd review: κ for the overall study set between individual reviewers ranged from 0.25 to 0.70 (all p<0.01); κ for diagnostic categories were 0.66 for LGD, 0.17 for ID and 0.40 for RA (all p<0.001). Diagnostic agreement reached unanimity in 8 cases and majority in 14. No majority was reached in 9 cases; 5 of these also had no majority on 1st review.

Intraobserver variability:

κ for overall study set between 1st and 2nd review ranged from 0.33 to 0.64 (p<0.004). In over half of cases (n ≥ 18), diagnoses remained unchanged. When diagnoses changed, reviewers were no more likely to upgrade than downgrade.

Conclusions: Review of amp bxs without clinical data showed fair and good interobserver agreement for diagnoses of RA and LGD, respectively. Interobserver agreement significantly improved for LGD when clinical information was provided; still, agreement remained fair for RA and poor for ID, and 29% of cases had no diagnostic majority. Given the potential clinical consequences of these diagnoses, further studies may be helpful in establishing reproducible diagnostic criteria and practice guidelines for the evaluation of dysplasia in amp bxs.

1740 "Oncocytic-type" of Intraductal Papillary Mucinous Neoplasm (IPMN): An Analysis of 25 Cases

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Background: The identity and clinicopathologic characteristics of intraductal oncocytic papillary neoplasm [IOPN; oncocytic-type of IPMN] have remained elusive, including the frequency and significance of invasive component (up to 61%, PMID: 25840549) and its biologic behavior.

Design: Clinicopathologic characteristics of 25 IOPNs showing the entity-defining morphology of arborizing papillae lined by 2-5 layers of cuboidal cells with oncocytic cytoplasm and prominent, eccentric nucleoli as well as intraepithelial lumina were analyzed. In 9, deep coverage, targeted next-generation sequencing of 300 key cancer-associated genes was also performed.

Results: F/M=16/9; mean age=61 (range, 36-95). In 39%, IOPN was discovered incidentally. The working diagnosis was "malignant tumor/ductal adenocarcinoma" in 40%. Eleven occurred in the head, and 6 in the tail. The mean tumor size was 5.1 cm (range, 1-14). Only 7 (29%) IOPN exhibited unequivocal invasion, mostly in the form of microscopic foci. In 2, invasive component was >2 cm. Invasion was mostly composed of small tubular units lined by oncocytic cells, or as individual cells within the stroma. In 3, the invasive component also revealed focal extracellular mucin production. Pancreatic duct margin was involved by IOPN in 3 intraductal and 1 invasive case. One case with >2 cm invasion had a LN metastasis and another one had liver metastasis at the time of diagnosis, which showed solid nests of oncocytic cells. Of 19 with follow-up, 1 died postoperatively. Four died of other causes at 13, 15, 16, and 18 years (only one had invasive component). Twelve patients were alive and free of tumor, although 1 patient with invasive IOPN had a recurrence 3.5 years later. The median survival for all patients was 5.8 years (range, 1-18), and 5-year survival was 100%. None of the 9 cases sequenced had the key molecular alterations including *KRAS* or *GNAS* mutations commonly detected in ordinary IPMNs, and only 1 case had *RNF43* and *PIK3R1* mutations. Interestingly, there were 4 repeatedly mutated genes, each in 2 of 9 typical IOPNs: *ARHGAP26, ASXL1, EPHA8,* and *ERBB4*.

Conclusions: IOPN is a distinct tumor type in the pancreas. It is often diagnosed as "malignant/adenocarcinoma" pre-operatively. Its molecular phenotype is strikingly different from ordinary IPMNs, lacking *KRAS* and *GNAS mutations*. Despite its morphologic complexity, often with extensive pagetoid spread to normal lobules creating invasive appearance, conventional invasion is seen only in 29% and usually as microfoci, and thus it is not surprising that it exhibits benevolent behavior.

1741 Long Noncoding RNA MALAT1 Sequesters miR-217 in Nucleus and Acts as a Oncogene in Pancreatic Carcinogenesis

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Background: Pancreatic ductal adenocarcinoma(PDAC) is one of the most lethal malignancies worldwide. Long noncoding RNAs(IncRNAs) have recently been discovered to play important regulatory roles in various kinds of tumors and variety of diseases. Long noncoding RNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) was first identified in lung cancer and was shown to play a important role in tumorigenesis, however, the functional roles of these transcriptes in pancreatic ductal adenocarcinoma are not thorughly understood.

Design: We selected IncRNAs as that were differentially expressed and correlated with ovarall survival in PDAC through re-analysis publically available databases. We analyzed three sets of high-quality pancreatic expression microarray data from the PubMed Gene Expression Omnibus datasets and found out a group of IncRNAs that were consistent differential in all the 3 expression sets of data. Data was compared with survival data using the Kaplan-Meier method and compared between groups by the log-rank test. Two cell lines were used in this study to check the biological role of MALAT1.

Results: We find that MALAT1 is upregulated in pancreatic tumors compared with non-tumor tissue and negative regulated by miR-217 through interaction with the putative miR-217-binding sites on MALAT1 seqence. We blocked MALAT1 function by small interfering RNA (siRNA). Knockdown of MALAT1 in cancer cells attenuated KRAS protein expression, which already verified to be a target of miR-217. The decreased KRAS protein in response to MALAT1 knockdown can be rescued by inhibiting miR-217 exprssion. MALAT1 and KRAS RNA are expressed in nucleus and cytoplasm respectively. We found the nucleus/cytoplasm ratio of miR-217 become small in response to MALAT1 knockdown, which suggesting a translocation for miR-217 from the nucleus to the cytoplasm. MALAT binded and seqestered miR-217 in nucleus to protect KRAS RNA from repression. We also found decreased phosphorylation level of MEK and ERK1/2 after MALAT1 knockdown in pancreatic cancer cells. In vitro and vivo knockdown of MALAT1 both reduced tumor cell growth and prolifertion. Also MALAT1 knockdown impaired tumor cell migration, invasion and cell cycle progression.

Conclusions: we propose that overexpression of MALAT1 promotes oncogenesis partially by enhancing activation of the KRAS/MAPK signaling pathway through directly interacting with miR-217.

1742 Significance of E-cadherin Expression in Pancreatic Intraepithelial Neoplasia and Ductal Adenocarcinoma

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Background: Pancreatic ductal adenocarcinoma (PDA) is a malignant tumor with poor prognosis. The aim of this study was to examine the expression of E-cadherin in both pancreatic intraepithelial neoplasia (PanIN) and PDA and their relationship to clinicopathologic characteristics.

Design: Eighty one cases with PDA, 25 normal pancreases, 14 PanIN-1A, 17 PanIN-1B, 16 PanIN-2, and 23 PanIN-3 were collected. Tissue microarray blocks were constructed, and immunohistochemical staining for E-cadherin was performed.

Results: All normal pancreatic ductal cells and acinar cells showed intact E-cadherin expression. Loss of E-cadherin expression was observed in 25.5% (12/47) of low-grade PanIN and 56.5% (13/23) of high-grade PanIN, respectively. High-grade PanIN showed significantly higher loss of E-cadherin expression than low-grade PanIN (P<0.05). In PDAs, 61.7% (50/81) showed loss of E-cadherin expression. High-grade PanIN and PDA showed significantly higher loss of E-cadherin expression than low-grade PanIN (P<0.01). Loss of E-cadherin in PDAs showed significant correlation with histologic grade (P<0.01). No significant correlation was observed between loss of E-cadherin expression and age, sex, tumor size, lymphovascular invasion, pT classification, and stage, respectively.

Conclusions: These findings suggest that loss of E-cadherin is usually observed in high-grade panIN-3 and PDA. Loss of E-cadherin expression may be a late event in pancreatic cancer progression.

1743 Molecular Profile of Pancreas Ductal Adenocarcinoma with CancerSCANTM Panel Analysis

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Background: Recent update in genome-based technologies revealed many molecular profiles of cancer those can be applied to diagnosis and therapeutic application. However cancer panel test may be a useful tool in aspect of cost-benefit management, such as Oncotype Dx for breast cancer. There is no cancer panel test for pancreas ductal adenocarcinoma. We designed cancer panel test (CancerSCAN™) and validated for pancreas cancer.

Design: Forty-seven formalin-fixed, paraffin-embedded pancreas carcinoma tissues were assayed on targeted deep sequencing using CancerSCAN™ to investigate mutational profiles of 83 cancer-associated genes.

Results: A total of 281 somatic point mutations and small indel were detected at an average of 6 per patient (range 1-14), the majority of which were nonsynonymous mutations (86.1%) including 178 mutations not reported in either COSMIC or TCGA. The most frequently mutated gene was *TP53* (n=40, 85.1%), followed by *KRAS* (78.7%), *NOTCH1* (27.7%), *SMAD4* (27.7%), *BRCA2* (25.5%), *NF1* (23.4%), *CDKN2A* (19.1%), *ATRX* (17.0%), *ARID2* (14.9%), *ATM* (14.9%), *BRAF* (14.9%), *STK11* (14.9%), respectively. Identified copy number variations were three cases, including deletion of ATRX, CDKN2A and amplification of FGFR3. In total, 103 somatic translocations of genes were detected at an average of 1 per patient (range 0-6). The majority of translocations were inter-chromosomal (n=91). The most frequently translocated gene was *TMPRSS2* on chromosome 21 (n=13, 32.5%), followed by *EWSR1* (27.5%), *BRAF* (22.5%), *NF1* (17.5%), *ATM* (12.5%), *ROS1* (12.5%), *MET* (10.0%), respectively. The involved genetically altered core pathways and regulatory processes in pancreatic cancers, we identified 4 groups including KRAS signaling (76.6%), DNA damage control (68.1%), TGF-β signaling (27.7%), and regulation of G1/S phase transition (23.4%). Survival analyses of these groups were statistically insignificant. In addition, there were no significantly associated clinicopathologic features with these groups.

Conclusions: This study provides diverse molecular profile of pancreas cancer, known to be important in pancreatic cancer and other genes at low individual prevalence. We found several mutations known as actionable targets to available inhibitors. This study may be applied for clinical applications for customized therapy.

1744 Intraepithelial Neoplasia as a Pitfall in Fine Needle Aspiration of Solid Pancreatic Masses

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Background: When ductal cells with cytological features of neoplasia are identified in a fine needle aspirate (FNA) of a solid pancreatic mass (SPM), they are presumed to represent pancreatic adenocarcinoma (AdCA). However, we have encountered cases which on resection had intraductal neoplasia, and not AdCA. Here we describe our experience with this subset of cases.

Design: Specimens from patients who underwent pancreatic FNA of a SPM that was interpreted as AdCA, whose subsequent resection showed intraductal neoplasia from 10/2008 to 12/2014, were selected. The tumor was entirely submitted. We reviewed the radiology and the FNA focusing on background, cellular architecture, nuclear details and mitoses.

Results: Seven patients were identified. The final diagnoses included: 1 neuroendocrine tumor (NET) with dilated PanIN 2, 1 autoimmune pancreatitis (AIP) with high grade intraductal papillary mucinous neoplasm (HGIPMN), 2 intraductal oncocytic papillary neoplasms (IOPN), and 3 HGIPMN. Besides the NET/ AIP, all masses were solid/ cystic and concerning for AdCA on imaging. Histologically, the solid areas correlated with fibrosis and chronic pancreatitis. The FNAs did not show coagulative necrosis or frequent mitoses. Background mucin was present in 2 HGIPMN and in the 2 IOPN. Abundant acute inflammation was seen in 2 HGIPMN. All cases showed crowding, loss of polarity and anisonucleosis. Feathering was a frequent finding. Two cases of HGIPMN showed single signet ring-like cells and small tufts, which histologically correlated with sloughing of the luminal epithelium. Both IOPN had single oncocytic cells, clusters and papillae. Cell blocks of both IOPN and of 1 HGIPMN showed papillary fronds lined by neoplastic cells in a clean background. The NET (0.7 cm) was not sampled, but the PanIN 2 showed cohesive clusters with pale chromatin, peripheral nucleoli, smooth nuclear membranes, and significant anisonucleosis. The remaining cases showed cohesive clusters, with enlarged nuclei, nuclear angulation, grooves, pale chromatin, and increased N/C.

Conclusions: Intraductal neoplasia may occur with a solid mass, such as AIP and NET, and can produce cytological features similar to those of AdCA. Single cells were not a reliable indicator of invasion. A solid component associated with a cystic mass is not always invasive cancer. Oncocytic neoplasms were problematic in our series. An intraductal component should be considered when there are well defined papillae, and when necrosis is absent. All patients had disease requiring resection and neoadjuvant therapy was not used, hence there was no impact on patient care.

1745 SMAD4 Inactivation as Assessed by Diligently Evaluated Immunohistochemistry Is Highly Associated with Adverse Prognosis in Pancreas Cancer

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Background: SMAD4 inactivation is frequent in pancreatic ductal adenocarcinoma (PDA), and SMAD4 immunohistochemistry (IHC) is a reliable surrogate to molecular analysis. Although several studies have suggested that SMAD4 inactivation is indicative of adverse prognosis, one large study found the contrary, such that a recent meta-analysis concluded that SMAD4 represents a "non-significant biomarker." The goal of this study is to verify SMAD4's prognostic significance.

Design: Tissue microarrays were constructed from 248 primary and 97 metastatic PDAs. SMAD4 IHC was performed with a monoclonal antibody (clone B-8, Santa Cruz, 1:100). Tumors were scored as intact (**any** cytoplasmic and/or nuclear staining) or lost (**completely absent** staining). Detailed clinical annotation was available for 168 tumors. For these, survival probabilities were estimated using the Kaplan-Meier method. Cox proportional hazards regression was used to assess the effects of clinicopathologic variables on progression-free (PFS) and overall survival (OS). Estimated effects are reported as hazard ratios (HRs) with 95% confidence intervals (CIs). All statistical testing was two-sided and assessed for significance at the 5% level.

Results: SMAD4 loss was detected in 34% of 232 primaries and 40% of 83 metastases (9% attrition). SMAD4 status was concordant in 88% of primary-metastatic pairs. SMAD4 loss in the primary was prognostically adverse on univariate and multivariate analyses of OS and PFS. Multivariate results are presented in the Tables.

Table 1			Overall Survival		
Covariate	Level	N	HR	CI	P
SMAD4 in Primary	Lost	45	2.07	1.35-3.17	<0.01
	Intact	95			
R Status	1	43	2.74	1.74-4.33	<0.01
	0	97			
Grade	High	46	1.64	1.05-2.56	0.03
	Low	94			
Adjuvant Therapy	No	37	2.22	1.39-3.56	<0.01
	Yes	103			

Table 2			Progression-Free Survival		
Covariate	Level	N	HR	95% CI	P
SMAD4 in Primary	Lost	49	1.56	1.06-2.30	0.02
	Intact	104			
R Status	1	47	1.85	1.27-2.71	<0.01
	0	106			
Grade	High	51	1.70	1.15-2.52	<0.01
	Low	102			

Conclusions: SMAD4 loss in primary PDA doubles a patient's risk of death. The hope is that this result will clear the way for the use of SMAD4 evaluation in future clinical trial design and assessment.

1746 Comparative Clinicopathologic Study of Biliary Intraductal Papillary Neoplasms and Papillary Cholangiocarcinomas

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Background: Intraductal papillary neoplasm of the bile duct (IPNB) has been widely accepted as a unique form of biliary neoplasm. However, the definition of IPNB remains to be standardized, as some pathologists call nearly all papillary neoplasms arising in the bile duct as IPNB, while others consider this diagnostic term for selected cases

Design: Papillary neoplasms of the bile duct were provisionally classified into two groups. IPNB was defined as biliary neoplasms that are regularly arranged in a high-papillary architecture along thin fibrovascular stalks, while the term "papillary cholangiocarcinoma (papillary CC)" was used for papillary adenocarcinomas that show predominantly intraductal growth but do not fit with IPNB because of their complex histologic architectures (e.g., mixed with solid-tubular growth or thick papillae). Clinicopathological features were compared between IPNBs (n=20) and papillary CCs (n=27) using immunohistochemistry and mutation analyses for *KRAS* and *GNAS*.

Results: In a consecutive cohort of primary biliary neoplasms in our institute, 15% showed predominantly intraductal papillary growth. About 1/3 of them (4.5% of the total) was IPNBs, while the remaining 2/3 (10%) were classified as papillary CCs. A majority of IPNBs were located in intrahepatic (60%) or hilar (35%) bile ducts, while papillary CCs occurred in extrahepatic (81%) or hilar (19%) ducts ($p<0.001$). Gross mucin was more commonly found in IPNBs than in papillary CCs (80% *vs.* 7%, $p<0.001$). In terms of tumor cell types, pancreatobiliary and intestinal type neoplasms were noted in both groups, while gastric and oncocytic type tumors were found only in IPNBs. Unlike papillary CCs, 48% of which had deep invasion beyond the duct wall, 60% of IPNBs were non-invasive or minimally invasive carcinomas. Immunophenotypes of tumor cells were also significantly different between the two categories particularly in terms of MUCs and CK20. A clustering analysis based on expressions of MUCs and CKs demonstrated two dominant clusters that were highly specific for either condition. Disease-free 5-year survival rates were around 81% for patients with IPNB and 40% for those with papillary CC ($p=0.03$). *KRAS* and *GNAS* appeared to be wild-type genotypes in all but one case of *KRAS*-mutated IPNB

Conclusions: Given the significant differences in clinicopathologic features between the two groups, the diagnostic term "IPNB" may be used for selected tumors that show regularly arranged papillary growth, separately from papillary CC.

1747 Quasimesenchymal Phenotype Predicts Distant Metastasis in Pancreatic Ductal Adenocarcinoma

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Background: Two pattern of disease are recognized in pancreatic ductal adenocarcinoma (PDAC), widely metastatic and locally aggressive disease. Epithelial- Mesenchymal transformation (EMT) has shown to be important in metastatic propensity in mouse PDAC models, although attempts to exploit EMT as a clinical biomarker have been met with limited success. Herein, we evaluate the predictive value of a quasimesenchymal (QM) gene signature and correlate the signature with metastatic potential.

Design: 158 patients were classified into Epithelial (E) and QM phenotypes using a probe set containing Epithelial (E) and Mesenchymal (M) signature probes on an RNA- in situ hybridization (ISH) platform. Custom designed QuantiGene ViewRNA (Affymetrix, CA) probes against E [CDH1, EpCAM, Keratin 5, Keratin 7, Keratin 8, Keratin18, KRT19; red chromogen] and M markers [FN1, CDH2, SERPINE1; blue chromogen] were evaluated on a single slide. EMT was defined as loss of E signal or gain of M signal in tumor cells. SMAD4 expression was analyzed using immunohistochemistry (IHC). Disease specific survival (DSS) and Metastasis free survival (MFS) probability curves were constructed using the Kaplan- Meier method, log-rank test was used to evaluate the statistical significance of differences. Only the variables that were significant on a univariate model were included in the multivariate analysis.

Results: Patients with QM phenotype had significantly shorter DSS (P=0.041) and MFS (P=0.0001) (Hazards ratio 9.1) than PDACs with an E phenotype. PDACs with SMAD4 loss had lower DSS (P=0.031) and MFS (P=0.001) than PDACs with intact SMAD4. However, the QM phenotype proved to be a more robust mean of predicting metastasis than SMAD4 status – area under the curve for QM=0.804, SMAD4 = 0.377. Furthermore, the E phenotype had a higher negative predictive value (NPV) for metastasis than intact SMAD4 (NPV E Vs. SMAD4 intact = 89% Vs. 68%). The predictive value for metastasis remained significant after adjusting for tumor stage and lymph node status in a multivariate model. QM tumors were more enriched for poorly differentiated morphology (P=0.016), however, tumor grade did not predict systemic metastasis.

Conclusions: The E and QM phenotypes have distinct clinical outcomes. The RNA ISH panel is superior to currently available markers in predicting metastatic potential of PDACs. We propose that the panel would prove a useful adjunct in optimizing therapy and stratifying patients on clinical trials.

1748 Choledochal Cysts in the West: Clinicopathologic Analysis of 84 Cases

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Background: Choledochal cyst (CC) is a clinically defined entity, the pathologic correlates of which are poorly characterized. Virtually all the literature is from Asia; there are few pathology-based Western studies.

Design: 84 resected CCs in the US were analyzed. In 66, the clinical findings were also reviewed by a Japanese gastroenterologist using Japanese criteria.

Results:

	WESTERN		ASIAN
	This Study (84 cases)	**MSKCC study (Katabi et al, 2014; PMID:25123074) (36 cases)**	S. Korea, Nation-wide/ Surgical study (Lee et al, 2013; PMID:22006877) (808 cases)
Mean age	48(18-77)	36(11-67)	42(18-82)
F/M ratio	5.7	5	3.8
Mean size (cm)	2.5	4.4	n/a
Proximal vs. Distal	64:19	n/a	n/a
Cyst type (Todani classification) (I:II:III:IV:V) %	88:0:3:3:6	71:9:0:14:6	68:1:1:29:1
Documented Pancreatobiliary (PB) Maljunction %	18 (None w/ ERCP)	64	71
BilIN3/CIS-only (w/o invasion) %	8	6	n/a
Invasive ca%	7	11	10

Of the 66 with imaging available, 9 were disqualified from being CC by Japanese criteria, and of the 39 with pre-op cyst typing, 10 were re-typed. 12 had been labeled "PB maljunction"; none had received ERCP (part of routine workup in Japan). 15 had features of duplication [separate duct on the wall(11) or complete muscular coat(4)]. One proved to be duodenal diverticulum. Pathologic findings: Mucosal hyperplasia-36 (43%): prominent glandular pattern-14, papillary configuration-4, peribiliary mucous gland hyperplasia-11; intestinal metaplasia-9; hyperplastic pseudostratification resembling BilIN-1/2(LGD)-14. Duct-centric lymphoplasmacytic inflammation-40 (dense-5, follicular-6); ulceration/erosion (with PMNs/granulation)-11; severe atypia mimicking dysplasia-9. High-grade dysplasia of non-tumoral type (BilIN-3/CIS) w/o invasion-7. One had intraductal papillary neoplasm (IPNB) with HGD w/o invasion. Invasive carcinoma in 6, all tubular/pancreatobiliary type. Cyst size was documented in 76: all 14 HGD/ca cases were in cysts >1 cm, and none of the 10 cysts <1cm had HGD/ca.

Conclusions: What is clinically designated as CC in the US is heterogenous pathologically, including duplications, mere dilatation of native ducts, even duodenal diverticula. There are substantial differences in the definition of CC in the East vs. West; about 15% of the cases in the US do not fulfill the Japanese criteria. CCs often show mucosal hyperplasia, duct-centric inflammation and atypia mimicking dysplasia. Carcinomatous change is seen in 16%; HGD/CIS (w/o invasion) in 8%, IPNB in 1%, and invasive carcinoma in 7%, thus resected CCs ought to be examined carefully to exclude these lesions.

1749 ATRX/DAXX Loss Is Highly Specific for Metastatic Pancreatic Neuroendocrine Tumors

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Background: Metastatic well-differentiated neuroendocrine tumors (NETs) arise from diverse anatomic locations, but share similar histologic and immunohistochemical features. However, 10 to 13% of metastatic NETs originate from an occult primary. Considering knowledge of the site of origin has important therapeutic implications, highly specific biomarkers to identify the source of a metastatic NET are required. Recently, loss of ATRX and/or DAXX has been identified in a significant proportion of metastatic pancreatic NETs (PanNETs), but has not been examined in metastatic NETs from other sites. The goals of this study were to analyze the prevalence, specificity and sensitivity of ATRX and/or DAXX loss in metastatic NETs.

Design: Immunolabeling for ATRX and DAXX was performed on 289 metastatic NETs from 114 patients. The sites of origin included 41 pancreas, 60 small intestine, 5 colorectum, 1 stomach and 7 lung. Metastatic sites were as follows: 196 liver, 47 mesentery, 15 non-regional lymph nodes, 10 soft tissue, 6 peritoneum, 4 ovary, 3 pancreas, 3 small intestines, 3 diaphragm/pleura, 1 colon, and 1 brain. The number of metastases examined per patient ranged from 1 to 12 (median 2, mean 3). Additionally, the corresponding primary PanNETs were also stained for ATRX and DAXX. Cases were scored as ATRX- and/or DAXX-negative in the absence of nuclear staining within neoplastic cells, while retained within surrounding non-neoplastic parenchyma.

Results: Metastatic PanNETs in 20 of 41 (49%) patients had ATRX and/or DAXX loss. In contrast, all metastatic NETs originating from the small intestines, colorectum, stomach and lung showed preserved expression of both ATRX and DAXX. Among metastatic PanNETs, all metastases from the same patient had either complete loss or preserved immunolabeling for ATRX and/or DAXX. No difference was identified between the status of ATRX/DAXX and distribution of metastatic sites. Expression of ATRX and DAXX within corresponding primary PanNETs mirrored their metastatic counterparts; however, for two cases ATRX and DAXX were preserved within the primary PanNET, but absent within all distant metastases.

Conclusions: Loss of ATRX and/or DAXX in metastatic NETs is a highly specific and moderately sensitive marker of pancreatic origin. Moreover, preserved expression of ATRX and DAXX in two primary PanNETs with loss in their respective metastases, and homogenous immunolabeling among metastases from the same patient, suggest ATRX and DAXX are involved in the metastatic progression of a subset of PanNETs.

1750 Gallbladder and Cystic Duct Invasion of Distal Bile Duct Carcinomas

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Background: In the current 7th American Joint Committee on Cancer (AJCC) staging system, gallbladder involvement of distal cholangiocarcinoma is defined as a T3. While, primary carcinoma of the cystic duct is applied not for extrahepatic bile duct cancer but for gallbladder cancer staging in the 7th edition AJCC system. The aim of this study was to investigate clinicopathological and prognostic significances of cystic duct and gallbladder invasion on the distal cholangiocarcinoma.

Design: A total of 200 patients with distal cholangiocarcinoma from May 2010 to June 2012 were collected from a single institution and compared with various clinicopathologic variables, including survival.

Results: Distal cholangiocarcinoma involved the pancreas (117/200, 59%), duodenum (54/200, 27%), and/or cystic duct (37/200, 19%). There was no invasion of gallbladder. In patients with cystic duct invasion, concomitant pancreatic and/or duodenal invasion was identified in 38% (14/37). Cystic duct invasion was associated with epicenter in mid common bile duct ($p < 0.0001$), resection marginal involvement ($p = 0.001$), and frequent nodal metastasis ($p = 0.031$) in distal cholangiocarcinoma patients. Median survival time of patients with and without cystic duct invasion was 25.1 months and 36.8 months, and there was no significant survival difference based cystic duct invasion ($p = 0.254$). But patients with pancreatic or duodenal invasion demonstrated significantly worse survival than those without pancreatic ($p < 0.0001$) or without duodenal invasion ($p = 0.002$), respectively. In the patients with cystic duct invasion, median survival time of those with and without concomitant pancreatic and/or duodenal invasion was 17.5 months and 43.1 months, respectively. The survival for patients with cystic duct invasion was not significantly different whether or not there was concomitant pancreatic and/or duodenal invasion ($p = 0.065$).

Conclusions: Although gallbladder invasion was not observed in distal cholangiocarcinoma, cystic duct invasion was observed in subset of distal cholangiocarcinoma patients. Distal cholangiocarcinomas with cystic duct invasion tend to have more lymph nodal invasion and margin positivity, but do not show prognostic significance like pancreatic and/or duodenal invasion.

1751 The Predictive Effect of Human Equilibrative Transporter 1 Stratified by Mismatch Repair Deficiency in Resected Pancreatic Ductal Adenocarcinoma Patients Treated With Adjuvant Gemcitabine

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Background: The predictive value of human equilibrative transporter 1 (hENT1) in pancreatic ductal adenocarcinoma (PDAC) patients treated with adjuvant gemcitabine remains ambiguous. We recently showed that mismatch repair (MMR) status is associated with adjuvant chemotherapy (AdjCTx) response in PDAC. Here, we report the predictive effect of hENT1 expression stratified by MMR status.

Design: A tissue microarray (TMA) from 246 resected PDAC patients acquired between 1987 - 2013 was stained using the SP120 antibody against hENT1 as well as four MMR proteins: MLH1, MSH2, MSH6, and PMS2. The predictive ability of hENT1 expression was quantified by modeling overall survival (OS) between the treated and untreated study arms in the four MMR/hENT1 factorials.

Results: MMR deficiency (MMRd) was found in 39 (15.8%) cases and of these, hENT1 was expressed in 18 (46%) of cases. This was contrasted with 132/207 (63.8%) of the MMR proficient (MMRp) cases expressing hENT1 ($p = 0.048$). AdjCTx was given in 24% of cases and its rate of application did not differ across the four MMR / hENT1 factorials ($p = 0.94$). Age, sex, grade, lymphovascular invasion, perineural invasion, pT-Stage, pN-Stage did not significantly differ across the four groups ($p \geq 0.39$). Multivariable OS analysis revealed that AdjCtx was only effective in the 53% of cases that were MMRp / hENT1+ (RR = 0.36 [95%CI 0.21 - 0.58]). A secondary multivariable OS analysis without MMR status revealed that only cases, which expressed hENT1, responded to adjuvant gemcitabine (RR = 0.41 [95%CI 0.25 - 0.64]).

Conclusions: This finding is congruent with some of the previous investigations of the predictive value of hENT1 expression in resected PDAC treated with AdjCTx. However, the addition of MMR status suggests that there may be a synergistic effect with regard to treatment response and that its inclusion into the emerging mosaic of predicitve biomarkers in PDAC has served to potentially identify a small group (7%) of hENT1+ cases and a larger group of MMRp cases (30%) that do not benefit from AdjCTx. This result suggests that this population of non-responders should be triaged for trials using novel targeted molecular therapeutics.

1752 Epigenetic Regulation through Differential MicroRNA Expression by the Cellular Components of the Tumor Microenvironment Influence the Phenotype of Pancreatic Cancer

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Background: Cellular interactions in the tumor microenvironment, including tumor-stroma interactions, play a major role in the neoplastic progression of many cancer types. This is especially true for pancreatic ductal adenocarcinoma (PDAC) which to date remains a highly lethal malignancy with rising incidence, characterized by a rich stromal component and treatment resistance. The presence of dissociative growing cancer cells (coined "tumor budding cells") at the tumor-host interface is an adverse prognostic factor in PDAC. Tumor budding phenotype shares molecular similarities with the experimental phenomenon of epithelial mesenchymal transition (EMT).

Design: Each six PDACs with and without EMT-like tumor budding phenotype (n=12) were studied. Of each specimen, RNA was extracted from main tumor, EMT-like cancer cells ("tumor budding cells") as well as juxta-tumoral and tumor-remote stroma, after laser capture microdissection of membrane slides. The expression of miR21, miR-210 and miR200b, miR-183, miR-203, miR-205 and miR-2017 was examined by qRT-PCR, using a pre-amplification protocol. Material from corresponding normal pancreatic tissue was used for normalisation. Results were compared with immunohistochemical markers of EMT.

Results: In PDACs with EMT-like tumor budding phenotype, miR-21 and miR-210 were overexpressed while miR-200b was downregulated by main tumor cells, EMT-like tumor budding cells and juxta-tumoral stroma compared to matched normal tissue. In cases without EMT-like tumor budding phenotype miR-21 was also overexpressed in main tumor cells as well as in stromal cells, but intersestingly, miR-200b and miR-210 showed the opposite picture with overexpression of miR-200b and reduced expression of miR-210. The expression of miR-200b showed a negative correlation with the ZEB1 protein expression in tumor and stromal cells. The expression of all other miRs was not found to be dysregulated.

Conclusions: Differential microRNA expression by tumor and stromal cells within the tumor microenvironment of the invasive front of pancreatic cancer may influence the PDAC phenotype by regulating EMT-like tumor budding. Our findings underline the importance of epigenetic regulation in the tumor microenvironment of PDAC and emphasize the need for developing novel targeted and individualized therapies for PDAC patients including microRNA-interference.

1753 Alternative Lengthening of Telomeres in Pancreatic Carcinomas with Acinar Differentiation

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Background: Pancreatic carcinomas with acinar differentiation are extremely rare and have a poor prognosis. Telomeres are maintained as neoplasms progress predominantly by activation of telomerase, and less commonly by the recombination-dependent alternative lengthening of telomeres (ALT) pathway. Activation of the ALT pathway has been observed in a variety of human cancers, including pancreatic neuroendocrine tumors, osteosarcomas, and glioblastomas. Carcinomas with acinar differentiation have not been previously evaluated for the presence of the ALT phenotype.

Design: Across 4 institutions, we collected 28 pancreatic carcinomas with acinar differentiation; 14 acinar cell carcinomas (pure ACCs) and 14 mixed acinar-neuroendocrine carcinomas (mixed ACCs), and constructed tissue microarrays. We performed telomere-specific fluorescent in situ hybridization to determine the ALT status of each case and correlated the results with clinicopathologic factors.

Results: ALT-positivity (ALT+) was observed in 18% of the carcinomas (5/28 cases; 1 pure ACC and 4 mixed ACCs), and ALT+ was more common in carcinomas without necrosis ($p=0.059$). However, ALT+ cancers were not associated with other clinicopathologic factors, including age and sex of the patients, growth pattern (expanding vs. infiltrating), tumor size, pT classification, and lymphovascular and perineural invasions. Interestingly, patients with ALT-positive tumors had better recurrence-free survival (median survival time, 71 months) than those with ALT-negative tumors (9 months) with marginal significance ($p=0.06$).

Conclusions: The ALT+ phenotype is observed in a subset of pancreatic carcinomas with acinar differentiation, including pure ACC and mixed ACCs, and these patients tended to have a better survival time than patients with ALT negative tumors.

1754 Alternative Lengthening of Telomeres in Primary Pancreatic Neuroendocrine Tumors Is Associated with Aggressive Clinical Behavior and Poor Survival, but Better Survival after Metastasis

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Background: Alternative lengthening of telomeres (ALT) is a telomerase-independent pathway for telomere maintenance in a subset of human cancers. Previous studies have demonstrated that the presence of the ALT pathway in pancreatic neuroendocrine tumors (PanNETs) is strongly associated with alterations in two chromatin remodeling proteins, ATRX and DAXX, and increased chromosomal instability.

Design: Telomere-specific fluorescent in situ hybridization was performed to determine the ALT status in a cohort of 278 surgically resected PanNETs. ALT status was compared with clinicopathologic factors.

Results: Overall, ALT-positive tumors were observed in 54 cases (19.4%). ALT-positive PanNET tumors were higher WHO grade, larger in size, and of higher pT stage than were ALT negative PanNETs. ALT positivity also correlated with lymphovascular and perineural invasion, lymph node and distant metastases, and patients with ALT+ primary PanNETs had a shorter recurrence-free survival. Interestingly, patients with distant metastases had significantly better survival when the tumors were ALT-positive compared to those with an ALT-negative tumor.

Conclusions: Primary ALT-positive PanNETs are associated with aggressive clinicopathologic behavior and have poor recurrence-free survival. Once ALT-positive clones establish as a metastatic foci, these cancers appear to grow more slowly in the new microenvironment, as patients with ALT+ tumors have a better prognosis than those with ALT negative.

1755 Neoadjuvant Therapy (NAT) for Borderline Resectable Pancreatic Cancer (BRPC): Comparison of Histologic Grading Systems of Residual Adenocarcinoma Following Chemotherapy (CT) Alone

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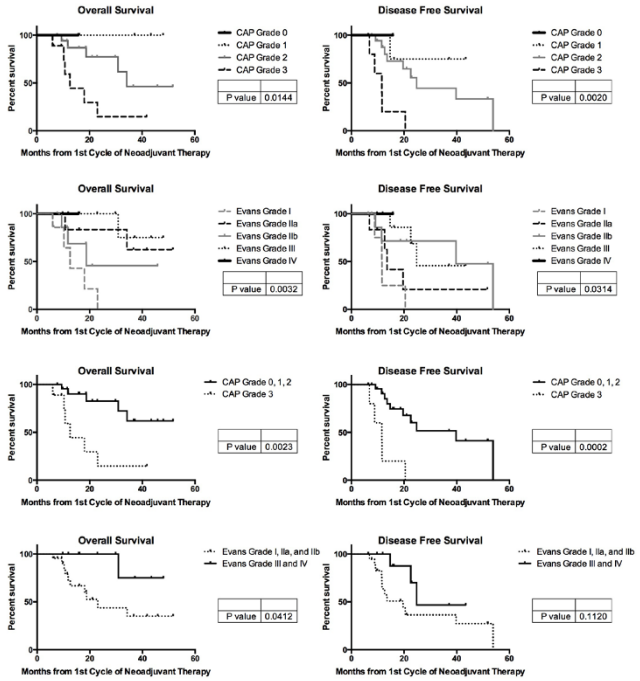
Background: NAT is recommended to increase feasibility of margin-negative(R0) resections in BRPC. Extent of residual tumor in posttreatment pancreatectomy specimens can be classify by proposed College of American Pathologists(CAP) and/or Evans grading systems, but which is more predictive of outcome for BRPC following neoadjuvant CT(NACT) w/o radiation (RT) is unknown.

Design: We conducted a retrospective review of 32 BRPC pts treated with NACT w/o RT followed by resection between 2010-2015. Log-rank test was used to test the association between pathologic response, comparing CAP and Evans grading systems, and patterns of recurrence and survival outcomes.

Results: Majority of pts(22) received neoadjuvant FOLFIRINOX(68.8%) vs gemcitabine-based regimens(31.2%). Overall, 28 pts(87.5%) achieved R0 resection and 18(56.3%) had node-negative disease. Angiolymphatic invasion, perineural invasion, and direct extension into adjacent structures were seen for 8(25%), 20(62.5%), and 22 pts(68.8%), respectively. With a median f/u time of 29.8 mo, 15 pts(46.9%) relapsed; including 8(53.3%) with both locoregional and metastatic recurrence. By Kaplan-Meier

analysis, median disease-free(DFS) and overall survival(OS) are 22.5 and 34.2 mo, respectively. Final pathologic tumor stages: ypT0=3.1%, ypT1=18.8%, ypT2=6.3%, ypT3=65.6%, and ypT4=6.3%.

One pt(3.1%) had pathologic complete response(CAP 0, Evans IV). Remaining grades classified: CAP 1=12.5%, 2=56.3%, and 3=28.1%; vs Evans I=21.9%, IIa=21.9%, IIb=25%, and III=28.1%. Both systems showed statistically significant associations with clinical outcomes (CAP: p=0.014 and p=0.002 for OS and DFS, respectively; Evans: p=0.003 and p=0.031). When each grading system was stratified into two tiers (CAP 0/1/2 vs CAP 3, and Evans I/IIa/IIb vs Evans III/IV), stratified CAP showed stronger statistical association for both OS(p=0.0023) and DFS(p=0.0002) than Evans(p=0.041 and p=0.112).



Conclusions: In this limited unique cohort of BRPC treated exclusively with NACT, pathologic response by both CAP and Evans grading systems is predictive of clinical outcomes. When each system is stratified into two tiers, CAP appears to be the superior method in terms of strength of statistical association with both OS and DFS.

1756 Undifferentiated Pancreatic Carcinomas Display Enrichment for Frequency and Extent of PD-L1 Expression by Tumor Cells

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Background: Pancreatic carcinoma is an aggressive malignancy with a dismal prognosis; thus, identification of novel effective therapies is clinically important. Studies have shown that expression of PD-L1 by tumor cells may predict response to targeted PD-1/PD-L1 inhibition. While pancreatic carcinomas have shown PD-L1 expression, the extent of PD-L1 expression has not been reported in detail and unselected conventional pancreatic ductal adenocarcinomas (cPDAC) have shown limited clinical responsiveness to anti-PD-L1 therapy. Specifically, detailed histologic analysis of undifferentiated pancreatic carcinomas for PD-L1 expression compared to cPDAC has not been performed.

Design: We identified 20 cases of undifferentiated pancreatic carcinoma (UPC) and 36 controls of conventional pancreatic adenocarcinoma (cPDAC). The cPDAC group included 8 well, 14 moderate, and 14 poorly-differentiated tumors. Immunohistochemistry for PD-L1 (E1L3N clone), CD3, CD20, CD68 and mismatch repair proteins was performed. Slides were scored for extent of membranous PD-L1 expression on tumor cells with ≥1% being positive. Correlations were examined by Fisher's exact test.

Results: PD-L1 expression was more frequent in UPC (n=10) than cPDAC controls (n=6) (50% vs 17%; p=0.01). The extent of PD-L1 expression was greater in UPC with 9 of 10 cases showing ≥10% tumor cells positive compared to 3 of 6 cPDAC controls (2 of which were poorly differentiated). UPC were characterized by sheets of pleomorphic tumor cells with frequent tumor giant cells and spindle cell areas; osteoclast-like giant cells were variably present (n=6). Gland formation was absent. Tumor infiltrating lymphocytes were not brisk in any UPC case. There was no histologic difference between UPC with or without PD-L1 expression. Each MSI-H tumor (n=3) showed PD-L1 expression including 2 UPC and 1 cPDAC. Correlation of PD-L1 expression with disease-specific survival is currently being analyzed.

Conclusions: UPC is enriched for PD-L1 expression both in frequency and extent relative to cPDAC controls. Our data also show that higher grade cPDACs are associated with an increased frequency of PD-L1 expression. We propose that the histologic subtype and tumor grade may aid in the selection of patients who have an increased likelihood of response to anti-PD-L1 and PD-1 therapy. Analysis of a larger cohort of UPC and control cases for validation of these findings is ongoing.

1757 Mutational Profiling of Oncocytic Intraductal Papillary Mucinous Neoplasms (IPMN-o)

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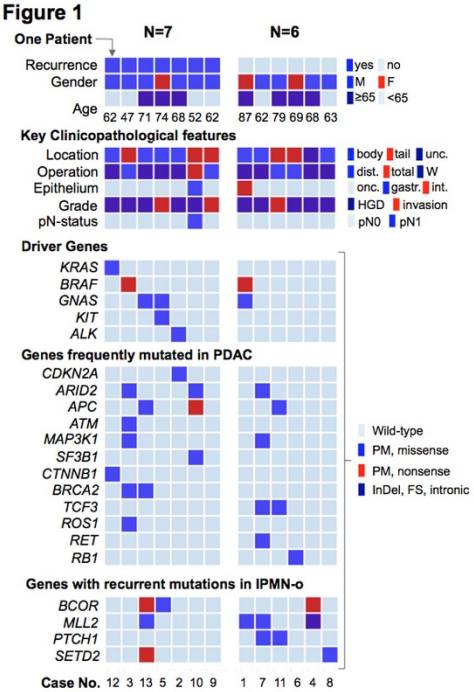
Background: The oncocytic subtype of intraductal papillary mucinous neoplasia (IPMN-o) is generally regarded as a clinically indolent subgroup. However, recurrences have been described. Here we compared the clinical and molecular profiles of non-recurrent and recurrent IPMN-o.

Design: We identified 13 IPMN-o by an archival search and macrodissected different areas from the same tumor (N=3 non-recurrent; N=1 recurrent) and comparison between original and recurrent (N=4 recurrent cases) to assess spatial and temporal heterogeneity. Genotyping was performed using the OncoPanel assay that by massively parallel sequencing using a solution-phase Agilent SureSelect hybrid capture kit and an Illumina HiSeq2500. We performed contingency testing by recurrence status and plotted key oncogenic as well as recurrent variants (found in more than one patient).

Results: 7/13 IMPN-o patients had a tumor recurrence as IPMN-o. Contingency testing between the two groups did not show significant differences (P-range: 0.46-1.0). Genotyping identified a total of 133 variants with a median of 6 variants/sample. The overall mutational burden was higher in the recurrent group (median: 12; range: 2-19) when compared to the non-recurrent group (median: 6.5, range 1-15) but this did not reach statistical significance (P=0.37; t-test). A mutually exclusive pattern of driver gene variants was seen in IMPN-o as well as the presence of additional variants in genes frequently mutated in pancreatic ductal adenocarcinoma (PDAC).

Figure 1



Comparison of the mutational pattern in recurrent lesions with the original IPMN-o revealed no consistent pattern, suggesting tumor heterogeneity. Given that all surgical excisions were initially complete (R0), the spatial (intratumoral, assessed in 4 of 13 cases) and temporal heterogeneity (between initial and recurrent lesions, assessed in 4 of 7 recurrent cases) suggests a mutational field effect affecting the pancreatic ductal tree.

Conclusions: Genotyping of IPMN-o shows key driver mutations as the principal pathogenetic pathways of pancreatic neoplastic progression and lack of a consistent mutational spatial or temporal spectrum suggest significant intratumoral heterogeneity.

1758 Comparative Study of Isocitrate Dehydrogenase 1 Mutations in Intraheptatic Cholangiocarcinoma by Immunohistochemistry and Pyrosequencing

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Background: Intrahepatic cholangiocarcinoma (ICC) is a hepatobiliary neoplasm with limited therapeutic options and poor prognosis. Recent studies have shown that isocitrate dehydrogenase (IDH1) mutations occur in approximately 25% of ICC and specific targeting of IDH1 with small molecule inhibitors is under investigation. IDH1 clinical testing algorithms are established for formalin fixed paraffin embedded (FFPE) glioma specimens and use anti-IDH^{R132H} immunohistochemistry (IHC) with reflex to DNA sequencing if IHC is negative. While DNA sequencing has effectively detected IDHmutations in ICC FFPE specimens, the utility of the IDH^{R132H} antibody for detecting mutations in ICC has not been established.

Design: ICCs diagnosed between 2003 and 2015 were identified from 29 patients (15 female and 14 male; age 29-80) from our institutional database. Blinded cases were evaluated by two GI surgical pathologists and representative slides were chosen for

anti-IDH^{R132H} IHC on all cases with appropriate controls. Tumor cell nuclear positivity from 5 high power fields was recorded and cases with more than 5% of cells staining were considered positive. Pyrosequencing for IDH1 was also performed in parallel on FFPE from each case.

Results: The average tumor size was 6.3 cm and the majority of cases were unifocal (n=26) within the hepatic parenchyma with a tumor stage of T1 (n=15), T2 (n=5) or T3 (n=9). Background liver demonstrated no significant abnormality (n=3), cholestasis (n=5) with mild to moderate portal inflammation (n=16) and/or macrovesicular fatty change (n=12). All 29 cases were negative for IDH^{R132H} staining by IHC. However, 2 cases (7%) had IDH1 mutations c.394C>T (p.R132C) and c.394C>A (p.R132S) by pyrosequencing.

Conclusions: IHC specific for the IDH1 R132H altered protein has proven useful in gliomas; a recent large study showed that 83% of IDH1 mutations are c.395G>A (p.R132H). However, both mutations found in our ICC cohort were for a different nucleotide (c.394) and resulted in different amino acid substitutions. While a larger cohort is needed to fully map the range of IDH mutations in ICC, our results suggest that IHC may not be useful for screening ICC. If IDH1 status is needed for clinical trial enrollment or prognostication, sequencing is more likely to identify the mutations present.

1759 Morphologic Characterization of Intrahepatic Cholangiocarcinomas (ICCs)

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Background: WHO 2010 classifies ICCs solely by grade. Further classification into two microscopic subtypes has been proposed: Perihilar large duct type (LDT) (mucin-producing ductal carcinomas similar to pancreatic ductal carcinoma often associated with KRAS mutations, chronic inflammation [CI] and precursor lesions, derived from peribiliary glands) and peripheral small duct type (SDT) (small fused glands formed by cholangiolar-type cells associated with IDH1 mutations, chronic hepatitis and cirrhosis, derived from canals of Hering). Our aim was to characterize ICCs in a Western patient population.

Design: 55 cases of CC (19 ICC and 36 ECC as controls) from 2000-2007 were reviewed. Morphologic features were recorded in a blinded fashion. After review, clinicopathologic data was collected.

Results: 13 (68%) ICCs were SDT and 6 (32%) LDT. Several histopathologic features were strongly associated with SDT or LDT (see table). 5 (38.5%) SDT had a mixed hepatocellular carcinoma component. Precursor lesions (BilIN/IPNB) and ductal CI were only noted in LDT. Cirrhosis was present in 31% SDT.

	SDT	N=13	LDT	N=42	P value
Gender	Male	10	Female	22	0.065
Age (mean)	61		64		0.323
Location	ICC	13	ECC	36	<0.0001
Histologic Feature					
Size of glands	Small	11	Large	32	<0.0001
Cell type	Cuboidal	12	Columnar	30	<0.0001
Cytoplasm Quantity	Scant	10	Abundant	28	0.008
Cytoplasm Quality	Amphophilic	7	Eosinophilic	34	0.002
Nuclear grade	High	13	High	28	0.024
N:C ratio	High	9	Low	29	0.022
Gland pattern	Anastomosing	7	Separate	39	<0.0001
Growth at periphery	Irreg pushing border	11	Infiltrative	36	<0.0001
Mucin	No	11	Yes	30	0.0007
Cellularity	High	13	Low	24	0.0002
Fibrosis	Central	9	Diffuse	35	<0.0001
Precursor	None	13	BilIN/IPNB	25	<0.0001
Ductal CI	No	13	Yes	31	<0.0001

Conclusions: Our data supports the classification of ICC into two types. Compared to data from Asia, we found a higher proportion of SDT in our population (p=.028, PMID 24406866), likely related to different risk factors (i.e., less hepatolithiasis in US). Several features reliably distinguish SDT from LDT ICCs, which is important, as LDT ICCs show worse prognosis and are associated with potentially detectable precursor lesions. More cases are being analyzing to substantiate the results.

1760 Clinicopathological Significance of MUC13 Expression in Pancreatic Ductal Adenocarcinoma (PDAC); the Positive Prognostic Marker and Possible Candidate for Molecular Targeted Therapy

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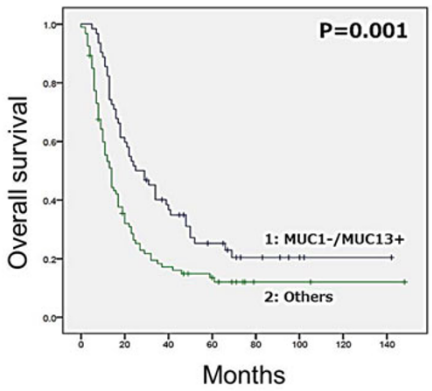
Background: PDAC is the fourth-leading cause of cancer-related death, and biomarkers that can predict prognosis and necessity of adjuvant chemoradiotherapy are desperately needed. According to the previously published results (Collisson EA, et al. Nat Med. 2011), MUC13 is one of the signature molecules of the classical type of PDAC. MUC13 is a recently identified transmembrane mucin which is normally expressed in the large intestine, trachea, kidney, small intestine, and gastric epithelium. Although it has been shown to be aberrantly expressed in ovarian and gastrointestinal cancers, only one study showed the association of MUC13 with PDAC (Chauhan SC, et al. Mol Cancer Ther 2011).

Design: We investigated the relationship between the immunohistochemical expression of MUC13 and other mucin-related proteins (MUC1, MUC4, MUC2, MUC5AC, and MUC6), trefoil factor family (TFF) 1, TTF3, morphological features of tumors and prognosis, using tissue microarrays of 155 formalin-fixed paraffin-embedded tissues of surgically resected PDACs. Morphological features were divided into 4 subgroups according to cytological atypia, tumor differentiation and stroma.

Results: The expression of MUC13 was correlated with tumor differentiation. Cluster analysis of MUC expression revealed two major clusters, MUC2/MUC4/MUC6+ (#1) and MUC1/MUC5AC/MUC13+ (#2). The expression of MUC13 and MUC1 in cluster #2 was significantly associated with tumor grade, however, MUC5AC was not. MUC13 in PDAC was associated with MUC5AC, TFF1 and TFF3. Decreased expression of MUC13 was correlated with high-grade tumor and lymphatic invasion. Moreover, MUC1+/MUC13- subgroup showed poor prognosis compared with MUC1+/MUC13+ and MUC1-/MUC13- subgroups.

Figure 1 MUC1-/MUC13+ subgroup shows better prognosis



Conclusions: Our study showed that the expression of MUC13 was significantly correlated with better prognosis. Although further studies are necessary, it suggests that MUC13 may serve as a prognostic marker of PDAC and may apply to the immunohistochemical subtyping for the classical type of PDAC susceptible to the selective molecular targeted therapy.

1761 Unique Mutational Signature of Pancreatic Neuroendocrine Tumors with Mismatch Repair Mutations

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Background: Pancreatic neuroendocrine tumors (PNETs) with mismatch repair (MMR) enzyme mutations are thought
to be rare entities, and the underlying mutational profile of PNETs with MMR mutations has not been characterized.

Design: In a study of 21 PNETs, we identified three PNETs with biallelic loss of function mutations in MMR genes (MMR-mutated PNETs) and compared their mutational profile to that of 18 PNETs without MMR mutations (non-MMR PNETs) using a 510 gene panel. Rare exonic and splice site mutations were considered for analysis.

Results: Compared to PNETs without MMR mutations, PNETs with MMR mutations were significantly more likely to harbor mutations in *TSHZ3* (3/3 vs 0/18, p=0.0008), *MEN1* (3/3 vs 1/18, p=0.003), *TRRAP, COL2A1*, and *PHLPP2* (2/3 vs 0/18, p=0.0143). Mutations in other genes associated with PNETs (*DAXX, ATRX, VHL, TSC2, PIK3CA, PTEN* and *TP53*) were not significantly different between the two groups. Certain genes were not mutated in the three MMR-mutated PNETs that were mutated in a subset of non-MMR PNETs (*PTPRD, PLCH2, COL1A1, HIF1A, NBN* and *KAT6A*), but these differences were not statistically significant in this small study.

Conclusions: In this cohort, MMR-mutated PNETs were more common than previously reported and have a distinct mutational profile compared to non-MMR PNETs. Study of additional MMR-mutated PNETs will determine whether this subset of tumors has a different prognosis or responds differently to therapy than non-MMR PNETs.

1762 Lymph Node Yield and Excision Margin Status in Pancreaticoduodenectomy (Whipple's) Specimens: A Three and a Half Year Audit Assessing the Impact of Neoadjuvant Therapy

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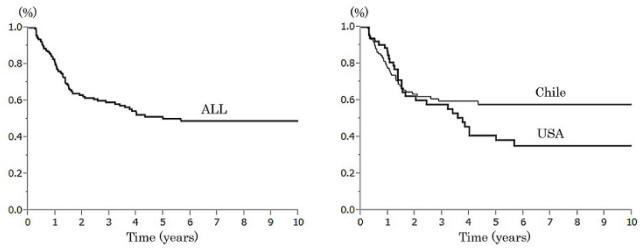
Background: Pancreaticoduodenectomy (PD) is the standard primary curative approach for resectable tumors of the pancreas, ampulla and distal biliary tract. Systematic pathologic examination of the PD specimen is essential for accurate staging and adjuvant treatment planning. Various neoadjuvant treatment (NAT) regimens are now commonly administered, but the effect on pathologic findings has not been well documented. The aim of this audit was to determine the effect of NAT regimens on lymph node yield (LNY), margin resection status and pathologic treatment response (PTR) in PD specimens.

Design: 214 PD specimens at St. Vincent's University Hospital between January 2012 and August 2015 were retrieved via a SNOMED search of the laboratory information system. Margin status, LNY and PTR data was obtained from pathology reports.

Definition of R0 status was based on Royal College of Pathologists pancreatic cancer dataset. PTR was assessed using the College of American Pathologists (CAP) tumour regression grade (TRG). Details of NAT were retrieved from the patient chart.
Results: 87 PD specimens contained pancreatic ductal adenocarcinoma and NAT was administered to 24 patients (9 FOLFIRINOX-based regimen, 6 various other combinations, 9 no details available) with all patients receiving concomitant radiation therapy. The mean LNY for NAT patients was lower than non-NAT patients (11.25 versus 15.24, p=0.001). R0 margin status was achieved in 51 (59%) PD cases including 19/24 (79%) of NAT cases (p=0.02) with 8/9 FOLFIRINOX-treated patients achieving a R0 resection. A favourable CAP TRG (0 or 1) was observed in only 3/24 (12.5%) cases.
Conclusions: Neoadjuvant therapy does reduce lymph node yield in pancreaticoduodenectomy specimens but has a positive impact on the R0 status. FOLFIRINOX-based regimens appear to have a more beneficial effect on resection margin status. Despite these significant treatment-related pathologic findings, the majority of cases did not demonstrate a favourable tumour regression grade score.

1763 Prognosis of T2 Gallbladder Carcinomas: An Analysis of 326 Cases Highlights a Prognosis Better Than the Current Impression in the West, but Incomparably Worse Than What Is Reported in Asia

Bahar Memis, Juan Carlos Roa, Takashi Muraki, Kee-Taek Jang, Enrique R Bellolio, Juan Carlos Araya, Miguel A Villaseca, Juan Sarmiento, Hector Losada, Shishir Maithel, David Kooby, Nevra Dursun, Humbert Polito, Sudeshna Bandyopadhyay, Pelin Bagci, Burcu Saka, Michelle Reid, Volkan Adsay. Emory University, Atlanta, GA; Pontificia University, Santiago, Chile; SMC, Seoul, Korea; University Frontera, Temuco, Chile; WSU, Detroit, MI.
Background: There are major differences in the reported prognosis of "T2" gallbladder carcinoma (GBC), with a 5-yr survival of <30 % in the West versus > 70% in Korea.
Design: Cholecystectomies with GBCs in authors files were evaluated for convincing perimuscular invasion (pT2) by using the Western criteria of invasion, and carefully excluding T1 cases extending to Rokitansky-Aschoff sinuses, as well as those showing involvement of serosal/hepatic surface (T3). An average of 7 slides per case were evaluated.
Results: 326 cases qualified as T2 GBC with the Western criteria for invasion (158 were from Chile, and 168 from North America and other continents). F/M = 3; mean age = 65 (vs 57 for Tis/T1 cases in the same database); mean size=2.6 cm. Disease specific survival when peri-operative mortality of 90 days was excluded was 59% at 3 yrs and 50% at 5 yrs (see KM curves). The patients in the US cohort appeared to show a steeper decline in the long-term follow up (after 5 yrs) than Chilean patients; however, this did not reach statistical significance (p=0.18). There was no significant difference between different cohorts in the extent/amount of T2 disease (both the mean size of carcinoma and the frequency of sub-stages of T2 appeared to be similar in Chile vs US).



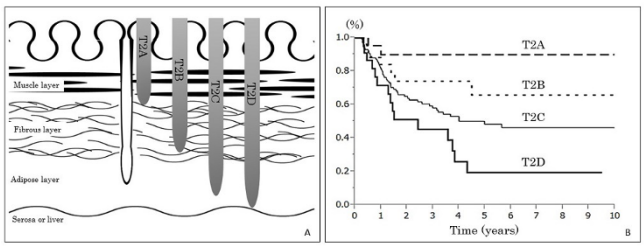
Conclusions: GBC with perimuscular invasion (T2) occurs about a decade older (65 vs 57) than "early" GBCs (Tis/T1), supporting the progression scheme. Defined by the Western criteria, 5-yr surv is 50% (fairly similar in both the Chilean and US cohorts), significantly better than the 30% reported in the US, which may be attributable to the under-sampling/under-staging phenomenon that we witness in our consultation practice in the US. However, it is also lower than the rates reported from Korea (5-yr >70%), which may be related to variability in the histopathologic criteria such as over-staging of RAS-involving Tis/T1 cases, or populational differences in its biologic behavior. Further studies are needed to clarify these issues.

1764 Not All T2 Gallbladder Carcinomas (GBC) Are Equal: Proposal for Sub-Staging of T2 GBC with Significant Prognostic Value

Bahar Memis, Juan Carlos Roa, Takashi Muraki, Ezgi Hacihasanoglu, Nevra Dursun, Burcu Saka, Enrique R Bellolio, Juan Carlos Araya, Juan Sarmiento, Hector Losada, David Kooby, Shishir Maithel, Michelle Reid, Brian Quigley, Alyssa Krasinskas, Volkan Adsay. Emory University, Atlanta, GA; Pontificia University, Santiago, Chile; University Frontera, Temuco, Chile.
Background: The vast majority of GBCs is discovered "incidentally" in cholecystectomies performed for chole-lithiasis/-cystitis, and mostly fall into the T2 category in the current TNM, although they present a wide spectrum in the amount of carcinoma encountered.
Design: 198 T2 GBCs in which adequate slides and follow-up were available to authors were analyzed for the extent of peri-muscular (peri-M) invasion on the GB wall.
Results: The extent of peri-M involvement

Substage class	Definition/Description	3 year survival (%)	5 year survival (%)
T2A (n:21)	Inv into Peri-M < 1. 5 mm depth (measured from deeper edge of the muscularis); mostly composed of a handful of glands in the Peri-M.	90	90
T2B (n:26)	Inv beyond 1.5 mm but confined to inner half (50%) or confined to the fibrotic component (in cases with established fibrotic-adiopocytic interphase).	74	66
T2C (n:128)	Inv into > 50% of the peri-M or the adipocytic component (in cases with established fibrotic-adipocytic interphase).	58	48
T2D (n:23)	Approximating the serosal surfaces, showing surrogate evidence of serosal involvement but w/o overt carcinoma on the surface.	45	19

	Chile (%)	US (%)
T2A (n:21)	15	6
T2B (n:26)	12	15
T2C (n:128)	64	65
T2D (n:23)	9	14



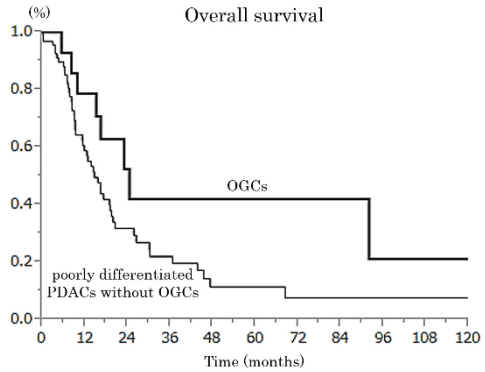
Conclusions: Pathologic sub-staging of T2 GBCs has significant prognostic value. The outcome of early/limited T2 cases (< 1.5 mm peri-M inv) is very good (5-year, 90%), closer to that of "EGBC" (Tis/T1) GBCs (PMID: 24022828); whereas, of those approximating the GB serosa, is dismal (5-year < 20%). There does not seem to be any significant geographic difference in the frequency of sub-stages (similar in Chile vs US).

1765 Undifferentiated Carcinoma with Osteoclast-like Giant Cells of the Pancreas: Clinico-Pathological Analysis of 20 Cases Highlights a More Protracted Clinical Course Than Currently Appreciated

Takashi Muraki, Gabriela Bedolla, Kee-Taek Jang, Pelin Bagci, Pardeep Mittal, Michelle Reid, Bahar Memis, Olca Basturk, Sudeshna Bandyopadhyay, Juan Sarmiento, Alyssa Krasinskas, Volkan Adsay. Emory University, Atlanta, GA; SMC, Seoul, Korea; MSKCC, NY, NY; DMC, Detroit, MI.
Background: Pancreatic undifferentiated carcinoma with osteoclast-like giant cells (OGCs) is a very rare tumor type. Its histogenesis and behavior remain controversial, with the current literature indicating that it is a highly aggressive neoplasm with a prognosis even worse than ordinary ductal adenocarcinomas (PDAC).
Design: 20 OGCs identified in the authors files were analyzed and contrasted with PDACs.
Results: F/M=13/7; Mean age, 56 (29-86; vs 63); Mean size, 5.9 cm (0.6-12.0; vs 3.4). All showed the classical OGC pattern including the 3 cell types: I. Osteoclasts, II. Pleomorphic/undifferentiated malignant cells (PCs), III. Background spindle-shaped histiocyte-like cells associated with hemorrhage (HLCs). These three cell types displayed different immunoprofiles.

	CK	CD68	p53	Ki67
Osteoclasts	0/12	12/12	0/12	0/12
PCs	5/12, focal	2/12, focal	8/12; >50%	9/12; >20%
HLCs	0/12	12/12	7/12; >50%	8/12; >20%

Nodular, pushing-border growth pattern was consistent, and 5 were arising in tumoral intraepithelial neoplasms (4 in MCN, 1 in IPMN) and 11 (55%) also showed prominent intraductal/cystic growth. Osteoid was seen in 7. LVI in 11 and PNI in 6. 15 (75%) had an invasive ductal/tubular adenoca (DA) component that ranged from microscopic to 80% of the tumor (mean, 23%). Despite their large size, LN mets were relatively uncommon (3/18 - 17% with LN sampling; vs 78% in PDAC). Biopsies of distant mets in follow up in 4 cases, 2 showed undiff pattern w OGCs. Survival of OGCs was significantly better than that of poorly differentiated PDACs without OGCs (n=95) (3-yr surv; 42 vs 22%, 5-yr surv; 42 vs 11%, median; 25 vs 15 mos, respectively. p=0.0287).



Conclusions: Pancreatic OGCs present with relatively large tumors and in slightly younger patients than PDACs. 25% arise in IPMN/MCN, and > 50% show intraductal/cystic polypoid growth or pushing-border infiltration as in sarcomatoid carcinomas in other organs. OGCs have a better prognosis than is currently appreciated in the literature, with estimated 5-yr survival of 42%.

1766 Pseudotumoral Pancreatitis: Clinicopathological Analysis of 93 Cases Resected with Pre-Operative Diagnosis of Pancreatic Ductal Adenocarcinoma but Proved to Be Inflammatory Conditions

Takashi Muraki, Gabriela Bedolla, Michelle Reid, Grace Kim, Alyssa M Krasinskas, Ipek E Seven, Bahar Memis, Field Willingham, Juan Sarmiento, David Kooby, Shishir Maithel, Pardeep Mittal, Volkan Adsay. Emory University, Atlanta, GA; UCSF, San Francisco, CA.

Background: Distinction of benign inflammatory conditions from malignancy is a well-known challenge in the pancreas.

Design: To determine the frequency and clinicopathologic associations of inflammation-induced pseudotumors, 1180 pancreatectomies performed with curative intent on presumed pancreatic ductal adenocarcinoma (PDAC) were evaluated. Suspected ampullary/duodenal/CBD cancers, biopsy specimens and consult cases were excluded. Imaging/clinical findings were verified for true mass formation (solid PDAC). Pseudotumors other than inflammatory nature and cystic pseudotumors were excluded.

Results: 93 pancreatectomies with a clinical diagnosis of PDAC proved to be pseudotumoral pancreatitis: M/F=1.6; mean age, 55 (vs 63 in PDAC), mean size 2.6 cm (0.8-6.5; vs 3.4 in PDAC), and location head 75%. Clinical h/o pancreatitis, alcohol and smoking was present in 38, 52 and 64, respectively, and hypertension in almost half.

I. Non-Specific Pancreatitis (n=45, 3.8%); 1) Alcohol-related, occurred in younger men. 2) Non-alcohol-associated, were smaller (<2cm, in 70%).

II. Paraduodenal (Groove) Pancreatitis (PDP) (n=26, 2.2%); occurred predominantly in men (73%) often with a h/o alcohol (76%)/tobacco (88%) and a relatively large mass lesion (> 2cm, in 81%). In addition to the characteristic accessory-duct/accessory-ampulla/groove oriented pseudotumors, these also often showed duct-centric inflammation (50%) and granulocytic epithelial lesions (56%), otherwise considered to be diagnostic of autoimmune.

III. Autoimmune Pancreatitis (AIP) (n=22, 1.9%); showing the distinctive fibrosis, obliterative periphlebitis, duct-centric plasma-cell-rich inflammation (positive for IgG4 IHC > 50/HPF). Clinical h/o of IgG4-related sclerosis in other organs was noted in 9, at time of resection.

Conclusions: What we propose to designate as pseudotumoral pancreatitis occurs in slightly younger patients than PDAC and often with h/o of alcohol/tobacco/hypertension, and constitutes 7.9% of pancreatectomies performed with a specific diagnosis of solid PDAC. PDP and AIP, two recognizable entities, account for more than 50%. Accurate sub-classification of resected PP cases as AIP, PDP or others is important because AIP cases are candidates for steroid therapy and may recur, and PDP cases have recently been shown to have significantly better performance status than ordinary pancreatitis.

1767 Clinicopathologic Associations of Paraduodenal (Groove) Pancreatitis, an Under-Recognized Entity: An Analysis of 47 Resected Examples with Emphasis on Imaging-Pathology Correlation

Takashi Muraki, Grace E Kim, Pardeep Mittal, Gabriela Bedolla, Michelle Reid, Bahar Memis, Alyssa M Krasinskas, Ipek Erbarut Seven, Hyejeong Choi, David Kooby, Shishir Maithel, Juan Sarmiento, Volkan Adsay. Emory University, Atlanta, GA; UCSF, San Francisco, CA; Ulsan University, Seoul, Korea.

Background: Paraduodenal (groove) pancreatitis (PDP) is poorly recognized by pathologists and its clinicopathologic associations remain to be fully unraveled.

Design: All 75 PDPs in the authors files were reviewed. 47 had a detailed preoperative imaging available for comparative evaluation and were analyzed in detail.

Results: General characteristics: M/F= 3.3; mean age, 50 (30-69); H/O alcohol 84%, smoking 95%, hypertension 59%. 61% operated with the primary diagnosis of periampullary/pancreatic cancer. Pathologically characterized by thickening/ nodularity of the duodenal wall (especially in the region of accessory ampulla) and adjacent pancreas in the groove area, often forming a pseudotumor that is variably solid/cystic and revealed Brunner gland hyperplasia, exuberant myoid proliferation intermixed with scattered round pancreatic lobules (myoadenomatosis), variably sized cysts some with partial duct-epithelial lining (cystic dystrophy of heterotopic pancreas) as well as ruptured ducts associated with hypercellular reactive tissue (some forming a band around the ducts) and stromal deposition of acinar secretions associated with inflammatory/fibroblastic reaction. **Subtypes:** By imaging, 3 distinct types were identified. I. Solid-tumoral type, 1) forming a distinct band between duodenum and pancreas pattern (36%), commonly showing microabscess and often also showing duct-centric inflammation (29%) and granulocytic epithelial lesions (GELs) (35%). 2) forming irregular lesion typically spanning to the head to groove area pattern (19%), with less abnormalities elsewhere in the pancreas. II. Predominantry cystic type, 1) cyst in the groove area patern (15%), showing less keloid-like fibers and neural proliferation as well as inflammation/injury of the pancreatic head itself. 2) cyst in the only pancreas pattern (15%), more younger, female predominance and less alcohol/tobacco abuse. III. No mass-forming type (15%), showing less hemorrhagic/edematous change in the groove area.

Conclusions: PDP continues to be commonly misdiagnosed as pancreatic/periampullary cancer clinically because it forms a variably solid/cystic pseudo-mass in the accessory ampullary region and adjacent pancreas in the groove area. Duct-centric lymphoplasmacytic inflammation and GELs (findings believed to be features of autoimmune injury) are common findings in PDP. PDP comprises 3 clinically distinct types.

1768 Type 1 Autoimmune Pancreatitis (AIP) Confined to the Pancreatic Duct System with Massive Lobular Effacement: A Type of AIP That Causes Pancreatic Atrophy after Steroid Treatment?

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Background: Type 1 AIP is histologically characterized by diffuse involvement of the pancreatic parenchyme and stroma with lymphoplasmacytic infiltration and fibrosis. However, little has been discussed on variations of the lesion distributions and their clinical implications. In our experience, we encountered some peculiar type 1 AIP cases predominantly involving the pancreatic ducts and causing marked lobular atrophy. In order to clarify the pathological features of such cases, we carried out this study.

Design: 19 resection specimens with a pathological diagnosis of type 1 AIP were gathered. Hematoxylin and eosin-stained slides were evaluated on histological features of the duct system and lobules, and the presence or absence of peripancreatic inflammation and obliterative phlebitis.

Results: We identified 3 cases in which pancreatic ducts were selectively involved and pancreatic lobules were markedly atrophic (duct-centered type). Through the histological review, we identified two histological types of pancreatic duct lesions; one with a thick cuff composed of concentric infiltration of inflammatory cells without preexisting collagenous stroma (concentric pattern), and the other with lymphoplasmacytic infiltration partly involving the preexisting structure (diffuse pattern). All the 3 cases with the duct-centrered type showed the concentric pattern in the main duct and wall edema at the peripheral ducts. Pancreatic acinar cells in the lesions were almost completely absent in all of these cases, and peripheral ducts partially disappeared in 2. Inflammatory cells were scarce in the destructive lobules and peripancreatic region except for one case in which numerous lymphoid follicles were formed. Obliterative phlebitis was seen in only one case. Two other cases with the concentric duct pattern and 14 with the diffuse pattern revealed lobular and peripancreatic inflammation, of which the formers were more severely inflamed, and obliterative phlebitis was identified in every case.

Conclusions: This study indicates that there is a histological subtype of type 1 AIP in which inflammation was confined to the duct system causing a massive lobular effacement. This subtype may represent some type 1 AIP cases in which pancreatic atrophy occurs after steroid treatment. The concentric pattern of the duct lesions may be one reason why the lobules are markedly atrophic.

1769 The Diagnostic Utility of Pancreatic Biopsy for Inflammatory Bowel Disease-Associated Pancreatitis

Kenji Notohara, Shigeyuki Kawa, Toshiharu Ueki, Atsushi Kanno, Mamoru Watanabe, Tooru Shimosegawa. Kurashiki Central Hospital, Kurashiki, Japan; Shinshu University, Matsumoto, Japan; Fukuoka University Chikushi Hospital, Fukuoka, Japan; Tohoku University, Sendai, Japan; Tokyo Medical and Dental University, Tokyo, Japan.

Background: Type 2 autoimmune pancreatitis (AIP) is known to occur in patients with inflammatory bowel disease (IBD), but other causes of pancreatitis, such as adverse drug reactions, may also be involved. In most studies to date, investigation has been based on the histology of resected tissues or on clinical observations, with little discussion on biopsied tissues. In order to clarify the diagnostic utility of biopsy, we conducted a study with specimens gathered by a nationwide survey in Japan.

Design: Cases with both IBD and pancreatitis were gathered from members who belonged to reseach committees for Intractable Pancreatic Disease and Intractable Inflammatory Bowel Disease in Japan, respectively. Clinical information was gathered by a questionnaire, and histological slides were submitted for reevaluation. The histological patterns were classified based on a combination of types of predominant epithelial changes [A: remaining of acinar cells, B: acinar-ductal metaplasia (ADM), C: loss of the majority of the epithelium] and types of stroma and inflammatory infiltration (0: edema without inflammatory cells, 1: inflammatory cell infiltration, 2: fibrosis). A primary and, if present, a secondary pattern were recorded for each case. Intraglandular neutrophilic infltration (INI) was also evaluated.

Results: 23 patients, 18 with ulcerative colitis (UC) and 5 with Crohn's disease (CD), were included. The B-1 pattern was most frequent, and was observed in 9 patients as a primary and a secondary pattern, respectively. The C-1 pattern was secondarily more common, and was found in 8 patients. There was ADM with INI in 11 patients, with one patient having additional INI in the genuine duct (granulocytic epithelial lesion). The Pattern 2 (fibrosis) was seen in 5 patients, and was more common in CD (2/5)

than in UC (3/19). In one patient, eosinophils instead of neutrophils were found in the edematous lobules, suggesting an adverse drug reaction. Cytomegalovirus (CMV) inclusion was identified in another case.
Conclusions: All of these data are similar to what we demonstrated in the resected tissues with type 2 AIP (USCAP 2015), with ADM with INI with a preserved lobular configuration seeming to indicate type 2 AIP. Fibrosis seems more common than in non-IBD patients, and may predict a chronic clinical course. Biopsy was also informative in identifying one case each with an adverse drug reaction and with CMV infection.

1770 MicroRNA Expression in Pancreatic Ductal Adenocarcinoma: A Comparative Study to Predict Metastasis after Neoadjuvant Chemoradiation

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Background: Previous studies have reported the utility of microRNA (miR), small non-coding regulatory RNA which regulates gene expression, as diagnostic and prognostic markers for pancreatic ductal adenocarcinoma (PDAC). In this study, we tried to identify certain miR expression which may predict distant metastasis in PDAC patients who received neoadjuvant chemoradiation.
Design: Twenty-four PDAC patients who underwent neoadjuvant chemoradiation at our institution from 2002-2011 were divided into: study group (patients who developed metastasis) and control group (patients without metastasis). Five cases were excluded because of inadequate tissue on the formalin-fixed paraffin embedded (FFPE) blocks. For each sample, 7 unstained FFPE tissue sections (5 μm) were used for miRNA extraction using the miRNeasy FFPE Kit (Qiagen). Extracted samples were normalized to 10 ng/μL in RNAse-free water and stored at -80°C. Expression of miRs was evaluated using qRT-PCR. Reverse transcriptase reactions were carried out using the TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems) with primers specific for the following miRNAs: RNU6B (internal control), miR-10b, miR-21, miR-148a, miR-196a and miR-217. Real time PCR reactions were performed on the AB 7500 Fast Real-Time PCR System. Each PCR reaction was run in triplicate with the internal control. The expression level of each miRNA was calculated using the formula $\log_2(2^{-\Delta Cq})$; ΔCq was defined as the difference between the Cq value of the target miRNA and that of the internal control (Cq miRNA-Cq RNUB6).
Results: The study and control group consisted of 11 patients (M:F = 2:9; age 60.4±8.1 years) and 8 patients (M:F = 1:1; age 73.1±6.9 years), respectively. Patients in the study group developed metastasis to the lung (4), liver (3), abdominal wall/peritoneum (3), and bone (1). The miRs in both groups had similar expression patterns: miR-10b (underexpressed), miR-21 (overexpressed), miR-148a (inconsistent), miR-196a (underexpressed), and miR-217 (underexpressed). Further semi-quantitative stratification of the miR expression (based on the Cq value) also did not show any significant difference between the two groups.
Conclusions: Expression patterns of miR-10b, miR-21, miR-148a, miR-196a, and miR-217 in PDAC were not predictive of distant metastasis following neoadjuvant chemoradiation. Further studies are necessary to look for novel markers to predict the biological aggressiveness of PDAC.

1771 Impact of Training and Experience on Evaluation of Biliary Brushings (BDB)

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Background: While the low sensitivity and accuracy of BDB in assessing biliary pathology is well known the role of cytology training and signout (s/o) experience in this organ system has not been analysed.
Design: We evaluated impact of cytology training and GI pathology (GIP) experience on diagnosis (Dx) of 60 BDBs among 7 reviewers (3 board-certified cytologists (CYTOs) w/ 7, 10 and 11 yrs experience respectively, 2 GIPs (one w/o BDB s/o experience (GIP1); and 1 w/ (GIP2), 1 generalist (GEN) w/o CYTO/GI training, and a CYTO fellow). BDBs included 30 malignant (w/ histologic confirmation) and 30 benign (w/ resection/≥18 mths uneventful f/u).
Results:

	Sensitivity %	p value	Specificity %	p value	Accuracy %	p value
GI s/o experience v O	66 v 60	0.45	86 v 92	0.14	80 v 76	0.40
CYTO Boarded v O	59 v 72	**0.04**	94 v 86	**0.04**	77 v 79	0.65
Routinely s/o BDB (includes 2 CYTO, GIP2) v O	66 v 69	0.63	91 v 86	0.25	79 v 77	0.74
CYTO-GI (has GI s/o experience) v CYTO2	67 v 52	0.25	97 v 90	0.30	82 v 72	0.21
CYTO-GI v GIP1	67 v 79	0.31	97 v 73	**0.01**	82 v 76	1
CYTO-GI v Fellow	67 v 60	0.59	97 v 97	1	82 v 78	0.65
GIP1 v GIP2	79 v 77	0.89	73 v 87	0.20	76 v 82	0.45
v, versus; O, others.						

For the purpose of testing agreement, the Dxs rendered were compared to the consensus Dx made by at least 2 of 3 CYTOs.Interobserver agreement (IOA) was poor to fair (*K* 0.13-0.39) with fellow and CYTOs showing greatest agreement and GIP1 and CYTOs showing least (poor). IOA between 3 CYTOs and between GIPs was also fair.

Rater	% Agreement	Kappa	95% C.I.
CYTO1 vs 2	61	0.36	0.19-0.52
CYTO1 vs 3	53	0.28	0.15-0.41
CYTO2 vs 3	51	0.27	0.12-0.41
GIP1 vs CDx	51	0.32	0.18-0.44
GEN vs CDx	42	0.22	0.09-0.34
GIP1 vs CDx	26	0.13	0.03-0.21
Fellow vs CDx	60	0.39	0.24-0.54
GIP1 vs GIP2	34	0.22	0.09-0.36
GIP2 vs GEN	37	0.22	0.08-0.36
GIP1 vs Fellow	36	0.22	0.11-0.38
CDx, consensus Diagnosis			

Conclusions: Among pathologists, the level of IOA in evaluating BDBs is fair regardless of experience. While cytology training is associated with higher specificity in diagnosing malignancy this is at the expense of sensitivity, and suggests that CYTOs are more cautious in diagnosing malignancy unless the evidence is overwhelming. In contrast GIPs are more prone to calling things malignant (higher sensitivity) but with lower specificity. Experience in GI appears to improve overall performance of cytopathologists in evaluating BDB.

1772 Marked Geographic Differences in the Pathologic Diagnosis of Non-Invasive (Tis) vs Minimally Invasive (T1) Gallbladder Cancer: Santiago Consensus Conference Highlights the Need for the Unifying Category "Early Gallbladder Cancer" (EGBC)

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Background: Major geographic differences exist for T1 gallbladder cancer (GBC) with the reported proportion of T1 cancers among all GBCs ranging from 3 to 45%, and survival of T1 cancers ranging from 45% at 5-yr to 10-yr, 90%.
Design: 40 cases representing the spectrum of Tis/T1/early-T2 in GBC were reviewed independently, and discussed by international experts in a consensus conference in Santiago, Chile in Nov 2014.
Results: There was significant disagreement among experts from different continents in T-staging of early GBC. Of 19 cases classified uniformly as "non-invasive" by 7 US pathologists trained in different institutions, many were designated as "invasive (T1/T2)" by experts from other continents (range,11-89%, median, 37%;p trend <0.0001).

Interpretation of experts from other continents on cases uniformly designated as <u>non-invasive</u> by 7 Pathologists trained in the US (%)										
	SA1	**SA2**	**SA3**	**K1**	**K2**	**J1**	**J2**	**J3**	**E1**	**E2**
Non-invasive (%)	11	37	74	53	58	84			64	89
Invasive (%)	**89**	**63**	**26**	**47**	**42**	**16**			**36**	**11**
T1a	*37*	*16*	*21*	*47*	*26*	*16*			*26*	*11*
T1b	*26*	*31*	*0*	*0*	*16*	*0*			*5*	*0*
T2	*26*	*16*	*5*	*0*		*0*			*5*	*0*
SA-South American; K-Korean; J-Japanese; E-European.										

Conclusions: A significant proportion of cases designated as Tis (HGD/CIS/non-invasive) in the US would be diagnosed as T1/T2 in S. America and Asia. This suggests the need for the more unifying category of EGBC (Tis+T1), which is already being used in high risk regions, which essentially corresponds to the "intramucosal adenocarcinomas" of other GI organs, and it has been shown in international collaborative studies (with Western criteria) to have a 10-year survival of 90% (if T2 carcinoma is excluded by total GB sampling; PMID:24022828). In order to bridge the geographic gaps identified in this study, a sub-classification of these EGBCs by an approach similar to the Vienna classification for early gastric cancers was proposed at the Santiago meeting and is currently being tested for applicability.

1773 Stromal Changes Related to Therapy in Pancreatic Cancer

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Background: Dense desmoplastic stroma is a histologic hallmark of pancreatic cancer; however, it has received little attention until recently. Despite the recent success of chemotherapies, the survival benefit is modest. The poor survival benefit is attributed to poorly delivered chemotherapeutic agents due to hypovascular stroma composed of SMA positive activated (myofibroblastic) pancreatic stellate cells. Therefore, the focus of treatment has recently shifted from cancer cells to stroma. Preliminary data on stromal modification has shown contradictory results. Histologic changes in stroma related to treatment have not been described well in pancreatic cancer. The aim of this study is to assess the stromal response to neoadjuvant therapy and to correlate findings with residual tumor cellularity.

Design: We retrospectively reviewed slides from 93 patients (15 without neoadjuvant therapy (NoNeo), 78 with neoadjuvant therapy (Neo)) who underwent pancreatectomy for ductal adenocarcinoma from 2009 to 2014. Amount of collagen and myofibroblastic stroma were scored from 0-4 (1+: 1-25%, 2+:26-50%, 3+:51-75%, 4+:76-100%). Inflammation was scored from 0-2 (1+: 50-500 cells/10 hpf, 2+: >500 cells/hpf). Presence of neural hyperplasia was recorded. Arterial changes were scored from 0-3 (1+: 1 artery with change, 2+: 2-3 arteries, 3+:4-5 arteries). The most representative tumor slide was selected from 56 cases, and each slide was scanned with a scanning system. Area of fibrosis and tumor were selected and tumor stroma ratio (TSR=area of tumor mm^2/area of fibrous bed mm^2 x 100%) was calculated. ANOVA, Chi-square, and t-tests are used for statistical analysis.
Results: Average collagen scores in the NoNeo and Neo groups were 2.53±0.83 and 2.07±0.77. Collagen score was significantly lower in the Neo group (p=0.02). Average myofibroblastic stroma scores in the NoNeo and Neo groups were 1.80±0.77 and 2.36±0.81. Myofibroblast score was significantly higher in the Neo group (p=0.01). Arterial changes and neural hyperplasia were more frequent in the Neo group (p=0.0004, 0.0008). TSR minimal (0-2%), mild (2-6%), moderate to high (>6%) cases showed myofibroblast dominant cases as follows: 4/12 (25%), 11/21(52%) and 12/20(60%). Cases with more residual tumor showed more myofibroblast predominant stroma (p=0.04). There were no significant differences in inflammation between the two groups.
Conclusions: Myofibroblastic stroma is increased after neoadjuvant therapy, and treated tumors with higher residual cellularity are associated with higher myofibroblast scores. This suggests that myofibroblasts may contribute to therapy resistance.

1774 Concordance of Tumour Hypoxia in Resectable PDAC in Patients on the Pimo-Panc Clinical Trial and Patient Derived Xenografts Using Semi-Quantitative Image Analysis

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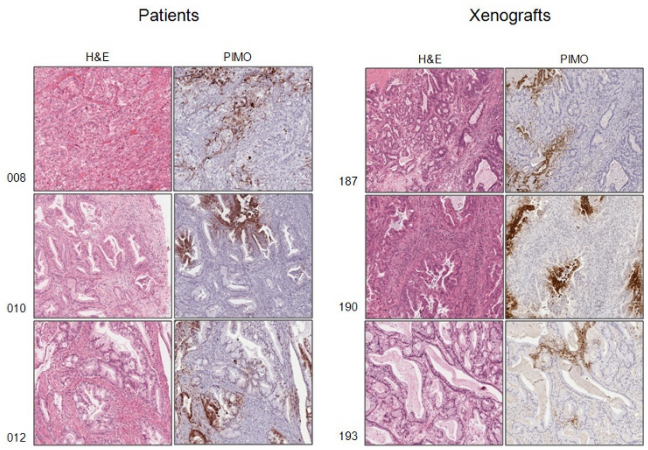
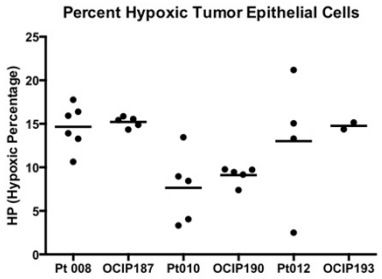
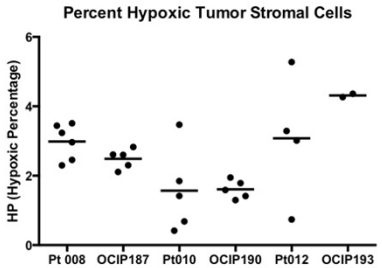
Background: Tumour hypoxia is attributed to the aggressive biology of pancreatic ductal adenocarcinoma (PDAC). We previously reported correlation of hypoxia with rapid tumour growth and metastasis in patient derived xenografts (PDX). We subsequently initiated Pimo-Panc trial (NCT01248637), where patients with resectable PDAC receive pre-operative pimonidazole. Anticipating a prognostic relevance of hypoxia, we developed protocols for semi-quantitative image analysis (IA) to provide objective observer-independent measures of hypoxia. We are evaluating the concordance of microenvironmental features in PDXs with those of the patient tumour.
Design: Pimonidazole IHC was performed on patient tumours. Hypoxic percentage (HP) in the epithelial and stromal compartments was obtained by analysis of IHC using 3 IA platforms based on pattern-recognition software; Definiens-Developer, Tissue Studio and Aperio's Genie. Sections from corresponding PDX models were analyzed similarly.
Results: The 3 IA platforms demonstrated HP in the range of 0-30% in the tumour, with higher levels of HP in epithelial (range=0-25%) vs stromal (range= 0-5%) compartment. Higher levels of HP were obtained by Genie vs Definiens, however HP rank ordered similarly. Furthermore, PDX models recapitulated the morphology in corresponding patient tumour. Mean HP in each patient and PDX was concordant. There was intra-tumoural heterogeneity in HP in the PDAC but not in PDXs.







Conclusions: Assessment of hypoxia in PDAC and corresponding PDXs using semi-quantitative IA is novel. The concordance of HP in PDX and patient tumour suggests these are highly relevant to study hypoxia-related tumour biology as well as diagnostic and therapeutic modalities.

1775 Clinicopathologic and Molecular Analysis of Pancreatic Ductal Adenocarcinoma in Patients with Lynch Syndrome

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Background: Although most pancreatic ductal adenocarcinomas (PDACs) are sporadic, ~10% may be due to an inherited syndrome. A subset of PDACs occurs in patients with Lynch syndrome; these cancers are particularly important to identify, given the growing impact of immunotherapy for mismatch repair (MMR)-deficient cancers. The goal of this study was to assess the histopathology, precursor lesions and genetic profile of PDACs arising in patients with Lynch syndrome.
Design: Nine resected PDACs from patients with Lynch syndrome were collected from 3 institutions. For each case, clinical information, gross findings and histologic sections were evaluated. Alterations in PDAC-associated genes were assessed by next generation sequencing and fluorescence *in situ* hybridization. Immunohistochemistry for Smad4 and the MMR proteins was also performed.
Results: The 5 females and 4 males ranged in age from 50 to 81 years (mean, 65.3 years). Six (67%) had a prior history of Lynch syndrome-associated cancers and 3 (33%) reported a family history of malignancy. Germline Lynch syndrome-associated alterations included: *MSH2* (n = 5), *MSH6* (n = 3) and *MLH1* (n = 1). The patients' tumors were predominantly located within the pancreatic body and/or tail (n = 8, 89%) and ranged in size from 0.5 to 14.0 cm (mean, 4.9 cm). Histologically, 7 (78%) were classified as tubular-type PDACs and the remaining were adenosquamous (n = 1) and medullary (n = 1). An associated intraductal papillary mucinous neoplasm (IPMN) was present in 6 (67%) cases. Four IPMNs were available for MMR immunohistochemistry and all lost expression of the protein corresponding to the germline MMR alteration. The PDACs were staged as follows: 1 was pT2N0, 3 pT3N0 and 5 pT3N1. Genetic profiling of all PDACs revealed somatic alterations in the following genes: *KRAS* (n = 6), *GNAS* (n = 4), *TP53* (n = 3), and *PIK3CA* (n = 3). *CDKN2A* deletions were detected in 4 (44%) PDACs. In addition, 3 (33%) lost Smad4 expression. Interestingly, 7 (78%) PDACs arose in association with an IPMN and/or harbored a *GNAS* mutation.
Conclusions: Lynch syndrome patients with PDAC frequently harbor germline *MSH2* or *MSH6* alterations. Although their tumors can be medullary, they are more often tubular in morphology and arise in association with an IPMN. As IPMNs are amenable to imaging, they represent ideal lesions for early detection strategies in these high-risk patients.

1776 A Clinicopathologic Study of ALK Rearrangements in Pancreatic Ductal Adenocarcinoma

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Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers with a 5-year survival of 6%. Current therapeutic regimens are largely ineffective and underscore the need for novel treatment strategies. Chromosomal rearrangements involving the anaplastic lymphoma kinase (*ALK*) and various partner genes are considered drivers of tumorigenesis, and have been identified in several neoplasms. In addition, the ALK protein inhibitors crizotinib and ceritinib have proven efficacy in patients with *ALK*-rearranged tumors. Therefore, the ALK fusion protein represents

an attractive target for directed therapy. As part of clinical care, we identified a PDAC harboring an *EML4-ALK* translocation, which prompted further evaluation of the prevalence and corresponding clinicopathologic features of *ALK*-rearranged PDACs.
Design: Comprehensive genomic profiling was performed on 1616 PDACs in the course of clinical care that examined 236 genes and 19 genomic rearrangements. A separate cohort of 498 resected PDACs on tissue microarrays was evaluated by both an *ALK* break-apart probe and ALK immunohistochemistry.
Results: Overall, 3 of 2114 PDACs harbored an exon 13 *EML4*-exon 20 *ALK* fusion gene. The patients consisted of 2 males and 1 female that ranged in age from 32 to 43 years. Morphologically, 2 cases were tubular-type PDACs and 1 was a colloid carcinoma. Of the 2 tubular-type PDACs, 1 showed prominent extracellular mucin. Besides the *EML4-ALK* fusion gene, other molecular abnormalities among the 3 tumors included a *BAP1* mutation; *STK11* mutation; and lastly, a *TP53* mutation, *CDKN2A/B* deletion and *MYC* gene amplification. However, *KRAS* and *SMAD4* alterations were absent in all 3 *EML4-ALK*-positive PDACs. Two cases developed distant organ metastases. One patient, who also harbored a *BAP1* mutation, was treated with crizotinib, but developed neutropenia. Upon switching to ceritinib, a reduction in serum CA19-9 from 315 to 23.5 units/mL (normal limits) and radiographic response within 2 months was observed. However, within 7 months, the patient developed resistance. Repeat molecular testing of a newly developed lung metastasis lacked the *BAP1* mutation, but now harbored a *PBRM1* mutation.
Conclusions: The frequency of *ALK* rearrangements in PDAC was 0.14%. *EML4-ALK*-positive PDACs were characterized by a young age, abundant extracellular mucin and absence of *KRAS* and *SMAD4* mutations. While *ALK*-rearranged PDACs were responsive to ALK inhibitors, resistance did develop over time.

1777 Bile Duct Brush and EUS FNA Pancreas in Pancreatic-Biliary Disease Management: A 5-Year Audit

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Background: Pancreatic and biliary malignancies are difficult diagnoses with high mortality. Accurate cytological diagnosis is essential for surgical intervention and/or chemotherapy/palliation. Investigative procedure choice is often based on multidisciplinary decision. Our data from 773 patients (573 bile duct brushings-BD and 398 EUS FNAP pancreas) investigated for pancreatic/biliary disease is presented.
Design: Tissue pathology laboratory computer records were searched for patients who underwent BD or EUS FNAP between Jan 2009 and April 2014. Follow up information (Clinical/Imaging/Pathology) was obtained (range 12-60 months). Nine patients were excluded due to lack of follow up. Cytology diagnostic categories atypical, suspicious and malignant were considered positive; insufficient and negative- negative
Results: Male:female ratio was 1:1, age range 6-98 years and the median age 68years. 629 patients had a single BD/EUS FNAP, 136 patients had 2-7 procedures. The cytology diagnoses were insufficient 56 (7.2%), negative 301 (38.8%), atypical 99 (12.8%), suspicious 91 (11.7%) and malignant 227 (29.3%). Neoplasia was confirmed in 497 patients (398 true positive, 99 false negative). 267 patients were benign (238 true negatives, 29 false positives). The sensitivity of cytological diagnosis was 80%, specificity 89.1%, diagnostic accuracy 83.2%, positive predictive value (PPV) 93.2% and negative predictive value (NPV) 70.6%. Specific cytological diagnoses of tumour included pancreato-biliary/metastatic/hepatocellular carcinoma, neuroendocrine tumour/carcinoma, lymphoma, solid pseudopapillary tumour and mucinous neoplasm. Chronic pancreatitis was not diagnosed on cytology. Histology was available in 29/56 (51.7%) insufficient, 85/301 (28.3%) negative, 51/99 (51.5%) atypical, 40/91 (43.9%) suspicious and 50/226 (22.1%) malignant; the proportion of malignancy being 12/29 41.3% insufficient (NPV 65.7), 37/85 43.5% negative(NPV 71.2), 30/51 58.8% atypical (PPV 77), 37/40 suspicious (PPV 92), 50/50 100% malignant (PPV 99.5) cytological categories. EUS FNAP had diagnostic accuracy of 84%, sensitivity 80.7%, specificity 90%, PPV 93% and NPV 72.2%. Clinical/radiological evidence of disease progression over 4-144 weeks confirmed malignancy in 22/136 (16.2%) patients with multiple inconclusive BD/EUS FNAP.
Conclusions: Combined BD and EUS FNA pancreas has a high diagnostic accuracy in pancreato-biliary malignancy. Surgical intervention for diagnosis is most useful in management of cytological categories insufficient, atypical or suspicious with proportionately increasing detection of malignancy.

1778 GATA-3 Expression in Pancreas Cancer

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Background: GATA-3 is a transcription factor critical to Th2 cell, breast ductal epithelial, and urothelial differentiation, and immunohistochemistry (IHC) for this protein has emerged as a key diagnostic adjunct in support of breast or urothelial origin. Expression in tumors from these sites tends to be diffuse/strong. Recently, one group reported GATA-3 expression in up to 40% of a small number of pancreas cancers, challenging the value of this marker. We performed a detailed expression analysis in large cohort of pancreatic adenocarcinomas (PDAs), using the two dominant, commercially available GATA-3 clones.
Design: Tissue microarrays were constructed from 248 primary and 97 metastatic PDAs (tumors arrayed in triplicate). Most of the metastatic cases involve regional lymph nodes excised at the time of Whipple and thus are matched to primaries. GATA-3 IHC was performed with the L50-823 and HG3-31 clones. Cases were score for extent (%) and intensity (0, 1+, 2+, 3+) of staining and an H-score (extent*intensity) calculated. Fisher's exact, Mann-Whitney, and Wilcoxon matched pairs tests were performed, as appropriate, with $p<0.05$ considered significant.
Results: Overall, GATA-3 immunopositivity was seen in 24% (81/331) and 11% (36/332) of tumors with the L50-823 and HG3-31 clones, respectively ($p<0.0001$). Expression tended to be weak and patchy, with mean and median H-scores for the various combinations of clone and primary vs metastatic status ranging from 8-44 and 1-8, respectively. L50-823-positivity appears stronger than HG3-31-positivity in primaries ($p=0.06$) but not metastases ($p=0.4$). In matched primary-metastatic pairs, GATA-3 positivity was stronger in metastases with both the L50-823 ($p=0.0002$) and HG3-31 ($p=0.049$) clones. Detailed expression data are presented in the Table.

	L50-823		HG3-31	
	% Positive	Mean/Median H-score (if positive)	% Positive	Mean/Median H-score (if positive)
Primary	23% (n=245)	15/4	8.5% (n=247)	8/1
Metastatic	28% (n=86)	44/8	18% (n=85)	23/5.5

Conclusions: Although GATA-3 immunopositivity is not uncommon in PDA, it was not as common as reported in one recent study, which utilized the L50-823 clone. As opposed to typical expression in breast and urothelial cancer, which in our experience produces H-scores in the 150-300 range, expression in PDA is weak and patchy. Attention to strength of positivity should prevent diagnostic confusion. We plan to investigate the specificity of this "low-level" GATA-3 positivity, by examining GATA-3 in a large series of upper GI and lung adenocarcinomas, which tend to be differential considerations in ("CK7-positive-only") metastases of unknown primary.

1779 The Prognostic Role of Desmoplastic Stroma in Pancreatic Cancer

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Background: Pancreatic ductal adenocarcinoma (PDAC) is characterized by an abundant desmoplastic stroma. We examined the prognostic value of stroma density and alpha-smooth muscle actin (αSMA) expression for activated pancreatic stellate cells in patients with resectable PDAC treated with surgery and adjuvant gemcitabine-based chemotherapy.
Design: FFPE-tissue originating from the pancreatectomy of 145 patients was immunohistochemicallystained for haematoxylin-eosin and Masson's trichrome to assess stroma density, and αSMA. Their expression was correlated with clinicopathological characteristics as well as overall survival (OS), progression-free survival (PFS), local progression-free survival (LPFS) and distant metastases free-survival (DMFS).
Results: After a mean follow-up of 20 months (range, 2-69 months), the median OS was 21 months and the 3-year OS was 35.7 %. In multivariate analysis, highly-dense stroma was an independent prognostic parameter for OS ($p=0.001$), PFS ($p=0.007$), LPFS ($p=0.001$) and DMFS ($p=0.002$), while αSMA expression lacked significance. Interestingly, highly-dense stroma retained significance for the four clinical endpoints only in early (pT1-2) but not late (pT3-4) stage tumors. Additionally, late pT-stage (pT3-4), the presence of lymph node metastases (pN+ vs pN0), perineural/neural invasion PNI and administration of adjuvant chemotherapy also correlated with prognosis in multivariate analysis. Altogether, stroma density constitutes an independent prognostic marker in PDAC patients treated with adjuvant chemotherapy.
Conclusions: Our findings highlight the dynamic complexity of desmoplasia and indicate that highly-dense stroma can prevent tumor progression. Further validation of the prognostic value of stroma as a biomarker and its role in in PDAC patients after adjuvant chemotherapy is warranted and will be performed in a prospective study.

1780 Genetic and Epigenetic Changes in Stromal Cells Regulate Tumor Progression and Emphasize the Genetic Complexity of the Tumor Microenvironment in Pancreatic Cancer

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Background: Tumor microenvironment is important for understanding the mechanisms of initiation and progression in cancer. It includes cancer cells, immune cells and stromal cells, mainly consisting of cancer associated fibroblasts. There is evidence that in pancreatic ductal adenocarcinoma (PDAC), a highly lethal malignancy with a rich stromal component, stromal cells are involved in neoplastic progression. Essential for progression and metastatic spread in cancer is the process of epithelial mesenchymal transition (EMT) which at the morphological level is thought to be reflected by the presence of dissociative growing cancer cells ("tumor budding cells") at the invasive front of many cancers including PDAC. Tumor budding is an independent prognostic factor in PDAC.
Design: Stromal cells were assessed at protein and mRNA level for EMT factors (SNAIL1, TWIST, ZEB1, ZEB2) and common tumor suppressors (SMAD proteins and PTEN) by mRNA-in-situ-Hybridization and immunostaining on multipunch tissue-microarrays containing 120 well-characterized PDACs. Additionally, analysis of specific miRNAs by qRT-PCR after RNA extraction from from juxta-tumoral and tumor-remote stroma by Laser Capture Microdissection was undertaken. Special attention was given to the stromal cells surrounding the EMT-like cancer cells at the invasive front.
Results: At the tumor microenvironment of aggresive PDAC, stromal cells surrounding EMT-like cancer cells overexpress EMT-markers (SNAIL1, TWIST, ZEB1 and ZEB2) at protein and mRNA level. A concommitant loss of SMAD proteins and PTEN in tumor and stromal cells was observed in a subset of PDACs and was associated with aggressive features like distant metastasis ($p=0.0045$), larger tumor size ($p=0.0112$) and worse overall-survival ($p=0.021$). Stromal cells with PTEN loss showed frequent chromosome-10 deletion and miR-21 overexpression. There was a differential

expression between juxta-tumoral and tumor-remote stroma concerning miR-21, miR-210, miR-200b and miR-203. Moreover, miRNA expression in stromal cells correlated with the expression of target proteins like ZEB1 (for miR-200b) and PTEN (for miR-21).
Conclusions: Stromal cells may regulate tumor progression of PDAC through genetic and epigenetic changes involving miRNA expression at the tumor microenvironment of the invasive front. Our findings emphasize the complexity of the tumor microenvironment in PDAC and underline the fact that alterations of stromal cells should be considered when designing targeted, personalized therapies for pancreatic cancer patients.

1781 Loss of MGMT Protein Expression in Pancreatic Neuroendocrine Neoplasms Does Not Affect Overall Survival

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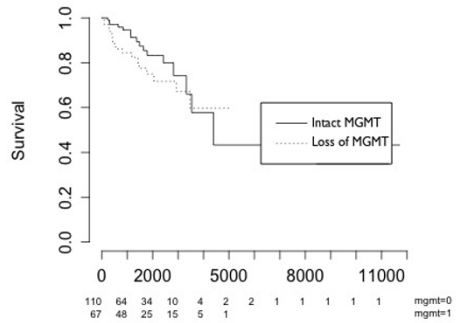
Background: Grading and prognosis of pancreatic neuroendocrine tumors currently relies on mitoses and Ki67 proliferation rate. There has been increased interest in additional immunohistochemical markers that may provide independent prognostic information, such as O^6-Methylguanine-DNA methyltransferase (MGMT), because it can potentially guide the use of alkylating chemotherapy agents. Prior studies have shown conflicting results as to whether loss of MGMT expression confers worse outcome.
Design: Pancreatic neuroendocrine tumors were identified from the pathology database of a large academic medical center (1996 to 2013) and compiled in a tissue microarray. H&E and Ki-67 stained sections were used to classify the lesions as well-differentiated neuroendocrine tumors, WHO grades 1 and 2 (WDNET), versus poorly-differentiated neuroendocrine carcinoma, WHO grade 3 (PDNEC). Immunohistochemistry for synaptophysin, chromogranin, and MGMT were performed on each tumor. MGMT protein expression was scored if an internal positive control was present. Loss of MGMT was scored if less than 1% nuclear staining was present in tumor cells. Kaplan-Meier analysis was used to calculate overall survival (OS). Cox proportional hazards models were used to assess significance of MGMT with survival.
Results: 170 primary pancreatic neuroendocrine neoplasmswere identified. Median age was 56 years, 93 (55%) were male, 162 (95%) were WDNETs, 8 (5%) were PDNECs. All cases were synaptophysin positive. The 5-year OS rate for the entire cohort was 78.5%. Loss of MGMT expression was seen in 67 tumors (39%). A univariate Cox regression analysis demonstrated that Grade 3 was associated with an increased hazard of death with a likelihood ratio of 21.9 ($p<0.0001$). Loss of MGMT expression has no significant effect on survival ($p=0.28$).



Conclusions: Overall, patients were predominantly young and had WDNETs. As expected, PDNEC had worse OS compared to WDNET. In a Cox regression analysis, the factor associated with increased hazard of death was PDNEC. Loss of MGMT immunohistochemical expression is not an independent predictor for worse survival.

1782 CD13hi Neutrophil-Like Myeloid-Derived Suppressor Cells Exert Immune Suppression through Arginase 1 Expression in Pancreatic Ductal Adenocarcinoma

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Background: Perineural invasion of tumor tissues is the cardinal feature of pancreatic ductal adenocarcinoma (PDAC). Heterogeneous myeloid-derived suppressor cells (MDSCs) are potent suppressors of antitumor immunity becoming a significant obstacle of cancer immunotherapy. It remains unknown what the role of MDSCs in perineural invasion of PDAC.
Design: First, we compared the pathological changes among chronic pancreatitis (CP) tissues, PDAC, and adjacent non-neoplastic pancreatic tissues (ANPTs). Second, we detected the profile of MDSC subsets in patients with PDAC by immunofluorescence double staining and flow cytometry. Third, we investigated the characteristics of two novel MDSC subsets including the phenotype, morphology, gene expressions and immune-suppressive function. Finally, we studied the relationship between these two novel MDSC subsets and tumor progression.
Results: We found that lymphocytic perineural cuffs were frequently identified in chronic pancreatitis (CP) tissues and adjacent non-neoplastic pancreatic tissues (ANPTs), but not in PDAC with perineural invasion. $CD11b^+$ $CD33^+$ MDSCs were infiltrated in PDAC tissues and perineural cuffs of ANPTs and the frequency of $CD11b^+$ $CD33^+$ $CD14^-$ $CD15^+$ neutrophil-like MDSCs (nMDSCs) but not $CD11b^+$ $CD14^+$ $HLA\text{-}DR^-$ monocyte-like MDSCs (mMDSCs) were increasing significantly in PBMCs of PDAC patients. By screening the phenotypes of nMDSCs, we identified two MDSC subsets including $CD13^{hi}$ nMDSCs and $CD13^{low}$ nMDSCs. They were similar in morphology with the characteristics of neutrophils, but $CD13^{hi}$ nMDSCs expressed higher levels of CD11b, CD33, CD16 and arginase 1 than $CD13^{low}$ nMDSCs. Importantly, $CD13^{hi}$ MDSCs suppressed alloreactive T cell responses via arginase 1 expression more effectively than $CD13^{low}$ nMDSCs. Furthermore, surgical treatment could reduce the number of $CD13^{hi}$ nMDSCs and switch $CD13^{hi}$ nMDSCs to $CD13^{low}$ nMDSCs with lower immunosuppressive activity.
Conclusions: We identified two novel MDSC subsets with different characteristics and immunosuppressive activity in PDAC and demonstrate the relationship between the quantity and quality of MDSC subsets and cancer progression, explaining the roles of MDSC subsets in immune escape and perineural invasion of PDAC.

1783 The Diagnostic Utility of TCTE3 as a Novel Immunohistochemical Marker for Pancreatobiliary Tumors

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Background: Adenocarcinomas from gastrointestinal (GI) tract and pancreatobiliary tree frequently share morphologic features. Several markers of pancreatobiliary differentiation have been described in the literature to help the differentiation; however, none have high specificity and sensitivity. T-complex-associated-testis-expressed 3 (TCTE3) encodes a dynein light chain in cilia and flagella in sperm. Here we introduce it as a novel pancreatobiliary marker and propose its diagnostic utility.
Design: A large panel of 224 adenocarcinomas from 10 different locations in the GI tract were studied, including GE junction (20), stomach (30), ampulla (16), pancreas (36), extrahepatic common bile duct (CBD), and gallbladder (15), cholangiocarcinoma (19), hepatocellular carcinoma (HCC) (20), small intestine other than ampulla (12), colon (32), and rectum (24). Tissue microarrays were constructed and tumors stained using a TCTE3 antibody (Sigma-Aldrich). Positivity was defined as $\geq 5\%$ tumor cells with unequivocal cell membrane/cytoplasmic staining. Histopathological data were correlated with the staining and analyzed by Chi-square test (JMP 11.0).
Results: TCTE3 expression was characterized by a strong , well defined cell membrane/cytoplasmic pattern in the normal pancreatobiliary epithelium, including large and small bile ducts/ductules in liver and pancreas and epithelium of gallbladder. It was not expressed in normal esophageal and gastric mucosa, hepatocytes, pancreatic acini and islets, small and large intestinal epithelium, or any non-epithelial cells. Positive TCTE3 stain was found in 86% of pancreatic duct adenocarcinomas, 47% of cholangiocarcinomas, 56% of ampullary adenocarcinoma, 27% of CBD/gallbladder adenocarcinomas, and 35% of GEJ adenocarcinomas. Only 3% of gastric and 6% of colon cancers exhibited positive staining. No expression was found in all the small intestinal (other than ampulla) cancers, rectal cancers, and HCCs. The staining positivity was not associated with grades or subtypes (for ampullary and cholangiocarcinoma). TCTE3's sensitivity and specificity for the diagnosis of pancreatic cancer in this current series was 86% and 83%.
Conclusions: The results demonstrate that TCTE3 may have diagnostic utility for pancreatic ductal carcinoma and in the separation of gastric cancer and small intestinal cancer from tumors of the pancreatobiliary tree. Correlation with intestinal marker and more investigation of non-GI tract tumors are needed to further strengthen the utility of TCTE3 as a specific pancreatobiliary marker.

1784 Non-Mucinous Epithelium Is a Common Finding in Mucinous Cystic Neoplasms of the Pancreas and Liver and May Represent a Precursor to the Mucinous Component

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Background: Mucinous cystic neoplasms (MCNs) can occur in the pancreas and liver. Classically, these cystic lesions are lined by columnar mucinous epithelium with underlying ovarian-type stroma. It has been proposed that cysts with OTS and non-mucinous epithelium be considered separate entities in both the pancreas (PMID 23596111) and liver (PMID 25792461). Using a large case series, we aimed to further characterize the histologic types of epithelium present in MCNs of the pancreas and liver.
Design: Cases collected from 1981-2015 with 2 or more slides containing lesion (average 13 slides, 22 sections of cyst) were reviewed (104 pancreatic, 32 hepatic). The percentage of mucinous and non-mucinous/biliary (NM/B) epithelium was recorded in 5% increments. Mucinous epithelium was defined as PanIN-like columnar cells with pale pink/clear apical mucin. Epithelial cells ranging from flat to cuboidal to short columnar without obvious mucin or goblet cells were classified as NM/B epithelium.
Results: The amount of NM/B epithelium present in each MCN ranged from 0-100%. 47% of cases had abundant ($\geq$55%) NM/B epithelium; these cysts were significantly smaller and none had HGD or invasion.

	# Cases (% Total)		HGD Only		Invasion +/- HGD		Age		Size	
% Non-Mucinous/ Biliary Epithelium	Pancreas	Liver	Pancreas	Liver	Pancreas	Liver	Pancreas	Liver	Pancreas	Liver
<5	6 (6%)	6 (19%)	1	0	4	3	57	58	9.4	12.8
5-10	24 (23%)	4 (12%)	3	0	6	0	45	56	9.3	11.0
15-50	22 (21%)	10 (31%)	3	1	1	0	40	44	7.7	11.4
55-85	27 (26%)	7 (22%)	0	0	0	0	47	50	4.8	12.5
90-100	25 (24%)	5 (16%)	0	0	0	0	45	48	4.1	6.2
			P=.007		P<.0001		P=NS		P<.0001	
P values are for ≤50% vs ≥55%.										

Conclusions: NM/B epithelium frequently occurs in MCNs of the pancreas and it can be the predominant component in some cases. Since mucinous and NM/B epithelia often occur together, there does not seem to be enough evidence to regard cases with predominantly NM/B epithelium as separate entities. Our study found that MCNs with abundant NM/B epithelium are significantly smaller than MCNs with abundant mucinous lining and we confirmed that these cases do not harbor HGD/malignancy. These findings suggest that mucinous change is a "progression" phenomenon in MCNs of the pancreas and liver and only when abundant mucinous epithelium is present is there a risk of progression to malignancy.

Pathobiology (including Pan-genomic/ Pan-proteomic approaches to cancer)

1785 Comprehensive Genomic Profiling of Lung Adenocarcinoma Identifies Patients with Genomic Alterations beyond Clinical Testing Guidelines Who May Benefit from Enrollment in Mechanism Driven Clinical Trials

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Background: The NCCN guidelines for lung cancer patients with metastatic disease recommend testing for *EGFR*, *BRAF* and *ERBB2* mutations, *MET* amplification and *ALK*, *ROS1* and *RET* rearrangements. *KRAS* is also a well-characterized driver of lung adenocarcinoma (AD) that predicts resistance to certain targeted therapies. However, many patients lack genomic alterations (GA) involving these established oncogenes. We therefore investigated the prevalence of other GA in lung AD using a comprehensive genomic profiling (CGP) test that can detect all classes of GA in hundreds of cancer-related genes.

Design: DNA was extracted from 40 microns of FFPE sections from 5371 consecutive clinical cases of lung AD from 2013-2015. CGP was performed using a hybrid-capture based next-generation sequencing assay to a median coverage depth of 554X. To identify patients who may benefit from investigational drugs that are available through clinical trials, we focused on genes enriched for GA in tumors with wild-type *EGFR*, *BRAF*, *ERBB2*, *MET*, *ALK*, *ROS1*, *RET* and *KRAS*.

Results: The mean age of patients was 63 years (range 13-88) and 55% were female. In this series, 5371 lung AD were tested by CGP and 2399 (45%) harbored at least one GA involving NCCN guideline genes while 1598 (30%) had mutated *KRAS*. Thirty-six genes were enriched for GA in the remaining cohort (1374 cases) without these known drivers. In this group, 402 (29%) harbored at least one GA involving known lung AD genes *NF1* (13%), *NRAS* (2.3%), *FGFR2* (1.3%), *HRAS* (0.7%), and *NTRK1* (0.7%) as well as potential drivers *RICTOR* (6.4%), *AKT2* (3.2%), *CRKL* (2.2%), *AXL* (1.6%), *MYCN* (1.5%), *MAP2K1* (1.2%) and *RAF1* (0.7%). Clinical responses to targeted therapies have been reported in patients with *NTRK1* and *FGFR* rearrangements and *CRKL* and *RICTOR* amplifications, among other GA listed above. Clinical trials that specifically target GA involving eight of these twelve genes are underway in lung AD.

Conclusions: Beyond detecting GA involving all seven driver oncogenes that are included in the NCCN guidelines and *KRAS* in 75% of lung AD patients, CGP identifies patients with other GA in a significant proportion of "pan-negative" cases who may benefit from enrollment in mechanism driven clinical trials for lung AD. Of note, there are currently hundreds of targeted therapies in development directed against GA in more than 150 distinct cancer-related genes.

1786 Integrative Immuno-Mapping of a Precision Cancer Medicine Cohort: Towards Establishing Predictive Molecular, Neoepitopes and TCR Signatures

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Background: Despite the clinical success of checkpoint blockade therapies, a systematic strategy to identify and evaluate fundamental determinants of clinical response to immunotherapy and its long-term prognosis is still lacking.

Design: To address this problem, we sought to generate a comprehensive immuno-mapping of genomically characterized metastatic biopsies from a precision cancer medicine (PM) cohort. The immuno-map of each tumor includes HLA restricted neoepitopes, immune-checkpoint modulators' expression, and cytotoxic T-cell (CTL) infiltration. Whole exome sequencing (WES) data was collected for ~350 PM samples across 32 different tumor types. Inferred HLA haplotypes and non-synonymous point mutations (NSPMs) were used to predict mutant neoepitope nanomers for MHC Class I molecules. A novel method to delineate and quantify the variable regions of T-cell receptors (TCR) in CTL's using RNA-seq data was applied for CTL infiltration and T cell repertoire assessment.

Results: In the PM cohort, approximately 50% of the global NSPMs (average=32 per tumor) resulted in at least one neoepitope. A significant correlation was found between the NSPMs and the neoepitope burdens ($R^2 \approx 0.99$), but this correlation did not extend to higher likelihood of strong binders (MHC Class I affinity <50nM). Neoepitope frequencies varied by tumor types, the highest median frequency was found in pancreatic tumors. A significant negative selection against the strong binding neoepitopes was observed in subsequent samples from metastatic tumors ($p<0.005$), suggesting possible immunoediting. A significant correlation between expressions of T-cell marker, CD8A, and the mapped TCR reads (density) was observed ($r=0.55$). TCR densities exhibited heterogeneity within and across tumor types (range=0.04-34 reads per million). Correlational clustering of TCR repertoires did not identify any enriched TCR clones to be tumor specific, highlighting widespread T cell clonal diversity. Although no strong correlation was found between the immunocheckpoint blockade proteins and TCR density ($r<0.50$), other immune response regulators were enriched e.g., leukocyte activation (FDR=0.05). No correlation was found between the number of neoepitopes and the TCR density.

Conclusions: Combining the three modalities mentioned above, our approach provides a panoramic immuno-map of patients with advanced cancer. We envision modeling a comprehensive immune-signature that would serve as a predictive biomarker to benchmark selection of patients best suited for immunotherapy.

1787 Frequency of KRAS Subtypes in Non-Small Cell Lung Cancer

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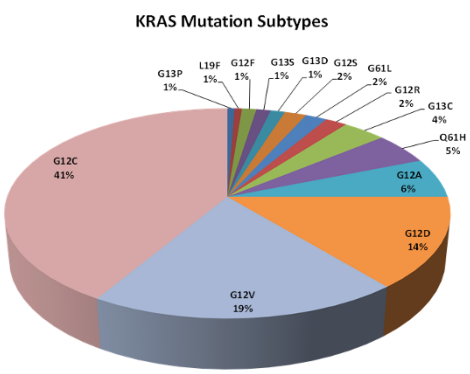
Background: It has been shown that KRAS mutant non-small cell lung cancers (NSCLCs) may vary in clinical outcome depending on which KRAS codon specific mutation is present. Shorter progression free survival has been associated with KRAS variants G12C and G12V, and cell lines with these variants depend to a greater extent upon the RAS/RAF/MEK/ERK signaling pathway. Consequently they may be more susceptible to MEK inhibition. Because different KRAS mutations are not biologically equivalent and may lead to different signal transduction cascades and altered drug sensitivity, we aimed to determine subtype specific KRAS mutation status in a NSCLC patient cohort at our institution.

Design: We tested 502 NSCLCs using a next generation sequencing 50 gene cancer hotspot panel that included the KRAS oncogene. DNA extracted from unstained tissue sections was used to prepare barcoded libraries and samples were multiplexed for sequencing on Ion Torrent 318v2 chips using the Ion PGM™ System. Variants were identified using the Variant Caller Plugin (v.4.0), and annotation and functional predictions were performed using Golden Helix SVS (v.8.3.4).

Results: The overall mutation rate in the KRAS gene was 32.7% among NSCLC cases studied (164/502). The most common KRAS mutations were G12C (41%) and G12V (19%) that are both considered RAS/RAF/MEK/ERK pathway dependent. The third most common subtype, G12D (14%), is known to act more through AKT phosphorylation. The remaining mutations included: G12A (6%), Q61H (5%), G13C (4%), and rarer subtypes: G12R, G61L, G12S (2% each), and G13D, G13S, G12F, L19F, G13P (1% each).



Conclusions: Based on this large mutation subtype-specific analysis, more than a half of KRAS mutant NSCLC patients could potentially benefit from the addition of a MEK inhibitor such as selumetinib to standard chemotherapeutic agents. It is especially important, considering that without MEK inhibition, G12C and G12V cancers seem to progress faster than all other KRAS subtypes or wild-type KRAS. Moreover, due to mutated KRAS, these patients will likely fail anti-EGFR therapies and have very limited therapeutic options.