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CONTENTS

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001

Cross-study reconciliation of SF-1 regulatory targets in adrenocortical carcinoma through in silico analysis

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Background
Steroidogenic Factor-1 (SF-1; *NR5A1*) is a nuclear receptor transcription factor crucial for the development of adrenal glands and gonads, as well as for steroid hormone production. Its overexpression in adrenocortical carcinoma (ACC) is associated with increased proliferation, poor prognosis, modulation of steroid production, and immune suppression.
Objectives
Three independent studies aimed to identify SF-1 regulatory targets in ACC using H295R cells, but comparisons of differentially expressed genes (DEGs) revealed poor overlap, with less than 10% of target genes shared. This study explores the reasons for this divergence and proposes a method to reconcile the findings using an *in silico* approach.
Methods
We reassessed *in vitro* raw data from the studies by Ferraz *et al*. (2011), Doghman *et al*. (2013), and Ehrlund *et al*. (2012), applying standardized analytical methods, including normalization, data preprocessing, and statistical tests. An *in silico* SF-1 regulon was used as an external reference for cross-study comparison. Additionally, we implemented a systematic approach to optimize the threshold for identifying shared differentially expressed genes (DEGs) among the studies.
Results
Our analysis revealed a consistent directional pattern across all phenotypes despite the low initial overlap in targets. We identified similar qualitative transcriptional signatures across all three studies, which led us to conduct a quantitative analysis using a systematic approach to threshold selection. This approach ultimately identified a common set of *NR5A1* targets shared among the studies.
Discussion/Conclusion
The findings suggest that the studies complement each other, providing a more comprehensive understanding of SF-1's regulatory role in ACC. Reassessing and standardizing the comparison between these three studies enhances the identification of SF-1 regulatory targets in ACC. In conclusion, the *in silico* methodology used here can be considered and assessed for feasibility, where the standard methods pose limitations to finding convergence in DEGs results, despite using the same cell line and similar technical approaches.
Keywords
SF-1, Adrenocortical Carcinoma, Gene Regulation, Bioinformatics, Threshold Optimization
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002

Evaluating the response of immunotherapy in metastatic adrenocortical carcinoma – a single institution retrospective review

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Background
Adrenocortical carcinoma (ACC) is a rare endocrine malignancy with a 5-year relative survival rate of 5.7% for those with metastatic disease. Surgical resection remains the only potential curative treatment, with chemotherapy and radiation providing variable benefits. In the last several years, clinical trials have investigated the outcome of immune checkpoint inhibitors (ICI) in this population.
Objective/Methods
Evaluate the response of patients with metastatic ACC undergoing immunotherapy at a single institution between 2020-2024 using RECIST best overall response in a retrospective review.
Results
Twenty-two patients (8M, 14F) with metastatic ACC received pembrolizumab. With respect to various treatment modalities, 16 patients (4-post neoadjuvant therapy) underwent surgical resection (14 high-grade disease via Ki-67 index), 15 patients received mitotane, 19 patients underwent cytotoxic chemotherapy, and 14 patients had either adjuvant radiation to the surgical bed or sites of distant metastases. Pembrolizumab was the initial systemic treatment modality in 9 patients and followed disease progression as second- and third-line agents in 11 and 2 patients, respectively. Two patients reached completion of a 2-year trial with either complete response (1) (CR) or partial response (1) (PR). Of the remaining 20 patients, 1 has not completed initial imaging post-therapy, 4 are actively on therapy (1 stable disease (SD), 3 thus far PD), and 15 patients ceased ICI secondary to hospice enrollment (8), functional performance decline (1), or PD (6).
Discussion
The overall response rate (ORR) was 10%, which is in line with published clinical trials that have investigated immunotherapy in patients with metastatic ACC (6-25%). However, RECIST does not capture heterogenous responses. The identification of a singular new lesion qualifies for PD, even when other target lesions may have CR/PR, which skews the results in favor of progression. Nonetheless, a continued long-term response has never been seen with other treatment modalities and therefore, further research is necessary to evaluate the clinical characteristics of a mixed response to immunotherapy, as has been done in other solid organ tumors, and determine the impact, if any, in the order of treatment with respect to cytotoxic chemotherapy and immunotherapy.
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003

Clinical presentation, management, and outcomes of patients with small ($\leq$ 4 cm) adrenocortical carcinoma: single-center retrospective cohort study

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Background
Data on outcomes of ACC $\leq$ 4 cm are scarce.
Objectives
To characterize presentation and outcomes of patients with ACC $\leq$ 4 cm.
Methods
Single-center cohort study of adults with ACC $\leq$ 4 cm, 2000-2024. Outcomes included overall survival (OS) and disease-free survival (DFS).
Results
In 32 patients (75% women) ACC was diagnosed at a median age of 61 years (range 21-83). ACC diagnosis (T0) was determined by the histopathology from adrenalectomy in 21 (65%), biopsy in 8 (25%), and presence of hormone excess in 3 (9%) patients with metastases. Following the earliest imaging that demonstrated a median adrenal mass size of 27 mm (range 9-40) and median unenhanced Hounsfield unit measurement of 31 (range 18-50), ACC was initially suspected in 15 (47%) patients. In 23 patients with at least two imaging studies 6 weeks apart prior to T0, median adrenal mass growth rate was 17 mm/year (range 0-57). Hormone excess was diagnosed in 22 (69%) patients: hypercortisolism in 11 (34%), hyperandrogenism in 4 (13%), mineralocorticoid excess in 4 (13%), and combined hypercortisolism and hyperandrogenism in 3 (9%) patients. Adrenalectomy was performed in 29 (91%) patients: laparoscopic in 20 (69%) and open in 9 (31%), with R0 resection documented in 25 (86%), R1 in 2 (7%), and Rx in 2 (7%) patients. Median tumor size at adrenalectomy was 37 mm (range 12-270). Oncocytic ACC was reported in 7 (24%), myxoid in 1 (3%), and oncocytic/myxoid in 1 (3%) patient. Median Ki-67 (n = 16) was 10% (range 2-51). At T0, staging was ENSAT stage I in 15 (46%), stage II in 4 (13%), stage III in 9 (28%), and stage IV in 4 (13%) patients. Patients were treated with mitotane (18, 56%), chemotherapy, immunotherapy and/or tyrosine kinase inhibitors (11,34%), and radiation (8, 25%). Patients were followed for a median of 3 years (range 0.06-20) post T0. In 28 (88%) patients with non-metastatic disease prior to adrenalectomy, metastases occurred in 10 (36%) at a median time of 0.9 years (range 0.2-3). Five-year OS was 63% and DFS was 56%.
Discussion/Conclusion
We demonstrate several factors associated with worse prognosis in patients with ACC $\leq$4 cm, including pre-operative biopsy, laparoscopic adrenalectomy, and Ki-67 $>$10%. A large multicenter study to understand gaps in diagnosis and treatment of ACC $\leq$4 cm is currently underway.
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004

Circulating cell-free DNA for adrenal masses discrimination: a pilot study

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Background

Ruling out malignancy in adrenal masses (AM) can be a clinical challenge, which may require numerous and/or invasive investigations. Recently, we showed that circulating cell-free DNA concentrations (ccfDNA-C) are higher in patients with adrenocortical carcinoma (ACC) compared to healthy subjects. However, ccfDNA is still poorly explored in other AM.

Objectives.

To explore the usefulness of ccfDNA-C measurement for AM discrimination.

Methods.

We enrolled 74 patients with adrenocortical adenoma (ACA, n =56), other benign AM (OB, n =4, oncocytic adenoma, ganglioneuroma, adrenal cyst, renal schwannoma), ACC (n =11), and adrenal metastases (MET, n =3, primary: papillary thyroid cancer, leiomyosarcoma, renal cell cancer). Clinical data, radiological parameters and blood samples were collected at the time of AM diagnosis. AM with initially indeterminate radiology (heterogenous appearance, unknown plain Hounsfield Units or plain Hounsfield Units > 10) and not associated with Cushing syndrome, primary aldosteronism, androgen excess or mixed hormonal secretion were labelled as "undefined AM" (n =31/74, 18 ACA, 4 OB, 6 ACC, 3 MET). ccfDNA was isolated with Cell3™ Xtract kit (Nonacus) and ccfDNA-C were measured with Quantus™ Fluorometer (Promega). We tested the diagnostic performance of our previously published ccfDNA-C healthy-derived cut-off (0.146 ng/µl) with logistic regression, positive (PPV) and negative predictive value (NPV) for recognition of malignant AM (ACC+/-MET).

Results.

Malignant AM as a whole (ACC+MET) showed higher ccfDNA-C compared to benign AM ($P = 0.03$). However, only ACC ccfDNA-C were higher than other AM types ($P = 0.003$). ccfDNA-C ≥0.146 ng/µl predicted ACC+MET (Odds Ratio (95% Confidence of Interval) (OR 3.884 (95%CI 1.146-13.171), $P = 0.025$), with PPV = 32.1% and NPV = 89.1%, and ACC (OR 10.421 (95%CI 2.054-52.864), $P = 0.001$), PPV = 32.1%, NPV = 95.7%. Among undefined AM, ccfDNA-C were comparable between benign and malignant lesions ($P = 0.078$). However, ccfDNA-C were higher in ACC than each adrenal tumour type when considering all groups separately ($P = 0.003$). ccfDNA-C ≥0.146 ng/µl predicted ACC+MET (OR 5.333 (95%CI 1.000-28.435), $P = 0.042$), with PPV=32.1% and NPV=95.7%, but it did not predict ACC ($P = 0.998$).

Conclusions.

In AM, high ccfDNA-C seems to be an ACC-specific characteristic and ccfDNA-C ≥0.146 ng/µl is confirmed to be a useful cut-off for discrimination of ACC. Further comparisons among larger cohorts of adrenal and non-adrenal tumours are needed.

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005

Outcome of combination chemotherapy for metastatic adrenocortical carcinoma

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Background

Etoposide, doxorubicin, cisplatin, and mitotane (EDP-M) is considered the firstline treatment for metastatic adrenocortical carcinoma (ACC). However, a considerable proportion of patients experience serious adverse events and impact of the EDP-M regimen on patient survival remains unclear. On that ground, it has been hypothesized that omitting doxorubicin from the EDP-M protocol would increase tolerability without a relevant loss of efficacy.

Objectives

To investigate the efficacy of EDP-M in terms of overall survival and objective response (by local criteria), and to compare it to that of patients treated with cisplatin, etoposide and mitotane (EP-M).

Methods

A multi-centric observational study based on a retrospectively cohort from the ENS@T registry. Patients enrolled were at least 18 years of age, had been diagnosed with advanced or unresectable ACC and started first-line chemotherapy treatment with EDP-M or EP-M between 2010 and 2020. Difference in overall survival (from start of chemotherapy) was examined using log-rank and cox-regression analyses including adjustment for relevant baseline parameters (age, Ki-67, oligometastatic disease, performance status, and disease stage) by using multiple imputation methodology.

Result

517 ACC patients from 17 centers were included, 392 received EDP-M and 125 EPM. Median Ki67 was 24% and 30% and the median age was 47 and 51 years in EDP-M and EP-M groups, respectively. Proportion oligometastatic ACC (Stage IVa and ≤5 metastases) was 36% (EDP-M) and 12% (EP-M) with 74% (EDP-M) and 76% (EP-M) of patients having WHO performance status 0-1. Overall survival for the EDP-M group was 20.6 months compared to 12.5 months in the EP-M group (hazard ratio 0.57 [95% confidence interval (CI) 0.46-0.7], $P < 0.0001$). The adjusted overall survival comparison resulted in in a hazard ratio of 0.6 (95% CI 0.47-0.75) in favor of EDP-M. An objective response was reported in 28% and 16% ($P = 0.017$) of patients receiving EDP-M or EP-M, respectively.

Discussion/Conclusion

This study validates findings of the FIRM-ACT study and confirms the activity of EDP-M in a real-world setting. EDP-M treated patients had more favorable outcomes and less aggressive disease characteristics as compared to EP-M treated cases. A randomized clinical trial would be needed to add further evidence on this topic.

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006

Phase 2 study of monotherapy with pembrolizumab for advanced adrenocortical carcinoma

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Background
Adrenocortical carcinoma (ACC) is a rare cancer with suboptimal response to chemotherapy. The role of immunotherapy in ACC management is evolving.
Methods
An investigator-initiated, open-label, phase 2 clinical trial was performed to ascertain the activity and safety of monotherapy with pembrolizumab (a humanized monoclonal anti-programmed cell death protein 1 antibody) in patients with advanced ACC. This study was part of a basket clinical trial (clinical trial registration ID: NCT02721732). Study participants were enrolled from August 15, 2016, till December 7, 2020. Pembrolizumab (200 mg) was administered intravenously every 3 weeks. The primary endpoint was the non-progression rate (being alive without progression) at 27 weeks, which was objectively assessed by an independent radiology team based on the immune-related Response Evaluation Criteria in Solid Tumors. Secondary endpoints consisted of adverse events assessed using the Common Terminology Criteria for Adverse Events (version 4.03).
Results
We enrolled 23 ACC patients (13 female [57%]) with a median age of 54 years (range, 31-78 years). Four cases were cortisol producing (17%). The median follow-up calculated by reverse Kaplan-Meier is 66.9 months (95% CI, 44.7-87.3 months). The median progression-free survival time was 4.0 months (95% CI, 2-6 months), and the median overall survival time was 15.5 months (95% CI, 6-23 months). Among 20 patients with evaluable response, 6 (30%) were alive without progression at 27 weeks. We saw no complete responses but did see partial responses in 4 patients (20%) with median duration of response of 20.9 months (95% CI non-evaluable). The clinical benefit rate was 30% (complete response, partial response, and stable disease at 27 weeks). Three treatment-related adverse events were grade 3 or higher and were potential side effects of pembrolizumab. No treatment-related deaths occurred.
Conclusions
Single-agent pembrolizumab in treatment of advanced ACC has potential for durable responses and a manageable safety profile.
Keywords
pembrolizumab, immunotherapy, adrenocortical carcinoma, adverse events
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007

Combination therapy of multi-targeted tyrosine kinase inhibitors and immune checkpoint inhibitors for advanced adrenocortical carcinoma

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Background
Adrenocortical carcinoma (ACC) is a rare malignancy with limited treatment options. There is limited data about the combined use of multi-targeted tyrosine kinase inhibitors (TKI) and immune checkpoint inhibitors (ICI) in ACC.
Methods
A retrospective study describing the activity and safety of the combination treatment of lenvatinib/pembrolizumab (LEN/PEM) and cabozantinib/pembrolizumab (CABO/PEM) in advanced ACC patients treated in a single institution.
Results
Twenty-four patients (13 females, 54.1%) received either LEN/PEM (17 patients, 70.9%) or CABO/PEM (7 patients, 29.1%) with a median age of 42.5 years (21.2-64.8 years) at the time of starting therapy. All patients failed prior therapy with median lines of prior treatment of 3 (range 1-8). Six patients (25%) had cortisol overproduction at the start of the combination therapy. The median follow-up time was 12.52 months (range, 0.62-72.87) and the median duration of the combination therapy was 5.4 months (range, 0.6-69.6 months). Median progression-free survival (PFS) and overall survival were 8.05 months (4.83-17.48, 95% CI) and 17.48 months (9.89-NA, 95% CI) respectively. The best responses included 3 (12.5%) partial response (PR), 11 (45.8%) stable disease (SD), and 9 (37.5%) progressive disease (PD). There was 1 non-evaluable patient for response. The disease control rate (DCR) was 58.3% and the overall response rate (ORR) was 13%. In two patients, the treatment was discontinued due to grade 3 ICI-induced pneumonitis and autoimmune hepatitis.
Conclusions
In the absence of established salvage therapy in ACC, the combination of multi-targeted TKI/ICI has been associated with clinical benefit in almost half of the subjects. Factors associated with response are worthy of further investigation to properly select patients for future trials testing the combined use of ICIs and TKIs in ACC.
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008

Clinical and genetic characteristics of patients with adrenocortical carcinoma and lynch syndrome

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Background
Lynch syndrome (LS), caused by MMR gene pathogenic variants (PVs), is the most common hereditary cancer syndrome observed in patients with ACC, but little is known about the clinical and genetic characteristics of patients with ACC and LS.
Objectives
To describe the clinical and genetic characteristics of ACC in patients with LS.
Methods
The EMR and local registries of patients at the University of Michigan were searched using defining keywords (e.g., 'Lynch Syndrome', 'adrenocortical carcinoma') and provider names through the EMERSE software. Patients with ACC and rare MMR germline variants were included in the study.
Results
31 patients with ACC and rare MMR germline variants were identified, of which, 30 patients (97%) had rare germline variants in one MMR gene, (20 [65%] PV [12 MSH2, 1 MLH1, 7 MSH6], 9 [29%] variants of uncertain significance/VUS [3 MSH2, 3 MSH6, 3 PMS2], and 1 patient had rare variants in 2 MMR genes (MSH6 [VUS] and PMS2 [benign variant]). 21 patients (68%) were diagnosed at stages I-III vs. 10 patients (32%) at stage IV. 18 patients (58%) had tumor mitotic rates > 20/50 per HPF and 16 patients (52%) had tumor Ki67 > 20%. 18 patients (58%) had hormone excess (5 cortisol, 5 androgen, 2 aldosterone, 6 cortisol and androgen). 5 patients (16%) had other LS-associated malignancies (4 of these patients with PV, 1 with VUS) and 22 patients (71%) had a family history of LS-associated malignancies (16 with PV, 5 patients with VUS, 1 patient with a benign variant). Only 3 patients (10%) met Amsterdam I criteria (all 3 with PV). 13 patients (42%) met Amsterdam II criteria, including ACC as a Lynch-associated cancer (12 PV, 1 VUS). The prevalence of LS in ACC patients was estimated as 3 - 4%.
Discussion
This analysis further supports the association of ACC with Lynch syndrome. Only a minority of patients fulfilled classical diagnostic criteria (Amsterdam I & II), justifying a general testing recommendation for all ACC patients regardless of family history.
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009

Characterizing the biology of ACC by longitudinal dual-PET imaging and multi-omics analyses

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Background
Adrenocortical Carcinoma (ACC) is a heterogenous disease characterized by variable degrees of adrenocortical differentiation and tumor growth rate: Both factors are important prognostic markers that are currently investigated on tumor tissue samples. As such we have a limited capacity to study ACC heterogeneity on the spatial level and to perform longitudinal measurements to document how ACC biology change over time. Molecular imaging may overcome these limitations with [^{18}F]FDG uptake reflecting ACC metabolism while [^{18}F]CETO is a new PET tracer targeting the adrenal cortex.
Aims
To study the use of dual-PET imaging as a mean to investigate ACC proliferation and adrenocortical differentiation over space and time.
Methods
A single-center prospective cohort study (PROGRESS ACC) that is aiming to include 20 patients with confirmed/suspected ACC before surgery or at disease recurrence/progression. Patients undergo PET/CT with [^{18}F]FDG and [^{18}F]CETO as well as collection of tumor tissue (surgery or biopsy) at study inclusion and at disease progression/recurrence. Tumor tissue is characterized by Whole genome sequencing and total-RNA sequencing with plasma samples undergoing targeted DNA sequencing.
Results
Four ACC patients have undergone dual-PET imaging and multi-omics characterization. The integrative analysis is ongoing: Two patients had tumors avid on both [^{18}F]FDG and [^{18}F]CETO imaging, while 2 patients had tumors avid on [^{18}F]FDG-PET but with negligible [^{18}F]CETO-uptake. The findings on PET-imaging could be validated in the gene expression dataset and by histopathology as shown in Table 1.
Table 1 with overview of results

Patient ID	Sample type	[^{18}F]FDG PET SUV_{max}	[^{18}F]CETO PET SUV_{max}	Ki-67 IHC	MKI67 RNA expression	CYP11B1 RNA expression
AC#1	Biopsy	21,2	26,9	38%	37.1	343.1
AC#2	Biopsy	10,7	171	8%	1.4	231.6
AC#3	Biopsy	29,3	4,12	25%	24.5	0.1
AC#4	Resection	12,8	3,2	10%	12.5	1.4

Legend: [^{18}F]CETO, para-chloro-2-fluoroethyletomidate; [^{18}F]FDG, Fluorodeoxyglucose; IHC, immunohistochemistry; PET, Positron emission tomography; SUVmax, maximum standardized uptake value.
Discussion
Dual-PET/CT imaging with [^{18}F]FDG and [^{18}F]CETO could be used to study ACC proliferation and differentiation *in-vivo*. Their combined use offers a unique potential for non-invasive analyses of ACC tumor biology. The PROGRESS-ACC study has so far included 9 ACC patients and the integrative analyses are ongoing.

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010

Dual inhibition of PLK1 and multiple CDKs: a novel approach for the treatment of adrenocortical carcinomas

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Adrenocortical carcinomas (ACC) are highly aggressive tumors with limited treatment options. Polo-like kinase 1 (*PLK1*) and cyclin-dependent kinases (*CDKs*) 1/2/4 are among the most overexpressed genes in ACC human samples. We have previously demonstrated the efficacy of the polo-box domain (PBD)-targeting PLK1 inhibitor (PLK1i) Poloxin in ACC cell lines (H295R, MUC-1, CU-ACC2). Here, we tested 1) the efficacy of Poloxin and the kinase domain (KD)-targeting PLK1i Plogosertib; 2) the efficacy of CDK1/2 inhibitor (CDKi) Dinaciclib, and CDK1-cyclin B1 inhibitor Cucurbitacin E; 3) the combinatorial effect of Plogosertib and Dinaciclib on cell proliferation. Experiments were carried out in four cell lines including H295R, MUC-1, and recently generated TVBF-7 and JIL-2266. Increasing drugs concentrations were used for 72h. Cell proliferation and apoptosis were assessed by BrdU incorporation and caspase 3/7 activity, respectively. Two-drugs combination data were analysed by the "SynergyFinder" tool. PLK1i Poloxin reduced cell proliferation at very high doses, reaching a maximum effect at 100μM ($P < 0.01$ in MUC-1 and TVBF-7; $P < 0.001$ in H295R and JIL-2266), and increased apoptosis at 10μM ($P < 0.05$ for all cell lines). At much lower doses, Plogosertib induced a dose-dependent reduction of cell proliferation ($P < 0.05$ at 100nM in MUC-1 and JIL-2266; $P < 0.01$ at 750nM in H295R and TVBF-7) and an increase of apoptosis ($P < 0.05$ for H295R, TVBF-7, and JIL-2266 at 1μM). CDKi Dinaciclib drastically reduced cell proliferation at low nanomolar concentrations ($P < 0.05$ at 20nM in MUC-1 and JIL-2266, and at 100nM in TVBF-7; $P < 0.01$ at 100 nM in H295R), and increased apoptosis ($P < 0.05$ in MUC-1, TVBF-7, and JIL-2266 at 200nM). Cucurbitacin E reduced proliferation in all ACC cells, but its effects were less pronounced than Dinaciclib. Synergistic inhibition of cell proliferation by combined treatment with PLK1i Plogosertib and CDKi Dinaciclib was observed in H295R ($P < 0.05$) and TVBF-7 ($P < 0.01$) cells. In conclusion, we identified Plogosertib and Dinaciclib as the most effective inhibitors on all cell lines, among those selected, therefore representing interesting novel treatment options for ACC. Moreover, the combination of these drugs showed a synergistic effect, suggesting a potential benefit of using both PLK1i and multi CDKi to increase therapeutic efficacy and to reduce potential side effects.

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011

In vivo CRISPR screening identifies tada2b as a key epigenetic regulator of immune response in adrenocortical carcinoma

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Background
Adrenocortical carcinoma (ACC) is an aggressive malignancy with poor survival outcomes and resistance to immune checkpoint blockade (ICB) therapy. Progress in understanding ACC immunobiology has been hindered by the lack of suitable models. We developed a genetically engineered mouse model of ACC (BPCre) targeting Wnt/β-catenin activation and p53 loss, which closely mimics aggressive human ACC, including epigenomic alterations and poor immune cell infiltration. Additionally, we derived a syngeneic ACC cell line (BCH-ACC3) that forms subcutaneous tumors with poor response to ICB, despite increased CD8 T cell infiltration, reflecting clinical outcomes in ACC patients.
Objective and Methods
To explore the role of epigenetic dysregulation in immune suppression in aggressive ACC, we performed an *in vivo* CRISPR loss-of-function screen targeting 936 chromatin regulators in the BCH-ACC3 cell line. To avoid immune recognition of CRISPR components, we utilized a lentiviral system with selective CRISPR antigen removal (SCAR) to transduce the cells with a pooled sgRNA library (6 sgRNAs per gene). After gene editing and immunogenic component removal, the cells were implanted into three mouse groups: immunodeficient (NSG), immunocompetent (WT-Bl6), and WT-Bl6 treated with ICB (anti-PD-1). Genomic DNA sequencing identified sgRNAs depleted under immune pressure.
Results
Comparison of the WT-Bl6 untreated and WT-Bl6 ICB-treated groups to the NSG group revealed numerous immune-sensitizing hits, including positive control genes and genes known to regulate tumor immunity. Notably, we identified *Tada2b*, a transcriptional adaptor protein essential for the acetyltransferase activity of the SAGA (Spt-Ada-Gcn5 acetyltransferase) complex, as a novel regulator of endogenous and immunotherapy-dependent antitumor immunity in ACC. *Tada2b* knockout (KO) tumors exhibited reduced growth compared to wild-type (WT) tumors, and ICB treatment resulted in greater tumor shrinkage and increased infiltration of activated CD8+ T cells in *Tada2b*-KO tumors.

Additionally, analysis of the ACC-TCGA cohort revealed that *TADA2B* expression negatively correlates with immune signatures such as pathways of TNFA, IL6-JAK-STAT3, IL2-STAT5, and interferongamma. These findings suggest that human ACC tumors with *TADA2B* expression are immune excluded.
Conclusions
These findings indicate that TADA2B modulates the immune response in ACC, suggesting that targeting TADA2B could be a promising strategy to overcome resistance to immunotherapy in ACC.

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012

PCDHGC3 hypermethylation as a diagnostic and prognostic biomarker in adrenocortical carcinomas

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Background
Our previous research highlighted the role of *protocadherin (PCDHC)* gene clusters in neuroendocrine tumors. We discovered a link between *de novo* methylation of the *PCDHGC3* gene and the development of metastases in pheochromocytomas/paragangliomas. Moreover, we identified *PCDHGC3* hypermethylation as a key factor in distinguishing gastrointestinal (GI) neuroendocrine carcinomas (GI-NECs) from GI-neuroendocrine tumors. Adrenocortical carcinomas (ACCs) are rare, aggressive tumors, and differentiating them from adrenocortical adenomas (ADs) can be challenging.
Objective
This study aims to assess whether epigenetic alterations of *PCDHGC3* could serve as clinically relevant biomarkers of malignancy in adrenocortical tumors.
Materials and Methods
The hypermethylation of the *PCDHGC3* gene promoter was analyzed in 50 ACCs and 12 ADs. Statistical analysis was performed using SPSS Statistics v29, and the ROC curve was employed to determine the predictive value for the diagnosis of ACC.
Results
The percentage of *PCDGH3* methylation in the 50 ACCs was statistically higher than in the 12 ADs: $2.7 \pm 2.5\%$ vs. $0.92 \pm 0.13\%$, $P = 0.014$, with an area under the curve (AUC) of 0.912 ± 0.038 (95% CI: 0.837–0.986). For a Youden index of 0.88, a methylation percentage $> 1.10\%$ showed an AUC of 0.94 ± 0.29 (95% CI: 0.883–0.997), with a sensitivity of 88%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 66.7% for differentiating ACCs from ADs. A positive correlation was found with ACC size ($r = 0.40$, $P = 0.005$), but not with the Weiss score, Ki-67, or age. The percentage of *PCDGH3* methylation in ACCs with ENSAT stage IV was higher compared to other stages ($4.1 \pm 4.1\%$ vs. $2.1 \pm 1.3\%$, $P = 0.021$). Kaplan-Meier analysis showed that time to metastasis development in ENSAT stages I-III was shorter in patients with methylation $>$ 1.10% (31 ± 10.4 vs. 13 ± 2.2 months, $P = 0.034$).
Conclusions
Our study suggests that *PCDGH3* hypermethylation could be a useful diagnostic biomarker for differentiating ACCs from ADs, and a prognostic biomarker for identifying patients at higher risk of disease progression.

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013

Discovery and characterization of OR-449, a potent antagonist to steroidogenic factor-1 (SF-1) and clinical candidate for treatment of ACC

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Background
SF-1, an orphan nuclear receptor, controls adrenal organogenesis and is linked to both the pathogenesis and progression of ACC by multiple, independent genomic observations, including somatic amplification in ~90% of cases of pediatric ACC. The SF-1 ligand-binding domain contains a binding pocket that accepts small molecule transcriptional antagonists.
Objectives
Identify potent and drug-like SF-1 antagonists, investigate pharmacology in both isolated cell and whole animal systems, select a development candidate, and evaluate safety in rodent and non-rodent studies suitable for submission of an Investigational New Drug application.
Methods
Novel small molecules were synthesized and characterized for potency and selectivity as SF-1 antagonists in transcriptional and biochemical assays and as inhibitors of proliferation in the rat Leydig tumor cell line R2C and in short-term dissociated cultures of the pediatric ACC tumor SJ-ACC3. Anti-tumor activity was first confirmed in R2C cell-derived xenografts in nude mice and subsequently in two separate pediatric ACC xenografts.
Results
We surveyed several SF-1 expressing cell lines to characterize pharmacology. Only R2C responded by inhibition of proliferation. Structure-activity correlations between inhibition of SF-1 transcriptional activity and DNA synthesis in R2C cells or dissociated SJ-ACC3 tumor cells were robust. A metabolically stable SF-1 antagonist, OR-689, blocked growth of an R2C tumor at 60 and 100 mg/kg upon oral dosing. OR-449, a highly selective SF-1 antagonist, progressively inhibited R2C growth at 3, 10 and 30 mg/kg and at 30 mg/kg blocked SJ-ACC3 xenograft growth and partially inhibited a second pediatric tumor, SW1939. A responsive adult ACC PDX was not identified but significant changes in tumor biomarkers were observed. In 28-day safety studies in mouse and dog, OR-449 demonstrated no serious adverse events at 200 mg/kg. OR-449 (60 mg/kg in rat at day 14 and 200 mg/kg in dog at day 21) did not inhibit ACTH-induced glucocorticoid levels.
Discussion
OR-449 is a potential medical therapy for adult and pediatric ACC. The pharmacological activity of OR-449 is more readily observed in tumor models than in normal adrenal tissue in these studies.

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014

Silent adrenal neoplasm masquerading as adrenocortical carcinoma in a patient with neurofibromatosis type 1

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Introduction
Adrenocortical carcinoma, Pheochromocytomas and Paragangliomas are rare tumors in the general population 1,2, and even more so in association with rare genetic syndromes such as Neurofibromatosis type 1 (NF1)3-6. Current guidelines do not recommend routine screening for these rare and malignant tumors in NF1 patients, posing a risk of missed diagnosis and potentially dangerous outcomes.
Case Presentation
We present a 49-year-old female diagnosed with NF1 at age 10, with a family history of NF1 in her mother. She presented with a subcutaneous thigh lesion that was biopsied and found to be a grade 2 Leiomyosarcoma. Workup with PET-FDG imaging revealed focal uptake in the right adrenal gland and discrete thyroid nodules bilaterally. The patient was asymptomatic, with no history of hypertension, rapid weight gain or weight loss, skin thinning, hyperglycemia, hypertensive spells, palpitations, sweating or panic attacks. Physical examination showed a vitally stable, well-nourished female, with some skin freckling and café au lait lesions, and with no focal signs of hypercortisolism. Further imaging with MRI showed a 3.8 cm right adrenal lesion with intermediate T2 signal enhancement and PETDotatate showed increased uptake of the lesion. Biochemical testing revealed a normal 1mg dexamethasone suppression test, and an elevated urine and plasma metanephrine and normetanephrine levels, more than three times the upper limit of normal. Based on imaging and biochemical testing, a diagnosis of pheochromocytoma was made and the patient was started on doxazosin 10 mg daily with a high-salt diet and underwent laparo **No table of contents entries found**. scopic right adrenalectomy without complication. Pathology confirmed a 4.1 cm malignant pheochromocytoma with capsular invasion. Evaluation of the thyroid nodules with fineneedle aspiration is pending.

Discussion
The patient had a silent clinical presentation, and imaging results were suggestive of an adrenocortical carcinoma. However, biochemical tests revealed a different diagnosis, which was further confirmed by pathology as malignant pheochromocytoma. Although adrenal pathologies are rare in patients with NF1, they are well documented and can lead to devastating outcomes if missed3-6. Current guidelines do not recommend routine screening for adrenal pathologies in patients with NF1. In our case, the diagnosis may have been overlooked if not for the incidental finding of leiomyosarcoma, which prompted further imaging and biochemical testing. While the patient's surgical course was uneventful, the same may not be true for all NF1 patients with undiagnosed pheochromocytomas. We recommend reviewing the guidelines on screening for adrenal tumors in all NF1 patients planning to undergo surgery and/or conception, to mitigate the risk of a pheochromocytoma crisis.
References
1. Kebebew E, Reiff E, Duh QY, Clark OH, McMillan A. Extent of disease at presentation and outcome for adrenocortical carcinoma: have we made progress? World J Surg. 2006 May;30(5):872-8. doi: 10.1007/s00268-005-0329-x. 2. Beard CM, Sheps SG, Kurland LT, Carney JA, Lie JT. Occurrence of pheochromocytoma in Rochester, Minnesota, 1950 through 1979. Mayo Clin Proc. 1983 Dec;58(12) :802-4. 3. Menon RK, Ferrau F, Kurzawinski TR, Rumsby G, Freeman A, Amin Z, Korbonits M, Chung TT. Adrenal cancer in neurofibromatosis type 1: case report and DNA analysis. Endocrinol Diabetes Metab Case Rep. 2014;2014:140074. doi:10.1530/EDM-14-0074. 4. Kollurage UA, Jayawikrama RS, Yasawardana AD, Atapattu N. Adrenal crisis in metastatic adrenal cortical carcinoma in an infant with neurofibromatosis type 1. *Sri Lanka J Child Health.* 2019;48:74–6. 5. Najafi-Semnani M, Rajabi-Moghaddam M, Abbaszadeh H. Adrenocortical carcinoma in a patient with neurofibromatosis type 1: A case report. Caspian J Intern Med. 2021 Fall;12(4):613-617. doi: 10.22088/cjim.12.4.613. 6. Gruber LM, Erickson D, Babovic-Vuksanovic D, Thompson GB, Young WF Jr, Bancos I. Pheochromocytoma and paraganglioma in patients with neurofibromatosis type 1. Clin Endocrinol (Oxf). 2017 Jan;86(1):141-149. doi: 10.1111/cen.13163.

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015

Bone metastasis model for the interrogation of ACC progression and therapeutic response: lessons learned from genitourinary cancers

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Background
Adrenocortical carcinoma (ACC) is the most common type of adrenal cancer. Although only 7% of ACC cases have bone metastasis, these patients have a poor prognosis and reduced quality of life due to severe skeletal related events. Establishing bone metastatic models that allow for the study of tumor progression and therapeutic regimen testing is an important translational research tool for clinical benefit.
Objectives
Specifically, during this symposium we would like to receive feedback from the ACC research community on the applicability and relevance of our approach to ACC.
Methods
Expand our clinically relevant models of bone metastases that encompass intravital multiphoton microscopy (iMPM), engineered bone window systems, *in vitro* bone mimetic environments, and spatial analysis of patient-derived tissues, to study ACC progression and response to therapy in bone.
Results
We generated *in vivo* models of cancer in bone based on intra-tibia injection of luciferase expressing cell lines or patient-derived xenografts (PDX) suitable to test the response to chemotherapy, radiation and anti-angiogenic therapy. Growth kinetics were followed, along with survival monitoring, and endpoint immunofluorescence analysis performed on select markers. We further created a tissue-engineered bone construct (TEBC) that, after directimplantation of cancer cells, is combined with an adjacent skin window, allowing for non-destructive intravital examination of tumor growth. iMPM displays both sensitivity and time-resolution to identify dynamic interactions between cancer cells and bone 3D adaptive niches, which support therapy response and resistance. To flank these *in vivo* dynamic analyses, we established a pipeline for *ex vivo* extraction of topological information related to the molecular and cellular niches involved in tumor progression and response to treatment in both mouse and human samples. In addition, we generated *ex vivo* bone mimetic environments to propagate PDXs and patient-derived cells in a bone-like environment and tested therapy response.
Discussion/Conclusion
The tuneability of our bone metastatic models make them an attractive platform for studying ACC bone metastasis.

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016

Chloroquine inhibits adrenocortical carcinoma cell survival independent of its effects on autophagy

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Background
Adrenocortical carcinoma (ACC) is a rare endocrine-related cancer with limited treatment options. The current therapeutic standard is mitotane, which is adrenolytic via unknown mechanisms. Many chemotherapeutic drugs are known to induce apoptosis and autophagy. The role of autophagy is of particular interest as it can promote either tumor cell survival or death.
Objective
We characterized the autophagic responses in H295R, CU-ACC1, and CU-ACC2 cell lines, hypothesizing that mitotane induces both apoptosis and autophagy and might synergize with autophagy inhibitors such as chloroquine (CQ) or bafilomycin (BAF).
Methods
Proliferation, MTS, and colony formation assays were performed to characterize ACC cell line sensitivity to mitotane. Western blots assessed cleaved PARP and LC3-II accumulation. Incucyte monitoring of caspase 3/7 activity determined mitotane-induced apoptosis with genetic silencing of critical autophagic regulator, ATG5, with or without autophagy inhibitors CQ and BAF.
Results
H295R, CU-ACC1, and CU-ACC2 show differential dose-dependent apoptotic and autophagic responses to mitotane. Silencing of ATG5 in mitotane-treated cells increased cell death in CU-ACC2, but not H295R. CQ and BAF synergized with mitotane in CU-ACC1 and CU-ACC2, but less in H295R. Genetic knockdown of ATG5 in CQ-treated cells did not further augment cell death. In addition, treatment with other autophagy inhibitors, VPS34-IN1 or SBI-0206965, did not increase caspase 3/7 expression.
Discussion
Mitotane induces autophagy differentially in ACC cell lines, correlating to their sensitivities to CQ. Autophagy is a targetable pathway in ACC. CQ, especially in combination with mitotane, is an effective treatment to induce tumor cell death. CQ induces cell death via autophagic and/or non-autophagic mechanisms. These data support the potential use of CQ in patients with ACC. Funded by VA Merit Review to MEW/KKV and laboratory funds

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017

Practice patterns, characteristics, and clinical outcomes for patients with ACC referred to a tertiary academic medical center

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Background
Adrenocortical carcinoma (ACC) is a rare, often fatal endocrine cancer. Standard of care is driven by expert opinion, which advocates for: multidisciplinary evaluation, early oncologic surgical resection, complete pre-operative hormonal/imaging work-up, and avoiding adrenal mass biopsy.
Objective
We characterized factors dictating practice patterns and clinical outcomes for patients seen at a tertiary academic medical center with high referral volume for ACC.
Methods
We retrospectively collected clinical data for 75 adult patients with ACC referred to WashU from 2000-2024; complete data currently available for 68 (91%).
Results
34/68 (50%) were female, median age 56 years (range 26-85), spanning all stages at diagnosis (34% I-II, 35% III, 31% IV). Median overall survival was 50.3 months, progression-free survival 7.8 months. 46/67 (69%) were diagnosed at community hospitals, though 41/57 (72%) received primary surgery in an academic setting (Chi-square $P = 2.54x10-15$). Type of hospital at diagnosis or

surgery was not associated with differences in survival. Only 29/60 (48%) saw endocrinology prior to initial intervention, associated with more complete hormonal evaluation (Wilcoxon t-test $P = 0.00037$). 43/50 (86%) with adequate hormonal work-up had secretory tumors, with the following major secretion patterns: 30% cortisol + androgen, 28% androgen, 22% cortisol. 22/67 (33%) received adrenal biopsy despite imaging concerning for ACC; 8/22 (36%) were nondiagnostic. Adrenal biopsy was associated with higher risk of death in univariate models (HR 2.8,95%CI:1.3-6.0), but not multivariate models including stage (HR 1.2, 95%CI: 0.43-3.3). 17/65 (26%) had an antecedent adrenal nodule, with first documentation ranging from 0.32-9.63 years (median 5.09) prior to ACC diagnosis. Longer antecedent periods were associated with higher risk of death, even in multivariate models including stage (HR 1.1, 95%CI:1.01-1.3).
Discussion/Conclusion
ACC is frequently diagnosed in the community prior to academic referral, typically for surgery. Patients were not consistently referred to endocrinology prior to surgery, leading to incomplete hormonal evaluation. Adrenal biopsies were common, nondiagnostic, but not independently associated with worse survival. Many patients had antecedent adrenal nodules, indolent for years before evolving to ACC. These observations highlight a need to disseminate knowledge about appropriate work-up for adrenal tumors across practice settings, and add to literature questioning adequate duration for surveillance of incidentalomas.

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018

Bilateral adrenal carcinoma: a rare case report

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Background
Adrenal cortical carcinoma (ACC) is a rare and aggressive neoplasm that frequently presents with nonspecific symptoms, complicating diagnosis. This case reports a 41-year-old female with a history of left adrenalectomy for oncocytic ACC, who subsequently developed a right adrenal lesion.
Case Presentation
The patient initially underwent left adrenalectomy after imaging revealed a large left adrenal mass measuring 3.1 x 2.8 cm with a precontrast Hounsfield unit of 24. A PET scan indicated a mass size of 2.5 cm with a standardized uptake value of 15.2. Postoperative pathology confirmed the diagnosis of oncocytic adrenocortical carcinoma. Functional studies for adrenal hormones and genetic screenings for tumor syndromes unremarkable. The patient received adjuvant radiotherapy to the left adrenal bed for local control. Eight years post-surgery, a follow-up CT scan showed no signs of recurrence, although an unchanged right adrenal adenoma was noted. However, subsequent MRI revealed a right adrenal lesion measuring 1.7 cm, characterized by T2 hyperintensity and heterogeneous enhancement. The accompanying PET scan demonstrated a non-FDG avid 2.5 x 1.7 cm right adrenal nodule. Laboratory evaluations, including urinary dopamine, metanephrines, DHEAS, dexamethasone suppression test remained within normal limits. Adrenal biopsy showed adrenal cortical proliferation, leading to a right adrenalectomy, which yielded post-surgical pathology favoring carcinoma (Ki 67 index 18%). The patient was then initiated on hydrocortisone and fludrocortisone.
Discussion/Conclusion
ACC is associated with a high recurrence rate and poor prognosis, necessitating careful monitoring following surgery. Imaging modalities such as MRI and PET scans are essential for evaluating adrenal masses, although non-FDG avid lesions can complicate diagnosis. The evolving role of genetic screening, particularly for syndromes like Li-Fraumeni and Lynch syndrome, is significant. This case highlights the complexities of managing ACC and underscores the importance of diligent monitoring, comprehensive clinical evaluation, and effective endocrinological management post-surgery.

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