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Scientific Programme

Wednesday 11th October 2023

14.00-17.00 **Adrenal Tumor Masterclass**

Chairs: Mercedes Robledo, Felix Beuschlein

14.00-14.30 Following guidelines or practical workarounds in primary aldosteronism ***(Marcus Quinkler)***

14.30-15.00 Adrenocortical cancer – time for personalized treatment options? ***(Matthias Kroiss)***

15.00-15.30 Mechanisms of transcriptional regulation – Goldilocks and the pathogenesis of adrenocortical carcinoma ***(Enzo Lalli)***

15.30-16.00 Data sharing, reality or (science) fiction? ***(Eva Ortega)***

16.00-16.30 Data sharing, reality or (science) fiction? (Part 2) ***(Fátima Al-Shahrour)***

16.30-17.00 **Round Table with the Founders of ENSAT:** How to develop a successful research network?

Chairs: Cristina Ronchi, Felix Beuschlein

ENSAT Founders: Xavier Bertagna, Massimo Manelli, Franco Mantero, Martin Reincke, Paul Stewart

17.00-17.30 **Coffee break**

17.30-18.30 **Harmonisation WG1 & WG2 meeting**

18.30-19.30 **Harmonisation WG3 & WG4 meeting**

19.30-20.00 **Harmonisation WG5 meeting**

20.30-22.00 **Welcome reception** (gathering of the Founders and the future of the Network)

Thursday 12th October 2023

08.00-09.00 **Harmonisation Management Committee meeting**

09.00-19.00 **ENSAT/Harmonisation Conferrence**

09.00-09.15 **Welcome address and practical information**

09.15-11.15 **Oral communications (ACC)**

Chairs: Enzo Lalli, Michaela Luconi

OR1: Ablation of Znrf3 & Trp53 induces metastatic adrenocortical carcinoma in mice ***(Diana Garcia-Garcia)***

OR2: Tumor microenvironment of adrenocortical carcinoma dissected by single-cell RNA-sequencing ***(Anne Jouinot)***

OR3: Cellular landscape of adrenocortical carcinomas at single-nuclei resolution reveals signature heterogeneity and feature of tumour aggressiveness ***(Barbara Altieri)***

OR4: Cancer cell xenograft in zebrafish embryos as a promising new experimental tool for drug screening in AdrenoCortical Carcinoma ***(Mariangela Tamburello)***

OR5: FSCN1 as a new druggable target in adrenocortical carcinoma ***(Carmen Ruggiero)***

OR6: Performance of targeted sequencing in paraffin-embedded samples for prognostic classification of adrenocortical carcinoma ***(Cristina Ronchi)***

OR7: Mitotane induces GDF15 expression in adrenocortical carcinoma - a potential therapy-induced mechanism of immune escape ***(Isabel Weigand)***

OR8: Neoantigens - a chance to improve immunotherapy in adrenocortical carcinoma? ***(Laura-Sophie Landwehr)***

OR9: Serum inflammation-based scores in a large cohort of adrenocortical carcinomas and adrenocortical adenomas: a comparative study and role of the hormonal secretion pattern ***(Alessandra Mangone)***

OR10: Prolonged Exposure To Target Mitotane Concentrations Is Associated With Better Recurrence-Free Survival In Patients With Adrenocortical Carcinoma On Adjuvant Treatment ***(Soraya Puglisi)***

OR11: EO2401 peptide immunotherapy + nivolumab in adrenocortical carcinoma (ACC); the Phase 1/2 EOADR1-19/SPENCER trial (NCT04187404) ***(Eric Baudin)***

11.15-12.15 **Coffee break**

Guided poster tour (ACC/PPGL)

Chairs: Michaela Luconi, Enzo Lalli (ACC)

Elena Rapizzi, Henri Timmers (PPGL)

12.15-14.00 **Oral communications (ACC/PPGL)**

Chairs: Matthias Kroiss, Massimo Terzolo

OR12: Specific characteristics of the genomic profile, tumor microenvironment and vascular structure of PPGLs with MAML3 fusions ***(Maria Monteagudo)***

OR13: Interaction between hypoxia signalling and kynurenine pathway in pheochromocytoma and paraganglioma (PPGL) ***(Nicole Bechmann)***

OR14: Evaluating microRNAs as circulating biomarkers for the prediction of malignant paraganglioma and pheochromocytomas ***(Tom Drossart)***

OR15: Monitoring metastatic pheochromocytomas and paragangliomas using plasma cell-free DNA sequencing ***(Carlota Arenillas)***

OR16: ROR-1 specific CAR-T cells with CRISPR/Cas9 mediated glucocorticoid receptor-knockout exert potent antitumor efficacy in advanced adrenocortical carcinoma ***(Marc Philipp Schauer)***

OR17: Testosterone, macrophages and senescence: novel therapeutic options for ACC? ***(Diana Garcia-Garcia)***

OR18: Identification of adrenocortical masses malignancy and aggressivity through radiomics: a pilot study ***(Lorenzo Tucci)***

OR19: Evaluation and Validation of an adapted pediatric S-GRAS-score ***(Verena Wiegering)***

OR20: Decrease in anticortisolic drug osilodrostat plasma exposure in patients treated with mitotane for an adrenocortical carcinoma ***(Louis Thomeret)***

14.00-15.00 **Lunch**

15.00-16.50 **Oral communications (PPGL)**

Chairs: Judith Favier, Rodrigo Toledo

OR21: Tracking pheochromocytoma evolution reveals multiple routes to metastasis, low intratumoral heterogeneity and an independent clonal origin in multiple disease ***(Ester Arroba)***

OR22: Single-cell chromosome and transcriptome analysis as a diagnostic tool to differentiate between benign and metastatic pheochromocytoma and sympathetic paraganglioma ***(Annika Berends)***

OR23: Heterogeneity of the tumor microenvironment across PPGL metastases ***(Bruna Calsina)***

OR24: Differences in the catecholamine secretion machinery in pheochromocytomas and paragangliomas ***(Nicole Bechmann)***

OR25: Recurrent disease in patients with sporadic parasympathetic head and neck paraganglioma **(*Susan Richter)***

OR26: Bone metastases and skeletal related events in pheochromocytoma and paraganglioma patients. International, retrospective study ***(Marta Lagana)***

OR27: Impact of surgical technique on hemodynamic instability during minimally invasive surgery for pheochromocytoma **(*Amir Hossein Chaman Baz)***

OR28: Responses to systemic therapy in metastatic pheochromocytoma and paraganglioma ***(Alessa Fischer)***

OR29: Sunitinib for malignant progressive pheochromocytomas and paragangliomas ***(Eric Baudin)***

OR30: EO2401 peptide immunotherapy + nivolumab in metastatic pheochromocytoma/paraganglioma (MPP); the Phase 1/2 EOADR1-19/SPENCER trial (NCT04187404) ***(Alfredo Berruti)***

16.50-17.45 **Coffee break**

Guided poster tour (APA/NAPACA)

Chairs: Sheerazed Boulkroun, Marcus Quinkler (APA)

Guido Di Dalmazi, Martin Fassnacht (NAPACA)

17.45-19.15 **ENSAT Registry**

19.15-20.15 **APA/NAPACA WG meeting**

Friday 13th October 2023

08.00-09.00 **ENSAT general assembly**

09.00-11.25 **Oral communications (APA/NAPACA)**

Chairs: Cristina Ronchi, Darko Kastelan

OR31: Whole blood transcriptomic signature of Cushing's syndrome ***(Maria Francesca Birtolo)***

OR32: Constitutional duplication of PRKACA gene is a cause of isolated Primary Pigmented Nodular Adrenocortical Disease (PPNAD): results of its systematic screening in Macro- and Micronodular Nodular Adrenal Hyperplasis ***(Patricia Vaduva)***

OR33: Identification and characterization of molecular heterogeneity in PBMAH by MALDI-MSI and RNA-Sequencing ***(Sharmilee Vetrivel)***

OR34: Study of somatic molecular heterogeneity in bilateral macronodular adrenocortical disease (BMAD) by NGS panel in a cohort of 26 patients ***(Florian Violon)***

OR35: Primary Unilateral Macronodular Adrenal Hyperplasia (PUMAH) With Concomitant Glucocorticoid And Androgen Excess due to KDM1A activation and constitute MC2R activation ***(Yasir Elhassan)***

OR36: Reconstitution of human adrenal cortex development and steroidogenesis, a new paradigm towards stem-cell based endocrinology ***(Kotaro Sasaki)***

OR37: Role of the mineralocorticoid receptor in the physiology of the adrenal cortex and the development of aldosterone producing adenomas ***(May Fayad)***

OR38: Single-Nuclei Analysis of Aldosterone-Producing Adenoma ***(Masanori Murakami)***

OR39: Modulation of calcium signaling “on demand” to decipher the molecular mechanisms responsible for primary aldosteronism ***(Bakhta Fedlaoui)***

OR40: The Association of Adrenal Steroids on the Metabolomic Differences in Primary Hyperaldosteronism vs Primary Hypertension ***(Zoran Erlic)***

OR41: Urine steroid metabolomics as a diagnostic tool in endocrine hypertension ***(Alessandro Prete)***

OR42: Inflammation-based scores in benign adrenocortical tumours are linked to the degree of cortisol excess ***(Vittoria Favero)***

OR43: The cardiometabolic risk in patients with non-functioning adrenal incidentaloma: an observational, retrospective and propensity score matched study ***(Mirko Parasiliti-Caprino)***

11.25-11.50 **Coffee break**

11.50-13.20 **ACC/PPGL WG meeting**

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Oral Presentations

OR1: ABLATION OF ZNRF3 & TRP53 INDUCES METASTATIC ADRENOCORTICAL CARCINOMA IN MICE

Diana Garcia-Garcia [1], James Wilmouth Jr [1], Julie Olabe [1], Iuliia Pinigina [1], Antoine Martinez [1], Pierre Val [1]

[1] *CNRS UMR6293,* GReD Institute, Clermont-Ferrand, France

Pan-genomic analyses have identified that the most aggressive subgroup of adrenocortical carcinoma (ACC) patients have overlapping alterations in the WNT/β-catenin pathway and the p53/RB signaling pathway. To investigate whether alterations in the p53/RB and the WNT/β-catenin pathway could work together to promote metastatic ACC in mice, we utilized Cre-loxP technology to mimic inactivation of ZNRF3 and TP53, the most commonly found alteration in each pathway in ACC patients. Using a combination of Kaplan Meier analysis, bulk RNA sequencing and immunohistochemistry, we demonstrate that adrenal cortex specific ablation of Trp53 and Znrf3 results in development of metastatic and lethal ACC over a 6-month time course. Consistent with ACC patients, metastatic spread is more frequent in female DKOs than male DKOs. This is associated with stronger infiltration of phagocytic tumor associated macrophages (TAMs) in male indolent DKOs compared to female indolent DKOs, further highlighting the anti-tumor effects of phagocytic TAMs in the adrenal cortex. In contrast, metastatic DKOs in both sexes have similar characteristics including high proliferation and low immune infiltration. Single nucleus RNA sequencing further shows that acquisition of aggressive features is associated with amplification of a population of proliferative cells characterized by expression of Mki67, Rad51 and Ezh2. These molecular features are also found in lung metastases, suggesting that these originate from dissemination of this set of cells. These data shed light on the underpinnings of metastatic dissemination and establish these mice as a good in vivo model to investigate novel therapeutic options.

OR2: TUMOR MICROENVIRONMENT OF ADRENOCORTICAL CARCINOMA DISSECTED BY SINGLE-CELL RNA-SEQUENCING

Anne Jouinot [1, 2], Yoann Martin [1], Thomas Foulonneau [1], Yanis Bendjelal [1], Phillip Calvet [1], Florian Violon [1, 3], Mathilde Sibony [1, 3], Daniel De Murat [1], Roberta Armignacco [1], Karine Perlemoine [1], Franck Letourneur [1], Brigitte Izac [1], Muriel Andrieu [1], Annabel Berthon [1], Bruno Ragazzon [1], Magalie Haissaguerre [4], Antoine Tabarin [4], Lionel Groussin [1, 2], Rossella Libé [1, 2], Jérôme Bertherat [1, 2], Guillaume Assié [1, 2]

[1] *Genomic and signaling of Endocrine Tumors,* Institut Cochin, INSERM U1016, CNRS UMR8104, Université Paris Cité, Paris, France
[2] *Endocrinology,* AP-HP, Hôpital Cochin, Paris, France
[3] *Pathology,* AP-HP, Hôpital Cochin, Paris, France
[4] *Endocrinology,* CHU de Bordeaux, Hôpital Haut-Lévêque, Pessac, France

Background: Molecular classification is important for diagnosis and prognosis of adrenocortical tumors (ACT). Transcriptome profiles identify two groups of ACC, “C1A” (“steroid” and “proliferation” signatures) and “C1B” (“immune” signature), of poor and better prognosis respectively. However, these signatures were characterized at the tissue level (“bulk”) and our knowledge of the cell composition of ACC is limited. The aim of this study was to dissect the tumor microenvironment composition in “C1A” and “C1B” ACC at the cell level. Methods: We performed single-nuclei RNA-sequencing (10x) of ~170,000 cells from human normal adrenal (N=4), benign ACT (N=14) and ACC (N=20) to construct a single-cell atlas of normal and tumoral adrenal cortex. This atlas was then used to estimate the cell composition of ACC (Cibersortx) in “bulk” transcriptome of 201 patients and to test its association with hormone secretion and outcome. Results: Tumor microenvironment was composed of fibroblasts (4.6% of total cells), endothelial cells (5.1%), myeloid cells (9.9%) and lymphoid cells (1.2%). ACC microenvironment showed tumor-associated signatures with cancer-associated fibroblasts (expressing PDGFRB and FN1), tumor-associated endothelial cells (ANGPT2 and VWF) and tumor-associated macrophages (CD163 and F13A1). Compared to “C1A”, “C1B” ACC were enriched in inflammatory “M1-like” macrophages. Deconvolution in bulk transcriptomes showed that this population is associated with non-cortisol secreting tumors (t-test $p<10\text{-}5$), longer disease-free survival (Logrank $p<10\text{-}5$) and longer overall survival ($p<10\text{-}7$). Conclusion: Inflammatory macrophages may be repressed by glucocorticoid

secretion, leading to immune system escape in "C1A" poor prognosis ACC. This may impact immunotherapy strategies in this cancer.

OR3: CELLULAR LANDSCAPE OF ADRENOCORTICAL CARCINOMAS AT SINGLE-NUCLEI RESOLUTION REVEALS SIGNATURE HETEROGENEITY AND FEATURE OF TUMOUR AGGRESSIVENESS

Barbara Altieri [1], David S. Tourigny [3, 2], Ali Kerim Secener [4], Silviu Sbiera [1], Marc P. Schauer [1], Panagiota Arampatzi [5], Sascha Sauer [4], Martin Fassnacht [1], Cristina L. Ronchi [6, 7]

[1] *Division of Endocrinology and Diabetes,* University Hospital of Würzburg, Würzburg, Germany
[2] *School of Mathematics, ,* University of Birmingham, , Birmingham, United Kingdom
[3] *Irving Institute for Cancer Dynamics ,* Columbia University, New York , United States
[4] *Molecular Medicine,* Max Delbrück Center, Berlin, Germany
[5] *Core Unit SysMed,* University of Würzburg, Würzburg, Germany
[6] *Institute of Metabolism and System Research,* University of Birmingham, Birmingham, United Kingdom
[7] *Centre for Endocrinology, Diabetes and Metabolism (CEDAM),* Birmingham Health Partners, Birmingham, United Kingdom

Background: Molecular mechanisms of adrenocortical tumorigenesis remain elusive. Aim was to investigate cellular heterogeneity of adrenocortical carcinoma (ACC) by single-nuclei RNA sequencing (snRNA-Seq), using adult normal adrenal glands (NAG, n=6) as reference. Methods: Single nuclei were isolated from 12 ACC samples, including 6 primary tumours, 3 recurrence and 3 metastasis from 8 patients. snRNA-Seq was performed using 1CellBio. Seurat R-package was used for data analysis. snRNA-Seq ACC signature scores was analysed against the TCGA- cohort. Results: The ACC tumour microenvironment was relatively devoid of immune cells compared to NAG, emphasising that ACC is an immunological cold tumour. The integrated analysis revealed 8 ACC specific subclusters, including six that were found across all samples (ACC 1-5 and ACC-M) and two specific to the oestrogen-secreting tumour (ACC E1-2). Particularly, ACC-M scored highest for gene sets “G2M_checkpoints”, “E2F_targets”, and “mitotic_spindle”, indicative of cells engaged in mitotic activity. DIAPH3 was the top marker and was significantly associated with poor survival in the TCGA-cohort (HR=8.0, 95%CI=3.3-19.3, $p<0.0001$), suggesting it represents a novel marker specific to mitotic cells. ACC-M signature was highly scored in COC3 subtype (associated with the most clinically aggressive tumours) compared to COC1 in TGCA. Two clusters (including one of the oestrogen-specific clusters) displayed a transcriptional signature of increased cholesterol

homeostasis, including many genes involved in early steroidogenesis. These two clusters were enriched in functional COC2-COC3 tumours from TGCA. Conclusion: Our snRNAseq analysis allowed the characterisation of the molecular heterogeneity of ACC and revealed specific cell populations associated with worse clinical outcome.

OR4: CANCER CELL XENOGRAFT IN ZEBRAFISH EMBRYOS AS A PROMISING NEW EXPERIMENTAL TOOL FOR DRUG SCREENING IN ADRENOCORTICAL CARCINOMA

Mariangela Tamburello [1], Andrea Abate [1], Elisa Rossini [1], Daniela Zizioli [2], Ram Manohar Basnet [2], Giovanni Ribaudo [1], Alessandra Gianoncelli [1], Guido Alberto Massimo Tiberio [3], Enzo Lalli [4, 5], Carmen Ruggiero [4, 5], Alfredo Berruti [6], Sandra Sigala [1]

[1] *Section of Pharmacology, Department of Molecular and Translational Medicine,* University of Brescia, Brescia, Italy
[2] *Section of Biotechnology, Department of Molecular and Translational Medicine, University of Brescia,* University of Brescia, Brescia, Italy
[3] *Surgical Clinic, Department of Clinical and Experimental Sciences, University of Brescia at ASST Spedali Civili di Brescia,* University of Brescia at ASST Spedali Civili di Brescia, Brescia, Italy
[4] *Institut de Pharmacologie Moleculaire et Cellulaire ,* CNRS UMR , Valbonne, France
[5] *Universite Cote d'Azur,* Universite Cote d'Azur, Valbonne, France
[6] *Oncology Unit, Department of Medical and Surgical Specialties, Radiological Sciences and Public Health,* University of Brescia and ASST Spedali Civili di Brescia, Brescia, Italy

Introduction: Despite the murine model has been widely used in in vivoexperiments, the zebrafish (Danio rerio) possesses unique advantages that make it a versatile and faster preclinical model for drug screening, especially in Adrenocortical Carcinoma (ACC) for whom preclinical models are scant. Our group developed the model of zebrafish embryos xenografted with ACC cells. Here we reported results obtained with this approach, to confirm the in vitro results obtained in ACC cells treated with different drugs. Methods: ACC cells were injected into the yolk-sac of 2-day-old wild-type or transgenic embryos. Xenografted embryos were exposed to the drugs directly added to the water. After 3 days, the drug effects on xenograft growth were scored, measuring the tumor areas of each group. Metastases-positive embryos (presence of at least one fluorescence dot outside the injection site) were counted. Results: We demonstrated the ability of abiraterone acetate, trabectedin and progesterone to significantly reduce the tumor area at non-toxic-concentrations. This experimental model allowed us to confirm in vivo that metastasis-derived cells were able to metastasize and that trabectedin and progesterone reduced the rate of embryos with metastasis. Moreover, metastasis formation was significantly reduced in H295R/TR-SF-1-xenografted embryos after fascin1knock-out or inhibition with G2-044. Conclusions; Even with some limitations, the model we developed offer a suitable and

expeditious animal model for the screening of potentially effective drugs, identification of dose toxicity, and determination of the most promising compounds for more advanced preclinical phases, especially in rare diseases such as ACC.

OR5: FSCN1 AS A NEW DRUGGABLE TARGET IN ADRENOCORTICAL CARCINOMA

Carmen Ruggiero [1, 2], Mariangela Tamburello [3], Elisa Rossini [3], Silvia Zini [3], Nelly Durand [1, 2], Giulia Cantini [4, 5], Francesca Cioppi [5, 6], Constanze Hantel [7, 8], Katja Kiseljak-Vassiliades [9, 10], Margaret E. Wierman [9, 10], Laura-Sophie Landwehr [11], Isabel Weigand [11, 12], Max Kurlbaum [11], Daniela Zizioli [13], Andrei Turtoi [14, 15], Shengyu Yang [16], Alfredo Berruti [17], Michaela Luconi [4, 5], Sandra Sigala [3], Enzo Lalli [1, 2, 18]

[1] *Institut de Pharmacologie Moléculaire et Cellulaire UMR 7275,* CNRS , Valbonne, France
[2] *Université Côte d'Azur,* Université Côte d'Azur, Valbonne, France
[3] *Department of Molecular and Translational Medicine, Section of Pharmacology,* University of Brescia, Brescia, Italy
[4] *Department of Experimental and Clinical Biomedical Sciences "Mario Serio", Endocrinology Unit,* University of Florence, Florence, Italy
[5] *Centro di Ricerca e Innovazione sulle Patologie Surrenaliche,* AOU Careggi, Florence, Italy
[6] *Department of Experimental and Clinical Medicine,* University of Florence, Florence, Italy
[7] *Department of Endocrinology, Diabetology and Clinical Nutrition,* University Hospital Zurich (USZ) and University of Zurich (UZH), Zürich, Switzerland
[8] *Medizinische Klinik und Poliklinik III,* University Hospital Carl Gustav Carus Dresden, Dresden, Germany
[9] *Department of Medicine, Division of Endocrinology, Metabolism and Diabetes,* University of Colorado Anschutz Medical Campus, Aurora , United States
[10] *Rocky Mountain Regional Veterans Affairs Medical Center,* Rocky Mountain Regional Veterans Affairs Medical Center, Aurora, United States
[11] *Department of Internal Medicine I, Division of Endocrinology and Diabetology ,* University Hospital, University of Würzburg, Würzburg, Germany
[12] *Department of Medicine IV,* University Hospital Munich, Ludwig-Maximilians-Universität München, Munich, Germany
[13] *Department of Molecular and Translational Medicine, Section of Biotechnology,* University of Brescia, Brescia, Italy
[14] *14Tumor Microenvironment and Resistance to Therapy Laboratory,* Institut de Recherche en Cancérologie de Montpellier, Université de Montpellier-INSERM U1194, Montpellier, France
[15] *15Platform for Translational Oncometabolomics,* Biocampus, CNRS-INSERM-Université de Montpellier, Montpellier, France
[16] *Department of Cellular and Molecular Physiology,* Penn State University College of Medicine, Hershey, United States
[17] *17Oncology Unit, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health,* University of Brescia and ASST Spedali Civili di Brescia, Brescia, Italy
[18] *INSERM,* INSERM, Valbonne, France

Adrenocortical carcinoma (ACC) is a rare endocrine malignancy with a high risk of relapse and metastatic spread. The actin-bundling protein fascin (FSCN1) is overexpressed in aggressive ACC and represents a reliable prognostic indicator. FSCN1 has been shown to synergize with

VAV2, a guanine nucleotide exchange factor for the Rho/Rac GTPase family, to enhance the invasion properties of ACC cancer cells. Based on those results, we investigated the effects of FSCN1 inactivation by CRISPR/Cas9 or pharmacological blockade on the invasive properties of ACC cells, both in vitro and in an in vivo metastatic ACC zebrafish model. Here, we showed that FSCN1 is a transcriptional target for β-catenin in H295R ACC cells and that its inactivation resulted in defects in cell attachment and proliferation. FSCN1 knock-out modulated the expression of genes involved in cytoskeleton dynamics and cell adhesion. When Steroidogenic Factor-1 (SF-1) dosage was upregulated in H295R cells, activating their invasive capacities, FSCN1 knock-out reduced the number of filopodia, lamellipodia/ruffles and focal adhesions, while decreasing cell invasion in Matrigel. Similar effects were produced by the FSCN1 inhibitor G2-044, which also diminished the invasion of other ACC cell lines expressing lower levels of FSCN1 than H295R. In the zebrafish model, metastases formation was significantly reduced in FSCN1 knock-out cells and G2-044 significantly reduced the number of metastases formed by ACC cells. Our results indicate that FSCN1 is a new druggable target for ACC and provide the rationale for future clinical trials with FSCN1 inhibitors in patients with ACC.

OR6: PERFORMANCE OF TARGETED SEQUENCING IN PARAFFIN-EMBEDDED SAMPLES FOR PROGNOSTIC CLASSIFICATION OF ADRENOCORTICAL CARCINOMA

Juliane Lippert [2], Ulrich Dischinger [2], Silke Appenzeller [3], Alessandro Prete [1], Stefan Kircher [4], Kassiani Skordilis [5], Yasir Elhassan [1], Barbara Altieri [2], Martin Fassnacht [2], Cristina Ronchi[1,2]

[1] *IMSR,* University of Birmingham, Birmingham, United Kingdom
[2] *Division of Endocrinology and Diabetes,* University Hospital of Wuerzburg, Wuerzburg, Germany
[3] *Core Unit Bioinformatics,* Comprehensive Cancer Centre Mainfranken, Wuerzburg, Germany
[4] *Institute of Pathology,* University of Wuerzburg, Wuerzburg, Germany
[5] *Dpt of Histopathology,* University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom

Adrenocortical carcinoma (ACC) is a rare aggressive malignancy with heterogeneous outcomes. We evaluated the added value of previously proposed DNA-based biomarkers (BMs), evaluated on routinely available formalin-fixed, paraffin-embedded (FFPE) tissue, compared to S-GRAS score that is the best known prognostic factor. We analysed 194 FFPE ACC samples, including a retrospective training cohort (107 cases) and a prospective validation cohort (87 cases). Targeted DNA sequencing and pyrosequencing were used to detect somatic single nucleotide variations and indels in ACC-specific genes and hypermethylation in PAX5. ENSAT tumour stage, age at diagnosis, symptoms at presentation, resection status, and Ki-67 index were combined to calculate the S-GRAS score. Endpoints were overall (OS), progression-free (PFS), and disease-free survival (DFS). Prognostic role was evaluated by multivariable survival analysis and discriminative performance compared by Harrell's C index. In the training cohort, two DNA-based BMs showed an independent prognostic role at multivariable analysis, i.e. alterations in Wnt/β-catenin and/or Rb/p53 pathways and hypermethylated PAX5 (both $P<0.05$ for PFS and DFS, HR=1.47-2.33). These BMs were merged with S-GRAS to obtain a combined (COMBI) score, ranging from 0 to 6. At comparative analysis, COMBI score was the best discriminant prognostic model in both training and validation cohorts, followed by S-GRAS score that was the second best (C index for OS=0.724 and 0.765, PFS=0.717 and 0.670, DFS=0.699 and 0.644, respectively). Conclusions: COMBI score improves prognostication of ACC beyond S-GRAS score. This approach may help to better individualise patient's

management, but larger prospective studies are needed before implementation in clinical practice.

OR7: MITOTANE INDUCES GDF15 EXPRESSION IN ADRENOCORTICAL CARCINOMA - A POTENTIAL THERAPY-INDUCED MECHANISM OF IMMUNE ESCAPE

Isabel Weigand [1], Tanja Anderlik [2], Jochen Schreiner [1], Hanna Remde [2], Otilia Kimpel [2], Eva Hoster [3], Florian Wedekink [4], Alexandra Triebig [1], Tanja Maier [1], Paul Schwarzlmüller [1], Laura-Sophie Landwehr [2], Jörg Wischhusen [4], Martin Reincke [1], Martin Fassnacht [2], Matthias Kroiss [1]

[1] *Department of Internal Medicine 4,* LMU University Hospital, Munich, Germany
[2] *Department of Internal Medicine I,* University Hospital, University of Wuerzburg, Wuerzburg, Germany
[3] *The Institute for Medical Information Processing, Biometry and Epidemiology,* LMU University, Munich, Germany
[4] *Department of Obstetrics and Gynecology,* University Hospital, University of Wuerzburg, Wuerzburg, Germany

Context: The only approved therapy for adrenocortical carcinoma (ACC) is mitotane but response rates are only ~20%. Response rates of ACC to immune checkpoint inhibition (ICI) are disappointing, likely due to an immunologically 'cold' tumour immune microenvironment. Growth/differentiation factor 15 (GDF15) is a cytokine that impairs tumoural immune infiltration. We previously found GDF15 to be induced in ACC cells upon mitotane treatment. Aim: To understand the potential impact of mitotane on ICI response by studying GDF15 secretion upon mitotane treatment in ACC in vitro and in patients. Methods: GDF15 was analysed by ELISA in four mock- and mitotane-treated human ACC cell lines, in 142 serum samples of ACC patients (94 prior and 48 during mitotane) and in 5 responders and 12 non-responders to ICI. Survival was correlated with serum GDF15. Results: Mitotane induced GDF15 secretion between 1.6±0.2 and 9.9±4.3 fold in all ACC cell lines. In 39 matched patient samples prior and after mitotane, GDF15 concentrations increased from 0.6±2.2 to 2.3±3.9ng/l, $p<0.0001$. Mitotane plasma concentrations significantly correlated with GDF15 levels (Spearman $r=0.58$). Importantly, patients with serum GDF15 <0.9 (median before mitotane) and <2.3 ng/ml (median during mitotane), respectively, had a significantly longer OS compared to patients with high GDF15. In the mitotane group, this association retained statistical significance after adjustment for known prognostic factors (HR: 1.41, 95%CI 1.15-1.73, $p=0.001$). Responders to ICI had lower GDF15 levels than non-responders ($p=0.081$).

Conclusion: Mitotane induces GDF15 and likely contributes to the poor response rates to ICI in ACC.

OR8: NEOANTIGENS - A CHANCE TO IMPROVE IMMUNOTHERAPY IN ADRENOCORTICAL CARCINOMA?

Laura-Sophie Landwehr [1], Silke Appenzeller [2], Laura Ölsner [2], Isabel Weigand [3], Silviu Sbiera [1, 2], Martin Fassnacht [1, 2, 4], Matthias Kroiss [3, 2]

[1] *Internal Medicine I, Endocrinology ,* University Hospital Würzburg, Würzburg, Germany
[2] *Comprehensive Cancer Center Mainfranken,* University of Würzburg, Würzburg, Germany
[3] *Internal Medicine IV, Endocrinology,* LMU University Hospital Munich, Munich, Germany
[4] *Clinical Chemistry and Laboratory Medicine,* University Hospital Würzburg, Würzburg, Germany

Adrenocortical carcinoma (ACC) is one of the most aggressive endocrine malignancies with poor prognosis in advanced stages. Effective treatments are lacking. The results of immune checkpoint inhibition were disappointing. For the development of novel immunotherapies, tumor vaccines and T cell-based treatments, target identification is essential. Tumor-specific mutant neoantigens that may be recognized by T cells in the context of major histocompatibility complex (MHC) I are promising candidates. We performed whole-exome sequencing in 10 ACC samples with matched blood controls. Somatic mutations were identified using an in house bioinformatics pipeline. By coupling POLYSOLVER for HLA typing with netMHCpan, in silico binding affinity of tumor-specific neoantigens to MHC was calculated for both peptide and HLA sequence information. Strong binding was defined as <0.5% rank, weak binding as 0.5-1.9% rank and no binding as >2.0% rank to MHC I. Across 10 ACC patients, we identified 1067 unique somatic mutations affecting 989 different genes. Binding affinity changed for 576 predicted neoantigens, while the mutant neoantigen load per patient ranged from 10 to 235 (mean 66.2) and was positively correlated with the Ki67 proliferation index (R2 0.56, 95%CI 0.54-3.37; p=0.013) and to an advanced ENSAT stage. This is the first study that demonstrates successful in silico neoantigen profiling in ACC. Mutant neoantigens were predicted to be present both in ACC with high and low tumor mutational burden. These data and ongoing additional whole-genome sequencing ACC analyses (n=70) hold potential to develop therapeutic cancer vaccines and T cell-based cancer immunotherapy in ACC.

OR9: SERUM INFLAMMATION-BASED SCORES IN A LARGE COHORT OF ADRENOCORTICAL CARCINOMAS AND ADRENOCORTICAL ADENOMAS: A COMPARATIVE STUDY AND ROLE OF THE HORMONAL SECRETION PATTERN

Alessandra Mangone [1], Vittoria Favero [2], Alessandro Prete [3, 4, 5], Yasir S Elhassan [3, 4, 5], Miriam Asia [5], Giovanna Mantovani [1, 6], Iacopo Chiodini [2, 7], Cristina L Ronchi [3, 4, 5]

[1] *Department of Clinical Sciences and Community Health,* University of Milan, Milan , Italy
[2] *Department of Biotechnology and Translational Medicine,* University of Milan, Milan, Italy
[3] *Institute of Metabolism and Systems Research,* University of Birmingham, Birmingham, United Kingdom
[4] *Centre for Endocrinology, Diabetes and Metabolism,* Birmingham Health Partners , Birmingham, United Kingdom
[5] *Department of Endocrinology,* Queen Elizabeth Hospital Birmingham, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom
[6] *Endocrinology Unit,* Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[7] *Unit of Endocrinology,* Ospedale Niguarda Cà Granda, Milan, Italy

Background: Serum inflammation-based-scores (sIBS) predict clinical outcomes in several cancers, including adrenocortical carcinoma (ACC). Furthermore, sIBS are altered in benign adrenocortical adenomas (ACA) and correlate with cortisol excess. It is unclear whether the extent of IBS alterations in ACC reflects tumour aggressiveness, steroid excess, or both. We investigated the relationship among IBS, malignancy, and steroid patterns in adrenocortical tumours. Methods: Retrospective, single-centre study of patients with ACA (n=429) and ACC (n=65) with available full blood count and hormonal evaluation at tumour diagnosis. Tumours were classified according to steroid pattern: aldosterone-producing adenoma (APA, n=54); cortisol-producing-adenomas with Cushing's syndrome (CS-CPA, n=22) or mild autonomous cortisol secretion (MACS-CPA, n=138); non-functioning-adenomas (NFAT, n=215); inactive-ACC (n=10); androgen-producing-ACC (n=9); MACS-ACC (n=28); CS-ACC (n=14). We investigated neutrophil-to-lymphocyte-ratio (NLR); platelet-to-lymphocyte-ratio (PLR); lymphocyte-to-monocyte-ratio (LMR); systemic immune-inflammation-index (SII); prognostic-nutrition-index (PNI); neutrophil-platelet-score (NPS). Results: ACC showed higher sIBS than ACA regardless of steroid secretion. NLR showed the best accuracy to distinguish ACC (median=6, IQR=3.1-8.7) vs ACA (median=2.4, IQR=1.7-3.3); $p<0.01$; AUC-

ROC=0.847. NLR>2.6 was the most sensitive cut-off (95%) to discriminate ACC from ACA, with suboptimal specificity (54%). NLR showed a correlation with cortisol levels after overnight 1mg-dexamethasone suppression test, both in ACC and ACA (R=0.639 and 0.290 respectively; p<0.001). Focusing on ACC, NLR varied according to the secretion pattern, increasing from inactive-ACC (median=2.8, IQR=2.5-3.3), to androgen-ACC (median=3.7, IQR=2.9-4.2), MACS-ACC (median=7.8, IQR=3.6-9.3), and CS-ACC (median=7.8, IQR=7.3-12.2). Conclusion: sIBS correlate with cortisol secretion both in ACC and ACA. ACC presented a higher degree of inflammation than ACA, probably reflecting cancer-specific rather than hormone-specific factors.

OR10: PROLONGED EXPOSURE TO TARGET MITOTANE CONCENTRATIONS IS ASSOCIATED WITH BETTER RECURRENCE-FREE SURVIVAL IN PATIENTS WITH ADRENOCORTICAL CARCINOMA ON ADJUVANT TREATMENT

Soraya Puglisi [1], Anna Calabrese [1], Vittoria Basile [1], Otilia Kimpel [2], Barbara Altieri [2], Anna Angelousi [3], Gregory Kaltsas [3], Urszula Ambroziak [4], Maria Luisa Appetecchia [5], Maura Arosio [6], Alfredo Berruti [7], Deborah Cosentini [7], Letizia Canu [8], Filippo Ceccato [9], Guido Di Dalmazi [10], Lucas Bouys [11], Rossella Libè [11], Karolina Nowak [12], Lucyna Papierska [12], James Pittaway [13], Tina Kienitz [14], Marcus Quinkler [14], Yasir Elhassan [15, 16], Cristina Ronchi [15, 16], Leonard Saiegh [17], Antonio Stigliano [18], Tina Dusek [19, 20], Darko Kastelan [19, 20], Martin Fassnacht [2], Massimo Terzolo [1]

[1] *Internal Medicine, Department of Clinical and Biological Sciences,* University of Turin, San Luigi Gonzaga Hospital, Orbassano, Italy
[2] *Division of Endocrinology and Diabetes, Department of Medicine, University Hospital,* University of Würzburg, Würzburg, Germany
[3] *Unit of Endocrinology, First Department and Department of Propaedeutic Internal Medicine, Laiko Hospital ,* National and Kapodistrian University of Athens, Athens, Greece
[4] *Department of Internal Medicine and Endocrinology,* Medical University of Warsaw, Warsaw, Poland
[5] *Oncological Endocrinology Unit,* Regina Elena National Cancer Institute – IFO IRCCS, Rome, Italy
[6] *Endocrinology Unit, Department of Clinical Sciences and Community Health,* University of Milan, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[7] *Medical Oncology Unit, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health,* University of Brescia, ASST Spedali Civili, Brescia, Italy
[8] *Department of Experimental and Clinical Biomedical Sciences,* University of Florence, Florence, Italy
[9] *Endocrinology Unit, Department of Medicine DIMED,* University-Hospital of Padua, Padua, Italy
[10] *Department of Experimental, Diagnostic and Specialty Medicine,* Alma Mater Studiorum-University of Bologna, Policlinico S. Orsola-Malpighi, Bologna, Italy
[11] *Service d'Endocrinologie, National Referent Center for Adenal Cancer, ENDOCAN-COMETE,* Hôpital Cochin-Port Royal, Assistance Publique des Hôpitaux de Paris, Paris, France
[12] *Department of Endocrinology,* Medical Centre for Postgraduate Education, Warsaw, Poland
[13] *Department of Endocrinology ,* St Bartholomew's Hospital, London, United Kingdom
[14] *Endocrinology in Charlottenburg,* Endocrinology in Charlottenburg, Berlin, Germany
[15] *Institute of Metabolism and Systems Research, College of Medical and Dental Sciences,* University of Birmingham, Birmingham, United Kingdom
[16] *Centre for Endocrinology, Diabetes and Metabolism,* Birmingham Health Partners, Birmingham, United Kingdom
[17] *Department of Endocrinology, Bnai-Zion Medical Center,* Ruth & Bruce Rappaport Faculty of Medicine, Technion, Haifa, Israel

[18] *Endocrinology, Department of Clinical and Molecular Medicine,* Sapienza University of Rome, Sant'Andrea Hospital, Rome, Italy
[19] *Department of Endocrinology,* University Hospital Centre Zagreb, Zagreb, Croatia
[20] *School of Medicine,* University of Zagreb, Zagreb, Croatia

Background: The management of adjuvant mitotane therapy in patients with adrenocortical carcinoma (ACC) is challenging. Plasma mitotane concentrations >14 mg/L have been associated with efficacy in the treatment of advanced ACC; however, data in the adjuvant setting are mixed. Moreover, there is no consensus on how to assess the optimal exposure to mitotane and all the proposed methods have inherent limitations. We have recently proposed a new method analogous to what is established for the anticoagulant warfarin, the time in target range (TTR). We aimed to evaluate whether the TTR is a factor influencing recurrence-free survival (RFS) in patients with ACC on adjuvant mitotane. Methods: This is an international, retrospective, cohort study undertaken in 18 centers in 8 countries, under the auspices of the European Network for the Study of Adrenal Tumors (ENSAT), including adult patients with ACC treated with adjuvant mitotane for at least 1 year following tumor resection, with the availability of at least 3 mitotane measurements per year. TTR was calculated with the Rosendaal method and expressed as the number of months with plasma mitotane concentration >14 mg/L. The following potential predictive factors for RFS have been investigated: patient sex and age, ENSAT stage, hormone secretion, resection status, Weiss score, Ki67 index, and TTR. Data are expressed as median and interquartile range. Results: From a total of 254 patients, 157 fulfilled inclusion criteria and were analyzed (F/M 94/63; age 49, 41-58 years), with a follow-up of 49 (33-92) months. The key baseline features were: 7.0% stage I, 69.4% II, 22.3% III, 1.3% IV; 51.6% secreting tumors; 87.2% R0, 2.6% R1, 10.2% RX; Weiss score 5 (4-7); Ki67 index 11 (5-20). All patients were treated with mitotane for 25 (22-36) months, with a TTR of 14 (6-21) months. At multivariate analysis, Ki67 index (HR 1.07, 95%CI, 1.02-1.12; $p<0.01$) and Weiss score (HR 1.7, 95%CI, 1.16-2.47; $p<0.01$) were associated with an increased risk of recurrence, while female sex (HR 0.14, 95%CI, 0.04-0.58; $p<0.01$) and TTR (HR 0.80, 95%CI, 0.70-0.90; $p<0.001$) were associated with a reduced risk. Conclusions: The present findings show that the patients who are exposed to plasma mitotane >14 mg/L for longer periods have better RFS. These findings provide indirect evidence of the value of adjuvant therapy with mitotane and support the importance of drug monitoring to guide dose adjustment in clinical practice and improve patient outcome.

OR11: EO2401 PEPTIDE IMMUNOTHERAPY + NIVOLUMAB IN ADRENOCORTICAL CARCINOMA (ACC); THE PHASE 1/2 EOADR1-19/SPENCER TRIAL (NCT04187404)

Eric Baudin [1], Salvatore Grisanti [2], C. Willemien Menke - van der Houven van Oordt [3], Harm Haak [4], Martin Fassnacht [6], Jaume Capdevila [5], Christelle de la Fouchardière [7], Vivek Subbiah [8], Camilo Jimenez [8], Jeffrey Yachnin [9], Dan Granberg [9], Gedske Daugaard [11], Matthias Kroiss [10], Deborah Cosentini [2], Livia Lamartina [1], Jan Fagerberg [12], Lucie Aubergeon [13], Laurent Chêne [13], Jean-Michel Paillarse [12], Alfredo Berruti [2]

[1] *Endocrine Oncology,* Gustave Roussy, Villejuif, France
[2] *Oncologia Medica,* Università degli Studi di Brescia, Azienda Ospedaliera Spedali Civili, Brescia, Italy
[3] *Medical Oncology,* Amsterdam UMC, Amsterdam, Netherlands
[4] *Medical Oncology,* Máxima Medisch Centrum, Eindhoven, Netherlands
[5] *Medical Oncology,* Hospital Universitari Vall d'Hebron, Barcelona, Spain
[6] *Internal Medicine,* University Hospital, Würzburg, Germany
[7] *Medical Oncology,* Centre Léon Bérard, Lyon, France
[8] *Medical Oncology,* The University of Texas MD Anderson Cancer Center (MDACC), Houston, United States
[9] *Endocrine tumors,* Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden
[10] *Medicine IV,* LMU University Hospital, Ludwig-Maximilians-Universität , Munich, Germany
[11] *Medical Oncology,* Rigshospitalet, Copenhagen, Denmark
[12] *Medical Department,* Enterome, Paris, France
[13] *Drug Discovery,* Enterome, Paris, France

Background: EO2401 was designed to expand pre-existing memory cytotoxic T cells recognizing specific protein sequences from gut bacteria which cross-react with tumor associated antigens (TAAs). EO2401 contains three synthetically produced CD8 HLA-A2 epitopes with mimicry to adrenal tumor-TAAs (IL13Rα2, BIRC5/survivin, and FOXM1) and the CD4 epitope UCP2. Methods: Patients received EO2401 (300 µg/peptide, q2weeklyx4 then q4weekly) with nivolumab (240mg q2weeklyx3 then 480mg q4weekly). Cohorts C2a and C2b included patients with study treatment delivered as 2nd/3rd and 1st line systemic therapy for metastatic disease. Immune testing was performed utilizing peripheral-blood-mononuclear-cells in ELISPOT after in vitro stimulation (IVS), or by tetramers ex vivo, or after IVS. Results: Thirty-three patients with ACC was recruited: C2a = 26 (79%) and C2b =7 (21%). Safety included the most common any grade AEs (irrespective of relationship): pyrexia (36% of patients), diarrhea (33%), fatigue (27%), injection site reaction (24%), anemia (24%), back pain

(21%), and AST increase (21%). Immune responses were seen in 92% and 87% of tested patients for expansions of T cells specific for mimic peptides, and TAAs (cross-reactive T cells). Specific T cells followed for up to 21 months. Strength of immune response correlated with progression-free survival (n=33; R=0.57, R2=0.33, p=0.004). Efficacy outcome with median survival follow-up 22.4 months included PR-rate=9%; disease-control-rate (PR+SD)=36%; duration-of-disease-control=median 6.5months (range 2.3-24.3); progression-free-survival=1.9months (0.4-24.3); survival=13.8months (1.0-27.1). The 12-/24-months survival rates were 58%, and 32%. Conclusions: EO2401/nivolumab was well tolerated with EO2401 adverse events limited to local administration site reactions and nivolumab events within expected range. EO2401 generated durable mimic and human TAA specific cytotoxic T cell immune responses; strength of immune response correlated with PFS. Survival on EO2401/nivolumab, despite 79% of patients having study treatment as 2/3rd line, like etoposide/doxorubicin/cisplatin-mitotane in 1st line (FIRMACT-study 12-/24-months survival 57%, and 31%). Based on outcomes a randomized study extension was initiated.

OR12: SPECIFIC CHARACTERISTICS OF THE GENOMIC PROFILE, TUMOR MICROENVIRONMENT AND VASCULAR STRUCTURE OF PPGLS WITH MAML3 FUSIONS

María Monteagudo [1], Bruna Calsina [1], Angel Mario Martínez-Montes [1], Alberto Díaz-Talavera [2, 1], Ester Arroba [1], Angel Fernández-Sanroman [1], Luis Javier Leandro-García [1], Cristina Montero-Conde [1, 2], Henri Timmers [3], Felix Beuschlein [4, 5], Nelly Burnichon [6, 7, 8], Anne-Paule Gimenez-Roqueplo [6, 7, 8], Alberto Cascón [1, 2], Mercedes Robledo [1, 2]

[1] *Hereditary Endocrine Cancer Group, Human Cancer Genetics Program,* Spanish National Cancer Research Centre (CNIO), Madrid, Spain
[2] *Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER),* Ministry of Science and Innovation, Health Institute Carlos III, Centro de Investigación Biomédica en Red (CIBER), Madrid, Spain
[3] *Department of Internal Medicine,* Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands
[4] *Medizinische Klinik und Poliklinik IV,* Klinikum der Universität München, Munich, Germany
[5] *Klinik für Endokrinologie, Diabetologie und Klinische Ernährung,* Universitätsspital Zürich, Zürich, Switzerland
[6] *Equipe labellisée par la Ligue contre le cancer,* University, PARCC, INSERM, F-75006 Paris, France
[7] *Genetics department, AP-HP,* Hôpital européen Georges Pompidou, F-75015 Paris, France
[8] *Rare Adrenal Cancer Network ,* COMETE, Paris, France

The genetic landscape of PPGLs is increasingly complex. One of the last identified recurrent genetic events associated with PPGL development and metastatic behavior are fusions of MAML3 gene (Fishbein L. et al 2017), which result in MAML3 chimeric proteins. Regarding molecular characterization of MAML3-PPGLs, transcriptome and methylome analysis of PPGLs has shown a specific signature related to an aberrant Wnt/β-catenin signaling activation. However, a comprehensive molecular and phenotypical characterization of MAML3-PPGLs has not been performed so far. The aim of this study was to decipher MAML3 fusions-driven molecular alterations and their association with the metastatic behavior of this genetic class. For this purpose, we analyzed multiple omics datasets and associated clinical data, and validated the findings with a battery of techniques. From the initial 779 PPGL series, 28 samples (3.6%) were confirmed to carry a MAML3 fusion through Sanger sequencing, FISH, PD-L1 IHC (Calsina B. et al 2023), qPCR, and a customized fusion-detection panel (NGS). The differential expression analysis of MAML3-PPGLs, followed by a functional enrichment analysis (GSEA), allowed us to identify several relevant altered processes. Among the Cancer

Hallmarks, the most significant were MYC-targets, Angiogenesis, EMT-transition and Immune-related pathways. Gene-level analysis revealed an overexpression of the MYC target HK2, associated with hypoxia and angiogenesis, and aberrant expression of the neuroendocrine-mesenchymal transition hallmarks (neuro-low: ENO2 and INSM1, and mesenchymal-high: SNAI1 and SNAI2). CD31-IHC revealed a particular and heterogeneous vascular pattern in MAML3-PPGLs, showing the highest vascularization rate with highly branched and elongated (large parallel arches) vessels compared to the other PPGL-genotypes. Finally, we explored by IHC the immunopopulations identified by RNA-deconvolution analysis: NK cells, CD8+ lymphocytes and CD163+/CD68+ macrophages. We performed a double staining with CD8+/perforin to distinguish CD8+ lymphocytes from NK/cytotoxic cells and observed that both immunopopulations are present in MAML3-PPGLs. Our results, together with previously described PDL1 expression, indicates that MAML3-PPGLs may be sensitive to immunotherapy. Our work contributes to a deeper understanding of the differential genomic, microenvironment and vascular characteristics of MAML3-related tumors, suggesting their suitability for combined therapies of antiangiogenic drugs and immunotherapy.

OR13: INTERACTION BETWEEN HYPOXIA SIGNALLING AND KYNURENINE PATHWAY IN PHEOCHROMOCYTOMA AND PARAGANGLIOMA (PPGL)

Gintarė Žygienė [1], Dennis Fröbel [1], Susan Richter [1], Mirko Peitzsch [1], Stephanie MJ Fliedner [2], Hans K Ghayee [3], Katerina Hadrava Vanova [4], Jiří Neužil [5, 6], Deepika Watts [1], Ben Wielockx [1], Denise Kaden [1], Catleen Conrad [1], Hermine Mohr [7], Graeme Eisenhofer [1, 8], Nicole Bechmann [1]

[1] *Institute of Clinical Chemistry and Laboratory Medicine,* University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany
[2] *University Cancer Center,* University Medical Center Schleswig-Holstein, Campus Lübeck, Lübeck, Germany
[3] *Department of Medicine, Division of Endocrinology,* University of Florida College of Medicine and Malcom Randall VA Medical Center, Gainesville, United States
[4] *Section on Medical Neuroendocrinology,* Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, United States
[5] *Institute of Biotechnology,* Czech Academy of Sciences, BIOCEV, Prague, Czech Republic
[6] *School of Pharmacy and Medical Science,* Griffith University, Southport, Australia
[7] *Institute for Diabetes and Cancer,* Helmholtz Zentrum München, Neuherberg, Germany
[8] *Department of Medicine III,* University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany

Pheochromocytomas and paragangliomas (PPGLs) with a pseudohypoxic phenotype (cluster 1 PPGLs) have a higher metastatic risk compared to cluster 2 PPGLs (kinase signalling activation). Increased stabilisation of hypoxia-inducible factor 2α (HIF2α) contributes to the pro-metastatic behaviour of cluster 1 PPGLs, but exact mechanism remains unclear. The kynurenine pathway (KP), a branch of tryptophan (TRP) metabolism, was found to be altered in numerous tumours. Kynurenine (KYN) interacts with the aryl hydrocarbon receptor (AHR), which binds the aryl hydrocarbon receptor nuclear translocator (ARNT) and initiates downstream AHR-ARNT signalling. As this process is potentially linked to hypoxia signalling, we investigated the crosstalk between KYN and pseudohypoxic pathways in PPGLs. Gene expression and KP-related metabolites were measured in PPGL tissues and pheochromocytoma cell lines (MPC) with or without Hif2α expression under normoxic and hypoxic (≤1% O2) conditions. MPC expressing Hif2α were previously shown to exhibit enhanced pro-metastatic behaviour. Tissue metabolomics revealed elevated KYN levels in cluster 1 versus cluster 2 PPGLs. In cluster 1 PPGLs, expression of the initial rate-limiting enzymes of KP, indoleamine 2,3-dioxygenase (IDO1) and tryptophan 2,3-dioxygenase (TDO2), was increased compared to

cluster 2 PPGLs. Expression of Hif2α in MPC cells was associated with elevated Ahr and Ahrr expression. KYN and TRP levels were in trend lower compared to cells without Hif2a expression. Extrinsic hypoxia in Hif2a expressing MPC cells did not affect Ahr expression, KYN or TRP levels, but upregulated Ahr target genes, Ahrr and Cdkn1a. However, further research is needed to elucidate the potential interaction between hypoxia and the KP in PPGLs and how it affects the aggressiveness of these tumours.

OR14: EVALUATING MICRORNAS AS CIRCULATING BIOMARKERS FOR THE PREDICTION OF MALIGNANT PARAGANGLIOMA AND PHEOCHROMOCYTOMAS

Tom Drossart [1], Alexandre Buffet [1, 2, 3], Ali Janbain [4], Chris Ottolenghi [5], Laurence Amar [6], Rossella Libé [7], Delphine Drui [8], Charlotte Lussey-Lepoutre [1, 9], Maxence Mancini [3], Timgad Lounis [6], Armelle Arnoux [4], Tchao Méatchi [10], Nelly Burnichon [1, 2, 3], Judith Favier [1, 3], Anne-Paule Gimenez-Roqueplo [1, 2, 3]

[1] *PARCC, U970,* Inserm, Paris, France
[2] *Génétique,* AP-HP, HEGP, Paris, France
[3] *Inserm,* Université Paris Cité, Paris, France
[4] *Unité de Recherche Clinique,* AP-HP, HEGP, Paris, France
[5] *Biochemistry, Metabolomics Unit,* AP-HP, Necker Hospital, Paris, France
[6] *Unité Fonctionnelle d'Hypertension Artérielle,* AP-HP, HEGP, Paris, France
[7] *Endocrinology Department,* AP-HP, Cochin University Hospital, Paris, France
[8] *Endocrinology Department,* Nantes University Hospital, Nantes, France
[9] *nuclear medicine department,* Pitié-Salpêtrière Hospital, AP-HP, Paris, France
[10] *Department of Pathology,* AP-HP, HEGP, Paris, France

Background: Circulating biomarkers are valuable tool in medical research and clinical practice as they offer a non-invasive and easy way to monitor disease and treatment response. Being able to predict the development of malignant pheochromocytomas and paragangliomas (PPGL) during diagnosis is crucial as it would drastically change the clinical care of affected patients. Recently, a 5-microRNA (miR) signature specific to metastatic PPGL was discovered. Objective: The aim of this prospective multicenter study is to evaluate a signature of 5 microRNAs as predictive biomarkers of the evolution of PPGL, as well as to test their robustness by evaluating its sensitivity and specificity to predict the metastatic status. Methods: Liquid biopsies of 183 patients were prospectively collected by 16 French hospitals. All samples were gathered and processed at Hôpital Européen Georges Pompidou (Paris) and circulating miRNAs were quantified by droplet digital PCR using TaqMan assay. Findings: Among five circulating miRNAs (miR-21-3p, miR-183-5p, miR-182-5p, miR-96-5p, miR-483-5p) the best classifier of metastatic status was found to be circulating miR-483-5p with an AUC at 0.64, a sensitivity of 67%, and a specificity of 63%. The other 4 miRs exhibited lower diagnostic performances, and any combination of multiple miRs did not improve the prediction of metastatic status. Interpretation: Circulating miR-483-5p is a promising biomarker, and used

in combination with other innovative biomarkers could be a decisive tool for the prediction of metastatic status of PPGL.

OR15: MONITORING METASTATIC PHEOCHROMOCYTOMAS AND PARAGANGLIOMAS USING PLASMA CELL-FREE DNA SEQUENCING

Carlota Arenillas [1], Anna Casteràs [1], Ana Belén Moreno [1], Jaume Capdevila [1], Jorge Hernando [1], Rodrigo Toledo [1]

[1] *Vall d'Hebron Barcelona Hospital Campus, Barcelona,* Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain

Background: Pheochromocytomas and paragangliomas (PPGL) are characterized by quiet genomes and usually indolent tumour growth, even when already metastatic (mPPGL). However, dormant mPPGL can switch to an aggressive phenotype through so far unknown molecular mechanisms. In the case of PPGL, needle biopsies are infrequently performed as they are unnecessary for the diagnosis or treatment of the patients and catecholamine-related crises. Therefore, the current genetics and genomics analyses of PPGLs remain limited mostly to benign, operable tumours, that may or may not become metastatic in the future. Aim: To apply non-invasive liquid biopsy sequencing analysis for characterize the genomic basis and monitor mPPGL patients. Results: Through the ENSAT and A5 networks, 130 plasma cell-free DNA samples from 86 patients were obtained: 67 patients with one time point, 10 patients with two time points, 6 patients with four time points, and 3 patients with five, six and eight time points, respectively. Shallow whole-genome sequencing (shWGS) was applied to all samples. The detection of circulating tumour DNA (ctDNA) was possible in 58,2% of the plasma samples and the monitorization of ctDNA levels could show the increase of tumour fraction (e.g. from 16% to 48%) at disease progression. Whole-genome duplication was detected in some cases. Whole-exome sequencing was performed in ctDNA-positive samples and the analyses are ongoing. Conclusion: Liquid biopsy analysis is a feasible technique for monitoring ctDNA in mPPGL patients and tracking tumour evolution. High values of ctDNA correlated with poor prognosis and detection of metastatic driver mutation and copy-number alteration events.

OR16: ROR-1 SPECIFIC CAR-T CELLS WITH CRISPR/CAS9 MEDIATED GLUCOCORTICOID RECEPTOR-KNOCKOUT EXERT POTENT ANTITUMOR EFFICACY IN ADVANCED ADRENOCORTICAL CARCINOMA

Marc Philipp Schauer [1, 2], Barbara Altieri [1], Rodrigo Redondo-Frutos [2], Peter Spieler [2], Tanja Maier [3], Daniel Oppelt [1], Matthias Kroiss [3, 1, 4], Silviu Sbiera [1], Justus Weber [2, 5], Martin Fassnacht [1, 5, 4], Laura-Sophie Landwehr [1, 5], Michael Hudecek [2, 5]

[1] *Department of Internal Medicine I, Division of Endocrinology & Diabetes,* University Hospital Würzburg, Würzburg, Germany
[2] *Department of Internal Medicine II, Center for Cellular Immunotherapy,* University Hospital Würzburg, Würzburg, Germany
[3] *Department of Internal Medicine IV, Division of Endocrinology & Diabetes,* LMU Hospital Munich, Munich, Germany
[4] *University of Würzburg,* Comprehensive Cancer Center Mainfranken, Würzburg, Germany
[5] These authors contributed equally, , Germany

Adrenocortical carcinoma (ACC) is a rare and aggressive endocrine malignancy with poor prognosis. It is characterized by endogenous glucocorticoid (GC) excess in 60% of the cases, which creates a hostile tumor microenvironment (TME) that could be one potential cause why immune checkpoint inhibitor therapy showed only modest results. To date no therapeutically relevant surface markers are known for ACC, which is why it has not already been considered for cell therapeutic interventions like CAR-T cell therapy. In this study, we evaluated ROR-1 expression by using qRT-PCR, qFACS, dSTORM, RNAscope and IHC in 5 ACC cell lines and in 197 ACC tissues. ROR-1 CAR-T cells were generated and tested in preclinical models. We show that ROR-1 is sufficiently and homogenously overexpressed in human ACC specimen (p=0.015), that it is strongly associated with GC excess and disease progression (p=0.009) and that its expression is susceptible by steroid inhibitor treatment. ROR-1 specific CAR-T cells recognize and effectively eradicate ACC tissues in vitro and in vivo. These effects can be effectively improved with CRISPR/Cas9 mediated human glucocorticoid receptor (hGR)-knockout CAR-T cells, which not only exert greater antitumor efficacy and CAR-T cell functionality (ROR-1-CART: 41.8% vs hGRKOROR-1-CART: 74.9 % specific lysis of NCI-H295R), but also are superior to pharmacological inhibition of the hGR or steroid synthesis. Our results identify ROR-1 as a new target antigen in ACC and reveal that

the deletion of hGR in ROR-1 CAR-T cells is an important modification to overcome hostile TME and is a superior therapeutic treatment approach in ACC.

OR17: TESTOSTERONE, MACROPHAGES AND SENESCENCE: NOVEL THERAPEUTIC OPTIONS FOR ACC?

Diana Garcia-Garcia [1], Julie Olabe [1], James Wilmouth Jr [1], Iuliia Pinigina [1], Antoine Martinez [1], Pierre Val [1]

[1] *CNRS UMR6293,* GReD Institute, Clermont-Ferrand, France

Adreno-Cortical Carcinoma (ACC) is a highly aggressive cancer. Treatment options are scarce and overall survival of metastatic patients remains low, with a median of <15 months. The WNT signalling inhibitor ZNRF3 is inactivated in 20% of ACC. Using a mouse model recapitulating Znrf3 inactivation, we showed that highly phagocytic macrophages prevented early phases of tumour progression in male Znrf3 KO mice. In contrast, female adrenals were devoid of these macrophages, allowing metastatic progression. Interestingly, phagocytic macrophage signatures were also more prominent in male ACC patients and were associated with better prognosis. Investigation of this sexual dimorphism showed that testosterone, by inducing a senescence-associated secretory phenotype, was responsible for recruitment of phagocytic macrophages and could induce regression of adrenal hyperplasia in female mice. This suggested that testosterone could have a potent tumoricidal role in ACC. In support of this hypothesis, our unpublished data show that testosterone treatment of Znrf3 KOfemales bearing aggressive tumours, results in inhibition of proliferation, tumour shrinkage and a marked reduction in metastasis. These observations are further confirmed in our novel aggressive ACC model, relying on the clinically relevant combined inactivation of Znrf3 and Trp53. Altogether, these data strongly suggest that testosterone treatment may constitute an interesting therapeutic option in the context of ACC. However, its virilizing side effects make it an unlikely option for women. Therefore, we are currently evaluating downstream targets of testosterone involved in stimulation of senescence and tumoricidal macrophages, as potential surrogates.

OR18: IDENTIFICATION OF ADRENOCORTICAL MASSES MALIGNANCY AND AGGRESSIVITY THROUGH RADIOMICS: A PILOT STUDY

Lorenzo Tucci [1], Giulio Vara [2], Ilaria Improta [1], Antonio De Leo [3], Saverio Selva [4], Caterina Balacchi [5], Donatella Santini [6], Cristina Mosconi [2], Valentina Vicennati [1], Uberto Pagotto [1], Guido Di Dalmazi [1]

[1] *Medical and Surgical Sciences Department, Endocrinology Unit,* Alma Mater Studiorum, IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy
[2] *Medical and Surgical Sciences Department, Radiology Unit,* Alma Mater Studiorum, IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy
[3] *Medical and Surgical Sciences Department, Anatomopathologic Unit,* Alma Mater Studiorum, IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy
[4] *Abdominal and Endocrine Surgery Unit,* IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy
[5] *Radiology Unit,* IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy
[6] *Anatomopathologic Unit,* IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy

Background: Adrenal lipid-poor adenoma (LPA) and adrenocortical cancer (ACC) may overlap in computerized tomography (CT). Radiomics recently emerged as new tool for malignant behavior identification. Aim: To assess radiomics utility for identification of ACC and LPA in adrenocortical masses with unenhanced (UE) CT scan attenuation≥10 Hounsfield Unit (HU). Methods. We retrospectively enrolled 50 patients, 38 radiologically defined LPA with 6-12 months of radiologic stability or benign histological exam (n=11), and 12 ACC. All patients underwent CT with UE scan, arterial (ACE), venous (VCE) and 15' delayed (DCE) contrast-enhanced phases. Radiomics was performed with LIFEx software (©LITO 2022-2023). To evaluate malignancy predictors (Weiss score≥3), we performed a first-step multivariate regression analysis (MRA) among single radiomics classes, and a second-step MRA to formulate predictive models with estimation of sensitivity, specificity, positive and negative predictive values. Results: In UE, surface-to-volume ratio (SVR) and Run Length Non-Uniformity (RLNU) predicted malignancy (Odds Ratio (OR)=2.718; 95%Confidence Interval(CI)=1.56-4.75; p<0.001), with 83.3% sensibility, 94.3% specificity, 83.3% PPV, 94.7% NPV. In ACE, SVR and Feret diameter predicted malignancy [OR=2.718; 95%CI=1.57-4.745; p<0.001], with 83.3% sensibility, 92.1% specificity, 76.9% PPV, 94.6% NPV. In VCE, SVR and compacity predicted malignancy [OR=2.719; 95%CI=1.54-4.79; p<0.001], with 83.3% sensibility, 92.1% specificity, 76.9% PPV, 94.5% NPV. In DCE, SVR and RLNU

predicted malignancy [OR=2.718; 95%CI=1.54-4.79; p<0.001], with 83.3% sensibility, 91.9% specificity, 76.9% PPV, 94.5% NPV. Conclusion: Radiomics can identify adrenal masses nature, even without CT contrast-enhanced phases. Among other radiomics parameters, SVR and HU intensity-based features seem to be powerful predictors of adrenocortical masses malignancy.

OR19: EVALUATION AND VALIDATION OF AN ADAPTED PEDIATRIC S-GRAS-SCORE

Verena Wiegering [1], Maria Riedmeier [1], Martin Fassnacht [2]

[1] *Department of Pediatrics, Division of Pediatric Hematology, Oncology and Stem cell transplantation, ,* University Hospital, University of Wuerzburg, Würzburg, Germany
[2] *Department of Medicine, Division of Endocrinology and Diabetes,* University Hospital, University of Wuerzburg, Würzburg, Germany

On behalf of all ENSAT-PACT members

Pediatric adrenocortical carcinoma (pACC) is rare and prognostic stratification remains challenging. We summarized clinical prognostic factors of pACC and determined the prognostic value of the pediatric scoring system (pS-GRAS) in adaption to the recommendation (S-GRAS) of ENSAT for the classification of adult ACC. Analysis on pACC patients of 33 available retrospective studies in the literature and validation of this scoring system in an international multicenter study with 263 patients. We searched the PubMed and Embase databases for manuscripts regarding pACC. pS-GRAS score was calculated as a sum of tumor stage, Ki67 index, resection status, age and hormone related symptoms generating ten scores and four groups (1: 0-2, 2: 3-4, 3: 5, 4: 6-9). Primary endpoint was overall survival (OS). We included 733 patients. Median age was 2.5 years and >85% of pACC showed hormone activity (mixed 50%, androgen 29%, glucocorticoid 21%). Androgen production was associated with a superior OS. Increasing age correlated with higher rates of inactive or only glucocorticoid-producing tumors, advanced tumor stage, and case fatality. Especially infants < 4 years showed more often low risk constellations with an increased OS for all tumor stages. The pS-GRAS score correlated strongly with clinical outcome: median OS was 133 months (95%CI: 36-283) in group 1 and 16 months (95%CI: 2.4-267) in group 4($p<0.05$). These results were validated in an international multicenter cohort. pS-GRAS score have a high predictive value in pACC patients and may serve as a helpful tool for risk stratification in future studies.

OR20: DECREASE IN ANTICORTISOLIC DRUG OSILODROSTAT PLASMA EXPOSURE IN PATIENTS TREATED WITH MITOTANE FOR AN ADRENOCORTICAL CARCINOMA

Louis Thomeret [1], Alicja Puszkiel [2], David Balakirouchenane [2], Lucas Bouys [1, 3], Jonathan Poirier [3], Annabel Berthon [1], Bruno Ragazzon [1], Anne Jouinot [1], Laurence Guignat [3], Laura Bessiene [3], Rossela Libe [3], Leopoldine Bricaire [3], Lionel Groussin [1], Guillaume Assie [3, 1], Benoit Blanchet [2], Fideline Bonnet-Serrano [4, 1], Jerome Bertherat [3, 1]

[1] *Team "Genomic and signaling of endocrine tumors", INSERM U1016-CNRS UMR8104, Paris-Cité University,* Institut Cochin, PARIS, France
[2] *Department of Pharmacokinetics and Pharmacochemistry,* Cochin Hospital, AP-HP, PARIS, France
[3] *Endocrinology department, reference center for rare adrenal disorders,* Cochin Hospital, AP-HP, PARIS, France
[4] *Hormonology department,* Cochin Hospital, AP-HP, PARIS, France

Introduction: The steroidogenesis inhibitor osilodrostat (OSI), indicated for the medical treatment of endogenous Cushing's syndrome, exhibits significant interindividual variability regarding the response to treatment (Pivonello et al., 2020). Plasma exposure may contribute to this variability. Our objective was to investigate the effect of concomitant use of mitotane (MIT), a potent inducer of CYP450 (3A4), on circulating OSI concentrations in patients treated for an adrenocortical carcinoma (ACC). Methods: Plasma OSI concentrations were determined every 4 hours over 24 hours by LC-MS/MS (Balakirouchenane et al., 2023) in 27 patients (19 with Cushing's disease, 4 with ectopic adrenocorticotropic secretion, 3 with macronodular bilateral adrenal hyperplasia and 1 with ACC) treated with OSI as a monotherapy ("OSI" group, 33 cycles), and in 3 patients treated with OSI in association with MIT ("OSI-MIT" group, 8 cycles) for ACC. Daily doses of OSI and plasma mitotanemia were expressed as median (min-max). The area under the OSI concentration curve (AUC-OSI) was used as pharmacokinetic endpoint. Results: The AUC-OSI was well correlated with the daily dose of osilodrostat in both the "OSI" group (Spearman r=0.8377; OSI 10 mg/day (2-40)) and the "OSI-MIT" group (Spearman r=0.8452; OSI 60 mg/day (20-60); mitotanemia 14.1 mg/L (0.5-26.6)). The AUC-OSI based on the daily dose was statistically decreased in the "OSI-MIT" group compared to the "OSI" group (medians: 11.92 vs 26.81 ng/mL.h; $p<0.001$). Discussion: Mitotane significantly decreases plasma exposure to osilodrostat in patients treated for a cortisol-

secreting ACC. Monitoring osilodrostat plasma levels could thus be particularly useful for therapeutic adaptation in these patients.

OR21: TRACKING PHEOCHROMOCYTOMA EVOLUTION REVEALS MULTIPLE ROUTES TO METASTASIS, LOW INTRATUMORAL HETEROGENEITY AND AN INDEPENDENT CLONAL ORIGIN IN MULTIPLE DISEASE

Ester Arroba [1], Bruna Calsina [1], Elena Piñeiro-Yáñez [1], Angel M Martínez-Montes [1], María Monteagudo [1], Angel Fernández-Sanromán [1], Alberto Cascón [1], Fátima Al-Shahrour [1], Mercedes Robledo [1]

[1] *Hereditary Endocrine Cancer Group,* Spanish National Cancer Research Center (cnio), Madrid, Spain

Metastatic pheochromocytoma (mPPGL) presents a heterogeneous range of clinical phenotypes, whose triggering molecular mechanisms are still unknown. We have recently described, in a large genomic profiled cohort, that high mutational load, microsatellite instability and somatic copy-number alteration burden are associated with ATRX/TERT alterations in the primary tumor, and are suitable prognostic markers for metastatic disease1. However, the alterations present in the metastases, explaining metastatic spread, are largely unstudied. Here, we analyzed 51 specimens (multiple primary tumors, relapses and metastases) from 20 patients with mPPGL, in order to explore intratumoral heterogeneity, tumor evolution and the phylogenetic relationships between tumors within the same single patient. Whole-exome sequencing was applied to the entire cohort, and mutational and copy-number alteration patterns have been mapped. We have traced the evolution of mPPGL by inferring the clonal structure in primary-relapse-metastases trios to study different patterns of metastatic spread (e.g. depending on rapid progression to multiple tissue seeding sites or stable disease), elucidating distinct models of metastatic dissemination. We have observed early and late divergence of primitive ancestral clones even within metastases from the same patient. Overall, we have identified that variant clones of high-impact consequences tend to be selected in the metastases. Moreover, the analysis of multi-regional primary tumors has revealed low intratumoral heterogeneity at the mutational level. The intra-individual primary tumor comparisons has also shown a lack of shared somatic alterations, supporting an independent clonal origin. This study reveals in fine detail the evolutionary landscapes of some interesting mPPGL cases.

OR22: SINGLE-CELL CHROMOSOME AND TRANSCRIPTOME ANALYSIS AS A DIAGNOSTIC TOOL TO DIFFERENTIATE BETWEEN BENIGN AND METASTATIC PHEOCHROMOCYTOMA AND SYMPATHETIC PARAGANGLIOMA

Annika Berends [1], René Wardenaar [2], Hilda van den Bos [2], Andréa Tijhuis [2], Thera Links [1], Richard Feelders [3], Leo Hofland [3], Schelto Kruijff [4], Karel Pacak [5], Diana Spierings [2], Michiel Kerstens [1], Floris Foijer [2]

[1] *Endocrinology,* University Medical Center Groningen, Groningen, Netherlands
[2] *University of Groningen,* European Research Institute for the Biology of Ageing, Groningen, Netherlands
[3] *Endocrinology,* Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands
[4] *Surgical Oncology,* University Medical Center Groningen, Groningen, Netherlands
[5] *Medical Neuroendocrinology,* Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Rockville, United States

Background: Approximately 10-20% of patients with pheochromocytoma or sympathetic paraganglioma (PPGL) develop metastatic disease, the majority of which present as metachronous lesions. Unfortunately, there is a lack of accurate markers to predict the biologic behavior of a PPGL at the initial diagnosis. Therefore, there is an unmet need to identify biomarkers that can reliably discriminate benign from potentially metastatic PPGL. Methods: We investigated tumor samples from patients with PPGL and a diagnosis of either benign or metastatic disease with synchronous or metachronous metastases and performed a comprehensive molecular analysis through application of single-cell whole-genome sequencing and transcriptome analysis, including variant detection analysis of RNA sequences. Results: PPGL displayed complex karyotypes with recurrent aneuploidies and substantial cell-to-cell karyotype variability, indicating ongoing chromosomal instability (CIN). Karyotype landscapes and intratumor karyotype heterogeneity was comparable between benign and metastatic tumors. Transcriptome analysis revealed several differences between benign and metastatic PPGL, including upregulated expression of genes associated with hypoxia, epithelial to mesenchymal transition and TNFa and TGFb signaling in metastatic PPGL. These differences were already detectable in primary tumor samples of initially benign-appearing PPGLs that developed metachronous metastases. Conclusions: Single-cell whole-genome analysis did not demonstrate significant differences between benign and metastatic PPGL. Transcriptome analysis, however, displayed several differences that were already detectable in primary tumor PPGL before

development of metastases. This finding could provide an important tool for improvement of patient stratification at initial diagnosis.

OR23: HETEROGENEITY OF THE TUMOR MICROENVIRONMENT ACROSS PPGL METASTASES

Bruna Calsina [1, 2], Ángel M Martínez-Montes [1], María Monteagudo [1], Elena Piñeiro-Yáñez [3], Ester Arroba [1, 3], Eduardo Gil [1, 2], Rocío Letón [1], Sara Mellid [1], María Currás-Freixes [2], Cristina Montero-Conde [1], Luís Javier Leandro-Garcia [1], Fátima Al-Shahrour [3], Alberto Cascón [1], Mercedes Robledo [1]

[1] *Hereditary Endocrine Cancer Group, Human Genetics Programme,* Spanish National Cancer Research Center (CNIO), Madrid, Spain
[2] *Familiar Cancer Clinical Unit, Human Genetics Programme,* Spanish National Cancer Research Center (CNIO), Madrid, Spain
[3] *Bioinformatics Unit, Structural Biology Programm,* Spanish National Cancer Research Center (CNIO), Madrid, Spain

The tumor microenvironment (TME) is important for tumor progression and anti-cancer therapeutic responses, and is being evaluated as a prognostic tool in many tumor types. Recently, in primary tumors, we have defined a general immunosuppressive microenvironment in metastatic pheochromocytomas, the exception being MAML3-related tumors expressing PD-L11. In this new study, we aimed to provide a portrait of the TME across individual metastases using a combination of gene expression data, manually scored immunohistochemistry and neoantigen prediction. For this, we have profiled n=21 metastases from 14 different patients, most of them paired with their primary tumors. Evaluation of the immune cell levels shows that metastases partially segregate by target organ, and reveals that metastases from the same patient show some similarities. We also examined the expression of immunomodulators (IMs) discovering that metastases still tended to cluster by metastasis site, although the ones from the same patient had very distinct IMs expression levels. Thus, these analyses effectively reveal heterogeneity of cell fractions and IMs expression across individual metastases, reinforcing the notion of a variable spatial architecture of the TME. On the other hand, our results also indicate a relatively homogeneous profile in tumors from the same patients, which differs when we take into account the organ in which the metastasis has developed. Further research will allow us to conclude whether the TME in PPGL metastases simply reflects metastatic organ site, or mirrors e.g. specific genomic features.

OR24: DIFFERENCES IN THE CATECHOLAMINE SECRETION MACHINERY IN PHEOCHROMOCYTOMAS AND PARAGANGLIOMAS

Sandra Jahnke [2], Gintarė Žygienė [1], Catleen Conrad [1], Martijn van Faassen [3], Ido P. Kema [3], Nan Qin [4, 5, 6], Maike Langini [4, 5, 6], Mirko Peitzsch [1], Marcus Quinkler [7], Martin Fassnacht [8], Svenja Nölting [9, 10], Stephanie M.J. Fliedner [11], Elena Rapizzi [12], Henri JLM Timmers [13], Christina Pamporaki [2], Giuseppe Opocher [14], Karel Pacak [15], Mercedes Robledo [16, 17], Stefan R. Bornstein [2], Graeme Eisenhofer [2], Nicole Bechmann [1]

[1] *Institute of Clinical Chemistry and Laboratory Medicine,* University Hospital Carl Gustav Carus, Medical Faculty Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany
[2] *Department of Internal Medicine III,* University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany
[3] *Department of Laboratory Medicine,* University Medical Center Groningen, University of Groningen, Groningen, Netherlands
[4] *Clinic for Hematology, Oncology and Clinical Immunology, Medical Faculty,* Heinrich Heine University, University Hospital Düsseldorf, Düsseldorf, Germany
[5] *Institute of Neuropathology,* Medical Faculty, HHU, UKD, Düsseldorf, Germany
[6] *Institue for Molecular Medicine I,* Heinrich Heine University, University Hospital Düsseldorf, Düsseldorf, Germany
[7] Endocrinology in Charlottenburg, Berlin, Germany
[8] *Department of Internal Medicine I, Division of Endocrinology and Diabetes,* University Hospital Würzburg, University of Würzburg, Würzburg, Germany
[9] *Department of Endocrinology, Diabetology and Clinical Nutrition,* University Hospital Zurich (USZ), and University of Zurich (UZH), Zurich, Germany
[10] *Department of Medicine IV,* University Hospital, Ludwig-Maximilians-University Munich, München, Germany
[11] *University Cancer Center Schleswig-Holstein,* University Medical Center Schleswig-Holstein, Lübeck, Germany
[12] *Department of Experimental and Clinical Medicine,* University of Florence, Florence, Italy
[13] *Department of Internal Medicine,* Radboud University Medical Center, Nijmegen, Netherlands
[14] *Department of Medicine,* University of Padua, Padova, Italy
[15] *Section of Medical Neuroendocrinology,* Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, United States
[16] *Hereditary Endocrine Cancer Group, Human Cancer Genetics Program, ,* Spanish National Cancer Research Centre (CNIO), Madrid, Spain
[17] *Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER),* Institute of Health Carlos III (ISCIII), Madrid, Spain

Pheochromocytomas and paragangliomas (PPGLs) are heterogeneous tumors with highly variable signs and symptoms and a diverse genetic background. The underlying pathogenic

variant (PV) determines the catecholamine phenotype and may further affect the secretory pathway among these tumors. Here, we hypothesized that pseudohypoxic cluster 1 PPGLs due to PVs in genes related to Krebs cycle (cluster 1A) or PVs in hypoxia-related genes (cluster 1B) exhibit differences in catecholamine secretion and excretion patterns compared to cluster 2 PPGLs with activation of kinase signaling pathways. Therefore, we measured catecholamines in tumor tissue (n=317), plasma (n=144) and urine (n=164) samples from patients with PPGLs and known genetic background. Cluster 1 PPGLs displayed reduced tissue catecholamine levels compared to cluster 2 PPGLs, with cluster 1A showing significantly lower levels than cluster 1B PPGLs. In contrast, cluster 1 PPGLs secreted significantly more catecholamines into plasma with associated higher rates of excretion into urine than cluster 2 PPGLs. Rates of tumoral catecholamine secretion and excretion of catecholamines into urine were higher in patients with cluster 1A PPGLs than cluster 1B PPGLs. Transcriptome and proteome analyses indicated differences related to catecholamine storage and secretion, which suggests a more immature catecholamine secretion machinery in cluster 1 PPGLs, particularly in cluster 1A, compared to PPGLs of cluster 2. Nevertheless, no correlation between catecholamine secretion and catecholamine-related signs and symptoms was found. This study provides further mechanistic insights into the genotype-phenotype relationship in PPGLs and revealed differences in catecholamine secretion in particular between cluster 1A and 1B PPGLs.

OR25: RECURRENT DISEASE IN PATIENTS WITH SPORADIC PARASYMPATHETIC HEAD AND NECK PARAGANGLIOMA

Susan Richter [1], Karel Pacak [2], Alexander Prejbisz [3], Felix Beuschlein [4], Hanna Remde [5], Mercedes Robledo [6], Graeme Eisenhofer [1], Henri J.L.M Timmers [7], Christina Pamporaki [1]

[1] *Department of Medicine III,* University Hospital Carl Gustav Carus at the TU Dresden, Dresden, Germany
[2] *Section on Medical Neuroendocrinology,* Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health (NIH), Bethesda, United States
[3] *Department of Hypertension,* Institute of Cardiology, Warsaw, Poland
[4] *Department of Internal Medicine IV,* University Hospital LMU, Munich, Germany
[5] *Department of Internal Medicine,* University of Würzburg, Würzburg, Germany
[6] *Hereditary Endocrine Cancer Group,* Human Cancer Genetics Programme, Spanish National Cancer Research Centre, Madrid, Spain
[7] *Department of Internal Medicine,* Radboud University Medical Centre, Nijmegen, Netherlands

Background: The prevalence of hereditary disease among patients with head/neck paragangliomas (HNPGL) is up to 50% and specific genetic alterations may increase the risk of recurrence. However, prevalence of recurrence in patients with sporadic HNPGL remains unclear. Objective: To investigate the prevalence of recurrence and the interval of the disease-free period among patients with sporadic HNPGL. Methods: This multicentric study included retrospective data from 209 patients with HNPGL at first diagnosis and follow up at least 12 months. Clinical information included sex, age at diagnosis, tumor location, size and catecholamine phenotype, genetic testing, primary tumor treatment and metastatic or recurrent disease. Patients with sporadic HNPGL were those with negative genetic test results. Results: Patients with sporadic HNPGL showed the previously reported features of predominantly female sex, older age and were more often with jugulotympanic than carotid body tumors. Prevalence of recurrence among patients with sporadic HNPGL was significantly lower than for those with hereditary disease (37vs60%, P=0.0015). Prevalence of metastatic disease was similarly low for both groups (9.3%vs10.6%). Among patients with sporadic HNPGL and recurrent disease, 71% were diagnosed within 10 years after primary tumor diagnosis compared to 54% for those with hereditary HNPGL. Metastases occured within 10 years for 67% and 80% of patients with sporadic and hereditary HNPGL respectively. Patients with partially or untreated primary HNPGL showed similar recurrence rates as fully treated. Conclusion:

Recurrence among patients with sporadic HNPGL is high enough to mandate long term (at least 10 years) follow up.

OR26: BONE METASTASES AND SKELETAL RELATED EVENTS IN PHEOCHROMOCYTOMA AND PARAGANGLIOMA PATIENTS. INTERNATIONAL, RETROSPECTIVE STUDY

Marta Lagana [1], Deborah Cosentini [1], Salvatore Grisanti [1], Hanna Remde [2], Madson Almeida [3], Sara Pusceddu [7], Timo Deutschbein [2], Gustavo Fagundes [6], Adelaide Pereira [6], Natalie Prinzi [8], Chiara Maria Grana [8], Nicola Fazio [8], Eleonora Corssmit [9], Alberto Bongiovanni [10], Letizia Canu [11], Kim Lim [12], Mohamed Amir Habra [13], Camilo Jimenez [13], Alfredo Berruti [1]

[1] *Oncology,* Spedali Civili Brescia, Brescia, Italy
[2] *Department of Internal Medicine I, Division of Endocrinology and Diabetes,* University of Würzburg, Würzburg, Germany
[3] *Endocrine Oncology Division, Cancer Institute of Sao Paulo State (ICESP), ,* University of Sao Paulo Medical School, sao paulo, Brazil
[4] *Division of Endocrinology and Metabolism,* Clinics Hospital, University of Sao Paulo Medical School, Sao Paulo, sao paulo, Brazil
[5] *Department of Medical Oncology,* Fondazione IRCCS, Istituto Nazionale dei Tumori, Milan, Italy
[6] *Endocrine Hypertension Unit University,* Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, sao paulo, Brazil
[7] *Radiometabolic Therapy Unit,* IRCCS European Institute of Oncology, Milan, Italy
[8] *Division of Gastrointestinal Medical Oncology and Neuroendocrine Tumors, ,* European Institute of Oncology, Milan, Italy
[9] *Department of Internal Medicine, Division of Endocrinology,* Leiden University Medical Center, leiden, Netherlands
[10] *Osteoncology and Rare Tumors Center (CDO-TR),* IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy
[11] *Department of Experimental and Clinical Biomedical Sciences,* University of Florence, florence, Italy
[12] *Department of Endocrinology,* St Bartholomew's Hospital, London; William Harvey Research Institute, Queen Mary University of London, london, United Kingdom
[13] *Department of Endocrine Neoplasia and Hormonal Disorders,* The University of Texas MD Anderson Cancer Center , Texas, United States

Pheochromocytomas and paragangliomas (PPGLs) are rare tumors that frequently metastatize to bone. This retrospective, observational, international, multicenter study, that recruited PPGLs patients with BMs to describe the frequency of SREs, such as pathological fractures, spine compression and hypercalcemia. Secondary aims were to define predictive factors for SRE development and prognostic factors. Data of 294 patients were analyzed. SREs occurred in 90 (31%) of patients, including 55 (19%) bone fractures, 47 (16%) spinal cord compressions, 11 (4%) hypercalcemias. Bone pain was reported in 144 patients (49%). Sixty-four patients

underwent bone surgery (22%) and 136 bone radiotherapy (46%). SRE frequencies resulted similar comparing US vs the European-Brazilian case series.Median overall survival was 5.2 years. In multivariate analysis, younger age of 48 years (hazard ratio (HR) 0.5, 95% confidence interval (CI): 0.2-0-9, P<0.005), PPGL of the head and neck (HR 0.4, CI:0.2-0.9, P< 0.005) and the absence of liver metastases (HR 0.6, CI:0.3-0.9, P< 0.005) were significantly associated with a lower mortality risk while the prognostic role of bisphosphonates and denosumab treatment just failed to attain the statistical significance (HR 0.6, CI:0.4-1, P = 0.057). Median time for development of true SRE was 4.2 months. Bisphosphonates or denosumab (HR 0.5, CI:0.3-0.8, P< 0.005) and metachronous bone metastases (HR 0.3, CI:0.2-0.6, P<0.005) correlated with a longer time to development of SREs.PPGL patients with bone metastases have a relatively long life expectancy and are at high risk of SREs. Bone resorption inhibitors could decrease the SRE risk and potentially improve outcome.

OR27: IMPACT OF SURGICAL TECHNIQUE ON HEMODYNAMIC INSTABILITY DURING MINIMALLY INVASIVE SURGERY FOR PHEOCHROMOCYTOMA

Amir Hossein Chaman Baz [1], Julie van de Wal [1], Simone Willems [2], Xiaoye Zhu [1], Frank d'Ancona [1], Henri Timmers [3], Johan Langenhuijsen [1]

[1] *Urology,* Radboudumc, Nijmegen, Netherlands
[2] *Anesthesiology,* Radboudumc, Nijmegen, Netherlands
[3] *Internal medicine, section Endocrinology,* Radboudumc, Nijmegen, Netherlands

Introduction: Surgical resection by either transperitoneal laparoscopic adrenalectomy (TLA) or posterior retroperitoneoscopic adrenalectomy (PRA) is the therapy for pheochromocytomas (PCC). PRA results in faster operating times with less blood loss and faster recovery, but is demanding due to smaller working space with higher CO2-pressures and may therefore result in more tumor manipulation and blood pressure fluctuations. We aim to investigate the impact of surgical technique on intraoperative hemodynamic instability in patients undergoing PCC surgery. Methods: We included patients undergoing either TLA or PRA for PCC from 2007-2022 from a prospective database. The primary outcome was the hemodynamic instability (HI) score and secondary outcomes were intraoperative hemodynamic parameters and drug administration. Results: We included 101 patients, 57 underwent TLA and 44 underwent PRA. The HI-score was higher in PRA compared to TLA (97 vs 46). In PRA there were more frequent (2-5 vs 1-3, p=0.025) and longer episodes of MAP<60 mmHg (5.6% vs 7.1%, p=0.013), and longer episodes of heart rate<50 bpm (9.9% vs 16.9%). TLA had a higher maximum systolic blood pressure (169 vs 157), longer episodes of heart rate>100bpm (31.6% vs 6.8%) and higher maximum heart rate (87 vs 77.5). PRA received more vasoconstrictive drugs (97.7% vs 78.9%) and fluid infusion (1027 vs 750 ml), while TLA received more vasodilating drugs (64.9% vs 38.6%). Conclusion: PRA is associated with higher hemodynamic instability than TLA due to hypotension necessitating vasoconstrictive drugs. This suggests that patients undergoing PRA or TLA could benefit from a patient-tailored preoperative α-adrenergic blockade.

OR28: RESPONSES TO SYSTEMIC THERAPY IN METASTATIC PHEOCHROMOCYTOMA AND PARAGANGLIOMA

Alessa Fischer [1], Simon Kloos [1], Hanna Remde [2], Ulrich Dischinger [2], Christina Pamporaki [3], Henri J L M Timmers [4], Mercedes Robledo [5], Stephanie M J Fliedner [6], Katharina Wang [10], Julian Maurer [10], Astrid Reul [1], Nicole Bechmann [7], Constanze Hantel [1, 3], Hermine Mohr [8], Natalia S Pellegata [8, 12], Stefan R Bornstein [1, 3], Matthias Kroiss [10], Christoph J Auernhammer [10], Martin Reincke [10], Karel Pacak [9], Ashley B Grossman [11, 13], Felix Beuschlein [10, 1], Svenja Nölting [1, 10]

[1] *Department of Endocrinology, Diabetology and Clinical Nutrition,* University Hospital Zurich (USZ) and University of Zurich (UZH), Zurich, Switzerland
[2] *Department of Internal Medicine I, Division of Endocrinology and Diabetes,* University Hospital, University of Würzburg, Würzburg, Germany
[3] *Department of Internal Medicine III,* University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany
[4] *Division of Endocrinology, Department of Internal Medicine,* Radboud University Medical Center, Nijmegen, Netherlands
[5] *Hereditary Endocrine Cancer Group.,* Spanish National Cancer Research Center (CNIO), Madrid, Spain
[6] *First Department of Medicine,* University Medical Center Schleswig-Holstein, Lübeck, Germany
[7] *Institute of Clinical Chemistry and Laboratory Medicine,* University Hospital Carl Gustav Carus, Dresden, Germany
[8] *Institute for Diabetes and Cancer,* Helmholtz Zentrum München, Neuherberg, Germany
[9] *Eunice Kennedy Shriver NICHD,* NIH, Bethesda, United States
[10] *Department of Medicine IV,* University Hospital, Ludwig-Maximilians-University Munich, Munich, Germany
[11] *Green Templeton College,* University of Oxford, Oxford, United Kingdom
[12] *Department of Biology and Biotechnology,* University of Pavia, Pavia, Italy
[13] *NET Unit, ENETS Centre of Excellence,* Royal Free Hospital, London, United Kingdom

Objective: The therapeutic options for metastatic pheochromocytomas/paragangliomas (mPPGLs) include chemotherapy with cyclophosphamide/vincristine/dacarbazine (CVD), temozolomide monotherapy, radionuclide therapies, and tyrosine kinase inhibitors such as sunitinib. The objective of this multicenter retrospective study was to evaluate and compare the responses of mPPGLs including those with pathogenic variants in SDHB, to different systemic treatments. Design¸Retrospective analysis of treatment responses of mPPGL patients (n=74) to systemic therapies. Methods: Patients with mPPGLs treated at six specialized national centers were selected based on participation in the ENSAT registry. Progression-free survival (PFS)

and disease-control rates (DCR) were evaluated based on medical records and tumor board recommendations. Results: Seventy-four patients with mPPGLs (79.7% progressive at baseline) received at least one line of systemic treatment. The DCR with first-line CVD chemotherapy was 80.0% (n=5, PFS 18 months; SDHB [n=2]: DCR 100%, PFS 18 months), with somatostatin peptide receptor-based radionuclide therapy (PPRT) 63.6% (n=22, PFS 17 months; SDHB [n=11]: DCR 72.7%, PFS 15 months), with 131I-meta-iodobenzylguanidine (131I-MIBG) 87.0% (n=24, PFS 43 months; SDHB [n=4]: DCR 100%, PFS 24 months), with sunitinib 85.7% (n=7, PFS 18 months; SDHB [n=3]: DCR 100%, PFS 18 months) and with somatostatin analogs 100% (n=6, SDHB [n=1]). The DCR with temozolomide as second-line therapy was 60.0% (n=5, PFS 10 months; SDHB [n=4]: DCR 75%, PFS 10 months). The results for patients with progressive disease were similar. Conclusions: We demonstrate in a real-life clinical setting that all current therapies show reasonable efficacy in preventing disease progression, with an especially good DCR for patients with SDHB tumors.

OR29: SUNITINIB FOR MALIGNANT PROGRESSIVE PHEOCHROMOCYTOMAS AND PARAGANGLIOMAS

Eric Baudin [1], FIRSTMAPPP GROUP [1], Martin Fassnacht [2]

[1] *Imaging endocrine unit,* Gustave Roussy, Villejuif, France
[2] *8. Department Internal Medicine I, Division of Endocrinology and Diabetes,* University Hospital, University of Würzburg, Würzburg, Germany

Background: No randomized trial has ever been performed in malignant pheochromocytomas and paragangliomas (MPP). Preclinical and first clinical evidence suggested beneficial effects of sunitinib. Methods: In this phase 2 randomized trial, we randomly assigned patients with progressive MPP to receive sunitinib (37.5 mg/d) or placebo, in a 1:1 ratio,. Randomization was stratified according to SDHB status and number of prior systemic therapies. Primary endpoint was the rate of progression-free survival (PFS) at 12 months according to real-time central review (RECIST 1.1). Key secondary endpoints were PFS, objective response rate, overall survival and safety (NCI CTCAE v.4). Based on a two-step Simon model, we aimed for the accrual of 74 patients, assuming a 20% improvement of 12 months-PFS rate from 20% to 40% to conclude that sunitinib is effective. Cross-over was allowed. Results: Seventy-eight patients (32% with germline SDHx variants; 60% with prior systemic therapies) were enrolled, 39 in each arm. The primary endpoint was met: PFS at 12 months was 35.9% with sunitinib and 18.9% with placebo. Median PFS was 8.9 (95% CI, 5.5-12.7) and 3.6 months (95% CI, 3.1-6.1), respectively. Partial response rate was 36.1% (95% CI, 20.8-53.8) with sunitinib and 8.3% (95% CI, 1.8-22.5) with placebo. Overall survival was not significantly different. Treatment was discontinued for adverse events in 14% and 0% of patients (sunitinib and placebo). Five deaths were reported (3 in sunitinib arm and 2 in placebo arm). Conclusion: Sunitinib becomes the medical option with the highest level of evidence of antitumor efficacy in progressive MPP.

(FIRSTMAPPP ClinicalTrial.gov number, NCT01371201)

OR30: EO2401 PEPTIDE IMMUNOTHERAPY + NIVOLUMAB IN METASTATIC PHEOCHROMOCYTOMA/PARAGANGLIOMA (MPP); THE PHASE 1/2 EOADR1-19/SPENCER TRIAL (NCT04187404)

Alfredo Berruti [13], Salvatore Grisanti [13], Martin Fassnacht [12], Vivek Subbiah [11], Camilo Jimenez [11], Christelle de la Fouchardière [6], C. Willemien Menke - van der Houven van Oordt [5], Harm Haak [1], Matthias Kroiss [9], Jaume Capdevila [7], Jeffrey Yachnin [8], Dan Granberg [8], Gedske Daugaard [10], Deborah Cosentini [13], Livia Lamartina [2], Jan Fagerberg [3], Lucie Aubergeon [4], Hélène Toussaint [4], Jean-Michel Paillarse [3], Eric Baudin [2]

[1] *Medical Oncology,* Máxima Medisch Centrum, Eindhoven, Netherlands
[2] *Endocrine Oncology,* Gustave Roussy, Villejuif, France
[3] *Medical Department,* Enterome, Paris, France
[4] *Drug Discovery,* Enterome, Paris, France
[5] *Medical Oncology,* Amsterdam UMC, Amsterdam, Netherlands
[6] *Medical Oncology,* Centre Léon Bérard, Lyon, France
[7] *Medical Oncology,* Hospital Universitari Vall d'Hebron, Barcelona, Spain
[8] *Endocrine tumors,* Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden
[9] *Medicine IV,* LMU University Hospital, Ludwig-Maximilians-Universität, Munich, Germany
[10] *Medical Oncology,* Rigshospitalet, Copenhagen, Denmark
[11] *Medical Oncology,* The University of Texas MD Anderson Cancer Center (MDACC), Houston, United States
[12] *Internal Medicine,* University Hospital, Würzburg, Germany
[13] *Oncologia Medica,* Università degli Studi di Brescia, Azienda Ospedaliera Spedali Civili, Brescia, Italy

Background: EO2401 was designed to expand pre-existing memory cytotoxic T cells recognizing specific protein sequences from gut bacteria which cross-react with tumor associated antigens (TAAs). EO2401 contains three synthetically produced CD8 HLA-A2 epitopes with mimicry to adrenal tumor-TAAs (IL13Rα2, BIRC5/survivin, and FOXM1) and the CD4 epitope UCP2. Methods: Patients received EO2401 (300 µg/peptide, q2weeklyx4 then q4weekly) with nivolumab (240mg q2weeklyx3 then 480mg q4weekly). Cohorts C3a included patients after prior systemic therapy, and for C3b patients, EO2401/nivolumab was 1st line systemic therapy. Immune testing was performed utilizing peripheral-blood-mononuclear-cells in ELISPOT after in vitro stimulation (IVS), or by tetramers ex vivo, or after IVS. Results: Fifteen patients with MPP was recruited: C3a = 11 (73%) and C2b =4 (27%). Safety included the most common any grade AEs (irrespective of relationship): injection site reaction (38%),

diarrhea (31%), asthenia (31%), injection site pain (31%), peripheral edema (31%), pyrexia (23%), anemia (23%), back pain (23%), decreased appetite (23%), weight decreased (23%). Immune responses were seen in 91% and 90% of tested patients for expansions of T cells specific for mimic peptides, and TAAs (cross-reactive T cells). Strength of immune response correlated with survival (n=15; R=0.57, R2=0.33, p=0.084). Efficacy outcome with median survival follow-up 17.9 months included PR-rate=7%; disease-control-rate (PR+SD)=73%; duration-of-disease-control=median 6.9months (range 2.6-19.6); progression-free-survival=5.0months (0.03-19.6); survival=not reached (0.03-23.6). The 12-/18-months survival rates were 57%, and 50%. Conclusions: EO2401/nivolumab was well tolerated with EO2401 adverse events limited to local administration site reactions and nivolumab events within expected range. EO2401 generated mimic and human TAA specific cytotoxic T cell immune responses; strength of immune response correlated with survival. One partial response was observed, and 18-months survival rate was 50%. Further follow-up will determine next steps.

OR31: WHOLE BLOOD TRANSCRIPTOMIC SIGNATURE OF CUSHING'S SYNDROME

Maria Francesca Birtolo [1], Roberta Armignacco [1], Nesrine Benanteur [1], Daniel De Murat [1], Laurence Guignat [2], Lionel Groussin [2, 1], Rossella Libé [1, 2], Franck Letourneur [1], Jérôme Bertherat [1, 2], Anne Jouinot [1, 2], Guillaume Assié [1, 2]

[1] *CNRS UMR8104, INSERM U1016, Institut Cochin, F-75014,* Université Paris Cité, Paris, France
[2] *Service d'Endocrinologie, Center for Rare Adrenal Diseases,* Hôpital Cochin, Paris, France

Background: Cushing's syndrome (CS) is associated with high morbidity and presents high interindividual variability. Easily measurable biomarkers, in addition to the hormone assays currently used for diagnosis, could better quantify the individual biological impact of glucocorticoids. The aim of this study is to identify such biomarkers through the analysis of whole blood transcriptome. Methods: Whole blood transcriptome was evaluated in 57 samples (35 in the training cohort; 22 in the validation cohort) classified in overt CS, mild CS, eucortisolism and adrenal insufficiency according to the clinical evaluation and 24-h urine-free cortisol. Total RNA was obtained from whole blood samples and sequenced on NovaSeq 6000 platform (Illumina). Both unsupervised (principal component analysis) and supervised (Limma) methods were used to explore transcriptome profile. Results: The transcriptomic profile discriminates samples with overt Cushing syndrome. Genes most associated with overt Cushing syndrome are enriched in pathways related to immunity, particularly in neutrophil activation. A prediction model of 1500 genes built on the training cohort demonstrated its discriminating value in the validation cohort (accuracy 0.73) and remains significant in multivariate analysis including the neutrophil rate (p=0.002). The prediction based on FKBP5 alone, a gene involved in glucocorticoid receptor signaling and one of the most overexpressed in overt Cushing syndrome, is comparable to the predictor based on 1500 genes (accuracy 0.68). Conclusion: Whole blood transcriptome reflects the biological action of glucocorticoids. FKBP5 could be a non-hormonal marker of Cushing syndrome.

OR32: CONSTITUTIONAL DUPLICATION OF PRKACA GENE IS A CAUSE OF ISOLATED PRIMARY PIGMENTED NODULAR ADRENOCORTICAL DISEASE (PPNAD): RESULTS OF ITS SYSTEMATIC SCREENING IN MACRO- AND MICRONODULAR NODULAR ADRENAL HYPERPLASIA

Patricia Vaduva [1], Florian Violon [1], Gerald Raverot [2], Stephanie Espiard [3], Amina Attia [1], Lucas Bouys [1, 4], Karine Perlemoine [1], Albin Chasavang [5], Sylvie Hieronimus [6], Marie Christine Vantyghem [3], Michel Polak [7], Bruno Ragazzon [1], Anne Jouinot [1], Eric Pasmant [5], Jerome Bertherat [1, 4]

[1] *Genomics and signaling of endocrine tumors,* Cochin Institute, Paris, France
[2] *Endocrinology,* Hospices Civils de Lyon, Bron, France
[3] *Endocrinology, Diabetology, Metabolism and Nutrition,* Lille University Hospital, Lille, France
[4] *Endocrinology, Reference center for rare adrenal diseases,* Cochin Hospital, APHP, Paris, France
[5] *Oncogenetics,* Cochin Hospital, APHP, Paris, France
[6] *Endocrinology, Diabetology and Reproductive medicine,* Nice university hospital, Nice, France
[7] *Endocrinology, Gynecology and Pediatric diabetology,* Necker Hospital, APHP, Paris, France

Objective: Constitutional duplications of the PRKACA gene locus have been described as responsible for adrenal Cushing. The objective here was to evaluate the results of its systematic screening in adrenal nodular disease and to describe the associated phenotype. Methods: Between 2020 and 2023, 440 consecutive index cases with macronodular or micronodular adrenal hyperplasia or Carney Complex (CNC) were genotyped with a targeted NGS panel including the exonic and intronic flanking regions of the ARMC5, MEN1, PRKAR1A (CNC) and PRKACA genes. Familial screening was then offered to relatives. Results: Constitutional duplications of PRKACA were identified in 5 index cases and 7 of the 11 screened relatives (sex-ratio=1 male/2 female), supporting the involvement of the PRKACA oncogene through a constitutional copy gain mechanism. All index cases had Primary Pigmented Nodular Adrenocortical Disease (PPNAD) responsible for Cushing's syndrome and ACTH-independent hypercorticism, diagnosed at a median of 20 years (min=9; max=32). They were treated by bilateral adrenalectomy. The adrenals were described as normal on conventional imaging in 3/5 cases, but iodocholesterol scintigraphy showed diffuse bilateral hyperfixation. No other

manifestation of the Carney complex was observed apart from PPNAD (median follow-up 11 years), except for testicular calcifications in 1/4 patients. Discussion: Constitutional duplication of PRKACA is a rare cause of PPNAD. It does not appear to be involved in other forms of nodular adrenal hyperplasia, nor is it frequently associated with other manifestations of CNC. Constitutional duplication of PRKACA should be searched in the absence of a pathogenic PRKAR1A variant in patients with PPNAD.

OR33: IDENTIFICATION AND CHARACTERIZATION OF MOLECULAR HETEROGENEITY IN PBMAH BY MALDI-MSI AND RNA-SEQUENCING

Sharmilee Vetrivel [1], Na Sun [2], Ru Zhang [1], Andrea Oßwald-Kopp [1], Sara Jung [1], Jess Baker [1], Annette Feuchtinger [2], Martin Reincke [1], Silviu Sbieria [3], Axel Walch [2], Anna Riester [1]

[1] *LMU Klinikum, Ludwig-Maximilians-University,* Endocrinology Medizinische Klinik und Poliklinik IV , München, Germany
[2] *Helmholtz Zentrum Munich,* Research Unit Analytical Pathology, München, Germany
[3] *Department of Internal Medicine I,* Division of Endocrinology and Diabetes University Hospital, University of Würzburg, Würzburg, Germany

Primary bilateral micronodular adrenocortical hyperplasia (PBMAH) represents a heterogeneous subtype of Cushing's Syndrome (CS). In the current study, MALDI MSI, LC-MS/MS and transcriptomics was used to characterize the cellular heterogeneity in PBMAH. The study included 22 PBMAH patients, mostly WT (15 of 22) for ARMC5 and KDM1A (hotspot genes). MALDI-MSI measurement was performed on the FFPE sections and for RNA, the marked tumor area was collected for RNA extraction and NGS. A panel of the 15 steroids in the steroidogenesis pathway and ACTH were quantified in peripheral blood samples by LC-MS/MS. To analyse the inter tumor heterogeneity based on MALDI MSI, kmeans clustering was done and 8 distinct metabolite subclusters with varying percentages of distribution among the samples were found. Cluster 1 was the dominant cluster (more than 75% of the area) in the 7 WT samples, Cluster 5 was the dominant cluster in 2 WT samples and Cluster 6 in 2 WT samples. There was no dominant subcluster in the mutants. The individual subclusters were found to show different levels of correlation to steroids and ACTH. Cluster 1, the dominant subcluster in 7 of the 17 WT samples was found to be positively correlated with ACTH and negatively correlated with cortisol, cortisone, deoxycorticosterone and progesterone. Co-ordination of the transcriptomic data with the MALDI-MSI data revealed various genes including Melanocortioctrophin-2-receptor (MC2R; log2FC=3.47,pval=0.029) to be significantly downregulated in these specific WT samples. The transcriptomic data is being further used to classify the heterogeneity based on metabolite specific pathway mapping.

OR34: STUDY OF SOMATIC MOLECULAR HETEROGENEITY IN BILATERAL MACRONODULAR ADRENOCORTICAL DISEASE (BMAD) BY NGS PANEL IN A COHORT OF 26 PATIENTS

Florian Violon [1], Lucas Bouys [1], Gaetan Giannone [1], Daniel De Murat [1], Patricia Vaduva [1], Karine Perlemoine [1], Annabel Berthon [1], Bruno Ragazzon [1], Mathilde Sibony [1], Jérôme Bertherat [1]

[1] *Endocrine tumors genomic and signaling pathway,* Institut Cochin, Paris, France

Bilateral macronodular adrenal disease (BMAD) is a genetically heterogeneous disease. Most cases are isolated. They are divided into three molecular groups depending on the patient's pathological variant causing the disease: ARMC5, KDM1A and no genetic cause known to date. ARMC5 and KDM1A function as tumor suppressor genes. Both a germline event and a somatic event lead to a bi-allelic inactivation of the gene resulting in the development of nodules. Several patients with bi-allelic ARMC5 inactivation showed heterogeneity of the second event from one nodule to another. Somatic events were never studied on several nodules from the same patient with bi-allelic KDM1A inactivation. The aim of this work was to study the mutational profile of different nodules from the same patient in a large cohort of BMAD to describe the somatic event of patients with pathological ARMC5 or KDM1A variants and to look for potential genetic alterations in patients with no known genetic cause. Patients and methods: 26 patients underwent surgery for BMAD at the Cochin Hospital between 2006 and 2021. Samples were obtained from formalin-fixed and paraffin-embedded material by macrodissection. Illumina NGS sequencing concerned a panel of 7 genes: ARMC5, GNAS, KDM1A, PDE8B, PDE11A, PRKACA and PRKAR1A. Results: 23 patients were analyzed, the DNA of the remaining 3 being too degraded. Of the 7 patients with a pathological ARMC5 variant, 6 had 2 to 8 pathological somatic variants of ARMC5 for 3 to 10 nodules sampled. The last patient had only one somatic event out of 5 samples. Of the 3 patients with a pathological KDM1A variant, each nodule had a loss of heterozygosity. In the 13 patients without a pathological ARMC5 or KDM1A variant, our analysis did not reveal any alteration on the panel of 7 genes studied. Discussion: Our study is the first to explore by NGS the mutational profile of different nodules from the same patient on a series of BMAD and the first to study this profile in patients with bi-allelic KDM1A inactivation. We confirmed the heterogeneity of ARMC5

second events and showed a high homogeneity of KDM1A alterations. The molecular mechanisms causing the disease in patients without a pathogenic ARMC5 or KDM1A variant remain to be discovered.

OR35: PRIMARY UNILATERAL MACRONODULAR ADRENAL HYPERPLASIA (PUMAH) WITH CONCOMITANT GLUCOCORTICOID AND ANDROGEN EXCESS DUE TO KDM1A ACTIVATION AND CONSTITUTE MC2R ACTIVATION

Yasir Elhassan [1, 2], Silke Appenzeller [3], Laura-Sophie Laura-Sophie Landwehr [4], Juliane Lippert [4], Dillon Popat [5], Lorna Gilligan [1], Edwina Goh [6], Salvador Diaz-Cano [7], Stefan Kircher [8], Susanne Gramlich [8], Robert Sutcliffe [9], Shakila Thangaratinam [6], Li Chan [5], Martin Fassnacht [3, 4], Wiebke Arlt [1, 11, 12], Cristina Ronchi [1, 2, 3]

[1] *University of Birmingham,* Institute of Metabolism and Systems Research, Birmingham, United Kingdom
[2] *Birmingham Health Partners,* Centre for Endocrinology, Diabetes and Metabolism, Birmingham, United Kingdom
[3] *University Hospital Würzburg,* Comprehensive Cancer Center Mainfranken, Würzburg, Germany
[4] *Department of Internal Medicine I,* Division of Endocrinology and Diabetes, Würzburg, Germany
[5] *Barts and the London School of Medicine, Queen Mary University of London,* Centre for Endocrinology, William Harvey Research Institute, London, United Kingdom
[6] *Obstetrics ,* Birmingham Women's Hospital, Birmingham, United Kingdom
[7] *Department of Pathology,* University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom
[8] *Department of Pathology,* University of Würzburg, Würzburg, Germany
[9] *The Liver Unit,* Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom
[10] *Birmingham Biomedical Research Centre,* University Hospitals Birmingham NHS Foundation Trust , Birmingham, United Kingdom
[11] *MRC Laboratory of Medical Sciences,* MRC, London, United Kingdom
[12] *Institute of Clinical Sciences,* Imperial College London, London, United Kingdom

We investigated a 33-year-old woman diagnosed during pregnancy with a 7cm unilateral adrenal mass associated with severe ACTH-independent glucocorticoid and androgen excess, a steroid phenotype usually indicative of adrenocortical carcinoma. Pregnancy had been achieved with in-vitro fertilisation on the assumption of underlying PCOS. Neonatal death occurred soon after emergency delivery due to foetal growth arrest at 26 weeks gestation. Histopathology after post-pregnancy unilateral adrenalectomy showed tumour-like macronodular adrenal cortical hyperplasia. Postoperatively, the clinical and biochemical phenotype resolved; the contralateral adrenal had normal size and morphology. The patient spontaneously conceived three months later and delivered a healthy baby. Germline mutations in ARMC5 were excluded. We performed whole-exome sequencing on four representative

hyperplastic cortical nodules. We detected several pathogenic germline variants, including p.G46S and p.R269Dfs*7 in KDM1A and p.M255I in the gene encoding for the ACTH receptor (MC2R). Copy number variation analysis showed clonally related nodules and demonstrated an additional somatic loss of the KDM1A wild-type allele on chromosome 1p36.12 in all nodules. RNA-sequencing on a representative nodule showed low/absent expression of KDM1A and a high expression of the gene GIPR compared to 52 adenomas and 4 normal adrenals, suggesting a similar pathogenic mechanism as recently described in primary bilateral macronodular adrenal hyperplasia associated with food-dependant Cushing Syndrome. Functional in-vitro analysis of the MC2R variant demonstrated constitutive activation of receptor activity. In conclusion, we present the first case of primary unilateral macronodular adrenocortical hyperplasia (PUMAH) associated with Cushing's syndrome and concomitant androgen excess and suggest pathogenic mechanisms involving KDM1A and MC2R.

OR36: RECONSTITUTION OF HUMAN ADRENAL CORTEX DEVELOPMENT AND STEROIDOGENESIS, A NEW PARADIGM TOWARDS STEM-CELL BASED ENDOCRINOLOGY

Kotaro Sasaki [1], Michinori Mayama [1], Keren Cheng [1]

[1] *Biomedical Sciences,* University of Pennsylvania, Philadelphia, United States

The adrenal cortex produces vital steroid hormones that maintain homeostasis. While steroid hormones produced from the fetal zone adrenal cortex are essential for both fetal development and maintenance of pregnancy, the molecular mechanisms leading to human adrenal cortex development and steroid synthesis remain poorly understood due to the paucity of model systems. Through progressive generation of fetal zone adrenal cortex-like cells (FZLCs) from human induced pluripotent stem cells through posterior intermediate mesoderm-like, and adrenal primordium-like states, we provide the first in vitro reconstitution of human adrenocortical fetal specification. Generation of FZLCs faithfully recapitulates human embryonic adrenal cortex specification as evidenced by histomorphological and ultrastructural analysis, transcriptional profiles and delta-5 steroid biosynthesis and occurs in an adrenocorticotropic hormone (ACTH)-independent manner, consistent with clinical observations. Furthermore, we demonstrate that FZLC generation is promoted by SHH and inhibited by NOTCH, ACTIVIN and WNT signaling and that steroid synthesis is amplified by ACTH/PKA signaling and blocked by pharmacologic inhibitors of delta-5 steroid synthesis enzymes. Finally, NR5A1 appear to self-stabilize its promoter activity and promote FZLC survival and steroidogenesis. Together, these findings provide a framework for understanding and reconstituting human adrenocortical development in vitro and pave the way for future cell-based therapies of adrenal insufficiency.

OR37: ROLE OF THE MINERALOCORTICOID RECEPTOR IN THE PHYSIOLOGY OF THE ADRENAL CORTEX AND THE DEVELOPMENT OF ALDOSTERONE PRODUCING ADENOMAS

May Fayad [1], Rami M. El Zein [1], Isabelle Giscos-Douriez [1], Fabio L. Fernandes-Rosa [1], Sheerazed Boulkroun [1], Maria-Christina Zennaro [1, 2]

[1] *Inserm U970, PARCC,* Université Paris Cité, Paris, France
[2] *Hôpital Européen Georges Pompidou,* Assistance Publique Hôpitaux de Paris (AP-HP), Paris, France

Introduction: Primary aldosteronism (PA) is the major cause of secondary arterial hypertension. The mineralocorticoid receptor (MR) binds aldosterone and is expressed in the adrenal cortex specifically in the zona glomerulosa (ZG) and in aldosterone-producing adenoma (APA). Given the role of MR in tissue remodeling and its expression in the ZG and APA, we hypothesized that aldosterone could be involved in the pathophysiology of APA through MR by an autocrine paracrine mechanism. To investigate this hypothesis, we sought to identify the role of MR on adrenal cortex structure and function and regulation of aldosterone production in the adrenal cortex. Methods and results: A mouse model was generated, expressing the Cre recombinase under Cyp11b2 promoter allowing specific expression of the Cre recombinase in the ZG. Those mice were crossed with Mrflx/flx mice. The adrenal phenotype of Cyp11b2+/Cre:Mrflx/flx (MRKOZG) mice was explored by morphological investigations. Genotyping of DNA from the adrenal cortex showed successful recombination in MRKOZG adrenals. MR expression was significantly reduced as shown by RT-qPCR and RNAscope. In MRKOZG mice, the adrenal cortex was disorganized and adrenocortical lineage appeared to be modified. In particular, ectopic expression of the fetal zone marker AKR1C1 in the zona fasciculata was observed in 12 weeks MRKOZG mice, suggesting abnormal transdifferentiation of adrenal cortex cells within the different zones. Conclusion: The identification of the underlying mechanisms of abnormalities observed in MRKOZG mice will allow to better understand cell lineage, differentiation and function of the adrenal cortex in relation to the development of PA.

OR38: SINGLE-NUCLEI ANALYSIS OF ALDOSTERONE-PRODUCING ADENOMA

Masanori Murakami [1], Kazunari Hara [1], Kenji Ikeda [1], Masato Horino [1], Rei Okazaki [1], Yoshihiro Niitsu [1], Akira Takeuchi [1], Jun Aoki [1], Kumiko Shiba [1, 2], Kazutaka Tsujimoto [1], Chikara Komiya [1], Yuki Nakamura [3], Yasuhisa Fujii [3], Tetsuya Yamada [1]

[1] *Molecular Endocrinology and Metabolism,* Tokyo Medical and Dental University, Tokyo, Japan
[2] *The Center for Personalized Medicine for Healthy Aging,* Tokyo Medical and Dental University, Tokyo, Japan
[3] *Urology,* Tokyo Medical and Dental University, Tokyo, Japan

Background: Recent advances in omics techniques have allowed detailed genetic characterization of aldosterone-producing adenoma (APA). Nevertheless, the pathophysiological intricacies of APAs have yet to be fully elucidated at the level of individual cells. Methods: The current study conducted single-nuclei RNA sequencing (snRNA-seq) of APAs and non-functional adenomas. Results: A total of 6,181 nuclei were successfully analyzed, comprising 2,188 from NF and 3,993 from APA. By comparing NF and APA, we identified multiple clusters specific to APA, with the aldosterone synthase gene (CYP11B2) serving as a marker. Pseudotime course analysis suggested that the relationships between these clusters reflect the differentiation status of tumor cells, as indicated by the expression levels of steroid synthesis enzymes.Notably, as the differentiation level increased, there was an accompanying rise in the expression levels of genes involved not only in aldosterone synthesis, such as CYP11B2 and HSD3B2, but also in the induction of cortisol synthesis, including CYP11B1 and CYP17A1, which are steroid synthesis enzyme genes. In contrast, the markers in the less differentiated population included genes associated with the WNT signaling pathway and AMPK signaling transduction pathway. Furthermore, using bulk RNA-seq data from 37 APA cases, we estimated the cluster composition of each case through deconvolution analysis based on snRNA-seq data, revealing heterogeneity in cluster composition even among APAs with KCNJ5 somatic mutations. Conclusions: snRNA-seq provides novel differentiation process of APA tumor, which will further support the understanding of APA pathophysiology.

OR39: MODULATION OF CALCIUM SIGNALING "ON DEMAND" TO DECIPHER THE MOLECULAR MECHANISMS RESPONSIBLE FOR PRIMARY ALDOSTERONISM

Bakhta Fedlaoui [1], Teresa Cosentino [1], Zeina Al Sayed [1], Isabelle Giscos-Douriez [1], Fabio Luis Fernandes-Rosa [1], Jean-Sebastien Hulot [1, 2], Chris Magnus [3], Scott M Sternson [3], Simon Travers-Allard [1, 4], Stephanie Baron [4], Maria-Christina Zennaro [1, 5], Sheerazed Boulkroun [1]

[1] *75015,* PARCC, INSERM U970, Université de Paris, Paris, France
[2] *75015,* CIC1418 and DMU CARTE, Assistance Publique Hôpitaux de Paris (AP-HP), Hôpital Européen Georges Pompidou, Paris, France
[3] *VA 20147,* Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, United States
[4] *75015,* Assistance Publique Hôpitaux de Paris (AP-HP), Hôpital Européen Georges Pompidou, Service de Physiologie, Paris, France
[5] *75015,* Assistance Publique Hôpitaux de Paris (AP-HP), Hôpital Européen Georges Pompidou, Service de Génétique, Paris, France

Primary aldosteronism (PA) is the most frequent form of secondary hypertension. Major advances have been made in our understanding of the disease with the identifications of germline or somatic mutations in ion channels and pumps. These mutations enhance calcium signaling, the main trigger of aldosterone biosynthesis. The objective of our work was to elucidate, using chemogenetic tools, the molecular mechanisms underlying the development of PA by modulating calcium signaling into the cells. We have developed an adrenocortical H295R-S2 cell line stably expressing a chimeric ion channel receptor formed by the extracellular ligand-binding domain of the a7 nicotinic acetylcholine receptor fused to the ion pore domain of the serotonin receptor 5HT3a named a7-5HT3. Its activation by a selective agonist named PSEM-817 leads to sodium entry into the cells and activation of calcium signaling. Treatment of a7-5HT3 expressing cells with increasing concentrations of PSEM-817 induced a significant increase in intracellular calcium concentrations, CYP11B2 mRNA expression, and aldosterone biosynthesis but did not affect neither cell proliferation nor cell death. Interestingly, gene expression analyses and steroid profiles highlighted the activation of different signaling pathways in response to Angiotensin II, K+ and PSEM-817. The exploration of adult mice expressing the a7-5HT3 specifically in the adrenal cortex, generated in our laboratory, is ongoing. These cell line and mouse model, in which we can modulate the

intracellular calcium concentration “on demand”, are useful tools for a better understanding of the alterations of intracellular ion balance and calcium signaling in the pathophysiology of PA.

OR40: THE ASSOCIATION OF ADRENAL STEROIDS ON THE METABOLOMIC DIFFERENCES IN PRIMARY HYPERALDOSTERONISM VS PRIMARY HYPERTENSION

Zoran Erlic [1], Robin Knuchel [1], Sven Gruber [1], Laurence Amar [3, 2], Maria-Christina Zannaro [2, 3], Paolo Mulatero [4], Martina Tetti [4], Alessio Pecori [4], Christina Pamporaki [5], Katharina Langton [5], Filippo Ceccato [6], Aleksander Prejbisz [7], Christian Adolf [8], Hanna Remede [9], Livia Lenzini [10], Michael Dennedy [11], Jaap Deinum [12], Emily Jefferson [13], Graeme Eisenhofer [5], Felix Beuschlein [1, 8]

[1] *Klinik für Endokrinologie, Diabetologie und Klinische Ernährung,* UniversitätsSpital Zürich (USZ) und Universität Zürich (UZH), Zurich, Switzerland
[2] *PARCC, INSERM,* Université de Paris, Paris, France
[3] *Unité Hypertension artérielle,* Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Paris, France
[4] *Division of Internal Medicine and Hypertension Unit, Department of Medical Sciences ,* University of Torino, Torino, Italy
[5] *Medical Clinic III, University Hospital Carl Gustav Carus,* TU Dresden, Dresden, Germany
[6] *UOC Endocrinologia, Dipartimento di Medicina DIMED,* Azienda Ospedaliera-Università di Padova, Padova, Italy
[7] *Department of Hypertension,* National Institute of Cardiology, Warsaw, Poland
[8] *Medizinische Klinik und Poliklinik IV, Klinikum der Universität München,* LMU München, Munich, Germany
[9] *Schwerpunkt Endokrinologie/Diabetologie, Medizinische Klinik und Poliklinik I,* Universitätsklinikum Würzburg, Würzburg, Germany
[10] *Clinica dell'Ipertensione Arteriosa, Department of Medicine-Dimed,* University of Padua, Padova, Italy
[11] *The Discipline of Pharmacology and Therapeutics, School of Medicine,* National University of Ireland, Dublin, Ireland
[12] *Department of Medicine, Section of Vascular Medicine,* Radboud University Medical Center, Nijmegen, Netherlands
[13] *Division of Population Health and Genomics, School of Medicine,* University of Dundee, Dundee, United Kingdom

Introduction: Targeted metabolomics (TM) reveal distinct metabolic patterns in primary aldosteronism (PA) and primary hypertension (PHT). It remains unclear whether these differences are due to hormonal excess or concomitant alterations of other adrenal steroids in PA. We aimed to analyze the impact of aldosterone and other adrenal steroids on discriminating metabolites in PA patients. Patients and Methods: Subgroup analysis of a retrospective European multicenter study, including 99 PA (mean age 48.0 years, 42.4% females) and 58 PHT (mean age 44.8 years, 19% female) patients. TM and adrenal steroid profiling were

performed in each patient. We used 44 metabolites and 12 metabolic indices (MIs) previously identified as discriminators between PA and PHT for statistical analysis. Multiple linear regression models included each metabolite and MI as a dependent variable, with aldosterone, other adrenal steroids, age, sex, BMI, and presence of DM as independent variables. Results: Significant associations were found between 23 metabolites and 6 MIs with adrenal steroids. Only 1 metabolite and two MIs were associated with aldosterone, while cortisone (16 metabolites), cortisol (6 metabolites), and DHEA (8 metabolites) had the most associations with the studied metabolites. Most MIs were associated with cortisone (4 out of 6), androstenedione, and DHEA (3 out of 6 each). Conclusion: Cortisone, cortisol and DHEA, rather than aldosterone, showed the greatest associations with metabolomic differences in PA patients. These findings suggest that the discernible metabolic patterns distinguishing PA from PHT primarily result from the concomitant secretion of cortisone and cortisol, along with other adrenal steroids.

OR41: URINE STEROID METABOLOMICS AS A DIAGNOSTIC TOOL IN ENDOCRINE HYPERTENSION

Alessandro Prete [1, 2], Lida Abdi [1, 3], Onnicha Suntornlohanakul [1, 4], Katharina Lang [1], Julien Riancho [5, 6], Aida Lazkani [7], Casper K. Larsen [5], Anne-Paule Gimenez-Roqueplo [5, 8], Alessio Pecori [9], Martina Tetti [9], Silvia Monticone [9], Lisa M. Müller [10], Christian Adolf [10], HJLM Timmers [11], Stephanie Hampson [13], Graeme Eisenhofer [14, 15], Filippo Ceccato [16], Felix Beuschlein [10, 17], Marek Kabat [18], Jerome Bertherat [19], Conall Dennedy [20], Eleanor Davies [13], Jaap Deinum [11], Martin Reincke [10], Gian Paolo Rossi [21], Paolo Mulatero [9], Laurence Amar [5, 6], Maria-Christina Zennaro [8], Peter Tino [22], Michael Biehl [23, 24], Angela E. Taylor [1], Wiebke Arlt [1, 3]

[1] *Institute of Metabolism and Systems Research,* University of Birmingham, Birmingham, United Kingdom
[2] *NIHR Birmingham Biomedical Research Centre,* University of Birmingham and University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom
[3] *Medical Research Council London Institute of Medical Sciences,* Imperial College London, London, United Kingdom
[4] *Endocrinology and Metabolism Unit, Division of Internal Medicine, Faculty of Medicine,* Prince of Songkla University, Hat Yai, Thailand
[5] *PARCC, INSERM,* Université Paris Cité, Paris, France
[6] *Unité Hypertension artérielle,* Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Paris, France
[7] *Centre d'Investigations Cliniques,* Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Paris, France
[8] *Service de Génétique,* Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Paris, France
[9] *Division of Internal Medicine and Hypertension Unit, Department of Medical Sciences,* University of Torino, Turin, Italy
[10] *Medizinische Klinik und Poliklinik IV, Klinikum der Universität München,* LMU München, Munich, Germany
[11] *Department of Internal Medicine,* Radboud University Medical Center, Nijmegen, Netherlands
[12] *Department of Laboratory Medicine,* Radboud University Medical Center, Nijmegen, Netherlands
[13] *School of Cardiovascular and Metabolic Health, BHF Glasgow Cardiovascular Research Centre,* University of Glasgow, Glasgow, United Kingdom
[14] *Institute of Clinical Chemistry and Laboratory Medicine,* University Hospital Carl Gustav Carus, Dresden, Germany
[15] *Department of Medicine III,* University Hospital Carl Gustav Carus, Dresden, Germany
[16] *UOC Endocrinologia, Dipartimento di Medicina DIMED,* Azienda Ospedaliera-Università di Padova, Padua, Italy
[17] *Klinik für Endokrinologie, Diabetologie und Klinische Ernährung,* UniversitätsSpital Zürich (USZ) und Universität Zürich (UZH), Zurich, Switzerland
[18] *Department of Hypertension,* National Institute of Cardiology, Warsaw, Poland
[19] *Department of Endocrinology, French Reference Center for Rare Adrenal Disorders, Hôpital Cochin,* Université Paris Cité, Institut Cochin, Paris, United Kingdom

[20] *The Discipline of Pharmacology and Therapeutics, School of Medicine,* National University of Ireland, Galway, Ireland
[21] *Internal & Emergency Medicine- ESH Specialized Hypertension Center, Department of Medicine-DIMED,* University of Padua, Padua, Italy
[22] *School of Computer Science,* University of Birmingham, Birmingham, United Kingdom
[23] *Bernoulli Institute for Mathematics, Computer Science and Artificial Intelligence,* University of Groningen, Groningen, Netherlands
[24] *Centre for Systems Modelling and Quantitative Biomedicine,* University of Birmingham, Birmingham, United Kingdom

Background: Identifying secondary causes of hypertension is key to offering targeted treatment. We tested the performance of urine steroid metabolomics (USM), the computational analysis of 24-hour urine steroid metabolome data by machine learning, for diagnosing endocrine hypertension. Methods: Mass spectrometry-based multi-steroid profiling was used to quantify the excretion of 27 steroid metabolites in 24-hour urine samples from 1400 hypertensive adults with and without endocrine causes (351 retrospectively and 1049 prospectively collected). Data were analysed by generalised matrix learning vector quantisation, using the retrospective cohort for training and the prospective for validation. Results: We included 610 patients with primary aldosteronism (PA; 110 retrospective, 500 prospective), 126 with phaeochromocytoma-paraganglioma (PPGL; 82 retrospective, 44 prospective), 83 with Cushing's syndrome (CS; 48 retrospective, 35 prospective), and 581 with primary hypertension (PHT; 111 retrospective, 470 prospective). Of the prospective patients with PHT, 188 had low renin levels. USM demonstrated high accuracy in identifying CS cases (area under the receiver-operating characteristics curve [AUC-ROC] 0.93), which showed higher urinary excretion of glucocorticoid and glucocorticoid precursor metabolites. USM yielded moderate accuracy in differentiating PHT from PA (AUC-ROC 0.73); however, the performance improved when comparing PA cases to low-renin PHT (AUC-ROC 0.86), with the major aldosterone metabolite - 3α,5β-tetrahydroaldosterone - being the most discriminatory. USM could not reliably differentiate PHT from PPGL (AUC-ROC 0.57). Conclusions: Urine steroid metabolomics is a non-invasive candidate test for the accurate diagnosis of hypertension secondary to cortisol and aldosterone excess, and can improve diagnosis and delivery of appropriate treatment in affected individuals.

OR42: INFLAMMATION-BASED SCORES IN BENIGN ADRENOCORTICAL TUMOURS ARE LINKED TO THE DEGREE OF CORTISOL EXCESS

Vittoria Favero [1,2], Alessandro Prete [2, 3], Alessandra Mangone [4], Yasir S Elhassan [2,3], Valentina Pucino [5, 6], Miriam Asia [3], Rowan Hardy [2], Iacopo Chiodini [1, 7], Cristina L Ronchi [2, 3]

[1] *Department of Biotechnology and Translational Medicine,* University of Milan, Milan, Italy
[2] *Institute of Metabolism and Systems Research,* University of Birmingham, Birmingham, United Kingdom
[3] *Department of Endocrinology,* Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom
[4] *Department of Clinical Sciences and Community Health,* University of Milan, Milan, Italy
[5] *Institute of Inflammation and Ageing,* University of Birmingham, Birmingham, United Kingdom
[6] *The Kennedy Institute of Rheumatology, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences (NDORMS),* University of Oxford, Oxford, United Kingdom
[7] *Unit of Endocrinology,* Ospedale Niguarda Cà Granda, Milan, Italy

Objective: Glucocorticoids play a significant role in immune modulation and regulation of inflammation. This is confirmed by multiple haematological alterations observed in patients with Cushing's syndrome (CS). However, little is known in patients with mild autonomous cortisol secretion (MACS). Serum inflammation-based scores (IBS) may reflect systemic inflammation and predict outcomes in several diseases. The aim of this study was to evaluate IBS in benign adrenocortical tumours with different degrees of cortisol excess. Design and Methods: A cohort of 375 patients was retrospectively evaluated: 215 patients with nonfunctioning adrenocortical tumors (NFAT), 138 with MACS (serum cortisol after 1-mg overnight dexamethasone suppression test>50 nmol/L and absence of CS features), and 22 with CS. We evaluated baseline cortisol profile and following IBS: Neutrophil-to-Lymphocyte Ratio (NLR), Lymphocyte-to-Monocyte Ratio (LMR), Platelet-to-Lymphocyte Ratio (PLR), and Systemic Immune-Inflammation Index (SII). Results: We observed a significant increase in leucocytes, neutrophils, and monocytes across the spectrum of cortisol excess, from NFAT over MACS to CS. NLR and SII were significantly higher in both MACS and CS when compared to NFAT ($p<0.001$ and $p=0.002$ for NLR and $p=0.006$ and $p=0.021$ for SII, respectively). Conversely, LMR was lower in MACS and CS than in NFAT ($p=0.01$ and<0.001, respectively), but also in CS compared to MACS ($p=0.007$). Conclusions: NLR, SII and LMR correlated with

the degree of cortisol excess in benign adrenocortical tumours and were altered in both patients with CS and MACS. These findings suggest that an immune dysregulation is present in MACS and may contribute to cardiovascular and metabolic comorbidities.

OR43: THE CARDIOMETABOLIC RISK IN PATIENTS WITH NON-FUNCTIONING ADRENAL INCIDENTALOMA: AN OBSERVATIONAL, RETROSPECTIVE AND PROPENSITY SCORE MATCHED STUDY

Mirko Parasiliti-Caprino [1], Fabio Bioletto [1], Gabriella Tomaiuolo [1], Anna Roux [1], Chiara Lopez [1], Martina Bollati [1], Matteo Procopio [1], Stefano Arata [1], Ezio Ghigo [1], Emanuela Arvat [1], Roberta Giordano [1], Mauro Maccario [1]

[1] *Endocrinology, Diabetes and Metabolism,* University of Turin, Turin, Italy

Introduction. Recent studies found an increased cardiometabolic risk in patients with Non-Functioning Adrenal Incidentaloma (NFAI), but all these data have low quality of evidence. Objective. To establish whether cardiometabolic risk and complications in NFAI patients can be associated to the presence of a non-secreting adrenal tumor, independently from potential confounding factors. Subjects and Methods. In this cross-sectional and retrospective study, all the NFAI patients referred to the University Hospital of Turin, between 2000 and 2022, were consecutively enrolled and compared with subjects not affected by adrenal disease. For each patient, clinical and biochemical data were evaluated, in addition to their cardiovascular (CV) risk scores and cardiometabolic outcomes. Results. A total of 2381 subjects were enrolled in the study (1137 patients with NFAI, including 831 with arterial hypertension and 306 with normal blood pressure values; 1244 non-NFAI, of which 1177 hypertensive patients and 67 normotensives). In the NFAI group, a significant increase of the CV risk was observed exclusively among subjects suffering from hypertension and it was calculated using risk scores (SCORE: 4.93±5.79% vs 3.94±6.77%, p=0.006; Framingham Risk Score: 11.03±9.32% vs 9.45±8.97%, p=0.002; Cuore project: 15.63±15.66% vs 11.46±13.42%, p=0.000). Moreover, the NFAI group presented globally higher AASI (Ambulatory Arterial Stiffness Index) values compared to the subjects with no adrenal mass, both among patients with arterial hypertension (0.47±0.16 vs 0.43±0.14; p=0.022) and among those with normal blood pressure values (0.45±0.14 vs 0.31±0.08; p=0.012). Regarding cardiometabolic complications, at multivariate logistic regression, NFAI proved to be independently associated with aortic ectasia (OR 2.779, 95% CI 1.287-6.001, p=0.009), correcting for age, sex, metabolic syndrome (MS) and previous CV events. To minimize the impact of differences between the NFAI and non-NFAI groups,

propensity score matching (1:1) was used. Even in this analysis, NFAI retained a statistically significant association with aortic ectasia (β=0.074, 95% CI 0.016-0.131, p=0.012). The matching variables were the same covariates applied in the logistic regression. Conversely, no significant associations with MS, type II diabetes, eGFR <60 mL/min/1.73m2, microalbuminuria, atrial fibrillation or hypertensive cardiomyopathy were found. Conclusions. The results of this study suggest the presence of an augmented cardiometabolic risk in patients affected by NFAI. Considering cardiometabolic complications, we described for the first time an association between NFAI and aortic ectasia. If these data will be confirmed in longitudinal studies, NFAI could be considered a condition of high cardiovascular risk and, therefore, patients with this disease could benefit from appropriate cardiometabolic follow-up and treatment.

Guided Poster Tour
(ACC)

PT1: WEE1 KINASE INHIBITOR, ADAVOSERTIB (AZD1775), IS A NOVEL POTENTIAL THERAPEUTIC STRATEGY FOR ADVANCED ADRENOCORTICAL CARCINOMA IN ADDITION TO STANDARD CHEMOTHERAPIES

Mariangela Tamburello [1], Silviu Sbiera [1], Tessa Holmstoen [3], Justus Weber [4], Juliane Lippert [1], Marc Philipp Schauer [1], Margaret E Wierman [3], Otilia Kimpel [1], Laura-Sophie Landwehr [1], Mario Detomas [1], Hanna Urlaub [1], Sonja Steinhauer [1], Constanze Hantel [5, 6], Sandra Sigala [2], Cristina L Ronchi [7], Michael Hudecek [4], Katja Kiseljak-Vassiliades [3], Martin Fassnacht [1], Barbara Altieri [1]

[1] *Division of Endocrinology and Diabetes, Department of Internal Medicine I, University Hospital,* University of Würzburg, Würzburg, Germany
[2] *Department of Molecular and Translational Medicine,* University of Brescia , Brescia, Italy
[3] *Division of Endocrinology, Metabolism and Diabetes, Department of Medicine,* University of Colorado Anschutz Medical Campus, Aurora, United States
[4] *Department of Internal Medicine II, Cellular Immunotherapy,* University Hospital Würzburg, Würzburg, Germany
[5] *Department of Endocrinology, Diabetology and Clinical Nutrition,* University Hospital Zurich , Zürich, Switzerland
[6] *Medizinische Klinik und Poliklinik III,* University Hospital Carl Gustav Carus Dresden, Dresden, Germany
[7] *Institute of Metabolism and System Research,* University of Birmingham, Birmingham, United Kingdom

Background: Adavosertib is a powerful inhibitor of WEE1 tyrosine kinase, which regulates cell cycle checkpoints by enabling DNA damage repair before mitotic entry. WEE1-inhibition could enhance existing DNA damage-based therapies currently used in adrenocortical carcinoma (ACC) care. Methods: WEE1 expression was evaluated by qRT-PCR in five ACC cell lines and adrenocortical tissues (n=19 normal adrenal glands/NAG; n=20 adenomas; n=52 ACC), and by immunohistochemistry in 114 ACC with known TP53 mutation. Adavosertib effect, alone or with cisplatinum or gemcitabine, was evaluated in vitro by cell viability assay and flow cytometry and in vivo in NCI-H295R-xenografted nude mice. Results: WEE1 levels were significantly higher in ACC compared to NAG and low levels were associated with better overall survival (HR=0.32,p=0.02). TP53-mutated ACC showed significantly stronger nuclear staining. In NCI-H295R, JIL-2266 and CU-ACC2 cells adavosertib induced cytotoxicity with IC50 of 1.2, 1.4 and 0.4 μM, respectively. CU-ACC1 and MUC-1 showed less sensitivity to adavosertib alone, but its effect was enhanced by cisplatin and gemcitabine, as found in the

other cell lines. In vivo, anti-tumoral effects were confirmed against NCI-H295R-xenografts. Adavosertib induced an increase in death and apoptotic cells in all cell lines with enrichment of cells in the S-phase. Moreover, a reduction in CDK1 phosphorylation and an increase in phosphorylated H2AX and cleaved PARP were observed. Conclusion: WEE1 is highly expressed in ACC tissues and targetable in ACC cell lines. Adavosertib exerts a cytotoxic effect enhancing in vitro and in vivo the efficacy of cisplatin and gemcitabine, giving a new pharmacological option in ACC.

PT2: TARGETING THE FERROPTOSIS-MACROPHAGE CROSSTALK IN ADRENOCORTICAL CARCINOMA (ACC)

Alexandra Triebig [1], Isabel Weigand [1], Sena Atici [2], Jose Pedro Friedmann-Angeli [2], Matthias Kroiß [1]

[1] *Department of Internal Medicine IV,* LMU Hospital Munich, München, Germany
[2] *Rudolf-Virchow-Center,* Univerity of Würzburg, Würzburg, Germany

Background: In adrenocortical carcinoma (ACC) cells steroid hormone synthesis has been shown to favor ferroptosis, a cell death mechanism characterized by excessive lipid peroxidation. Therapeutic activation of ferroptosis has been proposed as a treatment, but its impact on macrophages, an important component of the tumor immune microenvironment (TIME), is still unclear. Aim: To characterize the mediators and consequences of ferroptosis in ACC cell lines on macrophages. Methods: Prostaglandin E2 (PGE2) secretion in cell culture supernatants was quantified by LC-MS/MS and ELISA. Human peripheral blood mononuclear cells were isolated with Lymphoprep™ gradient and macrophages differentiated with GM-CSF/M-CSF and polarizing factors. Macrophages were characterized by western blotting, immunofluorescence and metabolic phenotyping. Phagocytosis of ferroptotic ACC cells by macrophages was analyzed by flow cytometry. Results: Treatment of the ACC cell line NCI-H295R with the ferroptosis inducer RSL3 resulted in increased secretion of the immune modulator PGE2. The release of PGE2 was completely blocked upon inhibition of both ferroptosis with the antioxidant Liproxstatin-1 and cyclooxygenase (COX) with celecoxib or diclofenac. Treatment of isolated macrophages with PGE2 lead to macrophage polarization towards an anti-inflammatory M2-like phenotype, which was characterized by high expression of CD163. Co-culturing of these macrophages with RSL3 treated NCI-H295R cells resulted in efficient clearance of ferroptotic ACC cells. Conclusion: Ferroptosis induction with RSL3 leads to the release of PGE2, an immune suppressor involved in M2-like polarization. ACC cells undergoing ferroptosis are phagocytosed by M2-like macrophages, which is expected to further maintain an anti-inflammatory TIME.

PT3: TOWARDS AN UNDERSTANDING OF THE MICROENVIRONMENT OF ACC: IMPACT OF STEROID HORMONES AND DRIVER PATHWAYS

Tanja Maier [1], Laura-Sophie Landwehr [2], Matthias Kroiß [1, 2], Martin Fassnacht [2]

[1] *Med4,* LMU Klinikum Muinch, Munich, Germany
[2] *Med1,* University Hospital Würzburg, Würzburg, Germany

Background: Immune checkpoint therapy response rate in adrenocortical carcinoma (ACC) is only ~15%. Glucocorticoid (GC) secretion is present in ~60% of tumours, associated with adverse outcome and has been associated with an immunologically cold tumoural microenvironment. On the other hand, activation of the Wnt/ß-Catenin pathway has been suggested to contribute to reduced immune infiltration. The aim of this work is to understand mechanisms limiting the response to immunotherapy and ultimately to overcome resistance. Aims: First, we aim to improve the understanding of cellular responses to glucocorticoid receptor (GCR) activation and antagonism and additionally understand the effect of tumoural steroid excess onto glucocorticoid related genes. Second, we hypothesize that activation of the Wnt/ß-Catenin pathway is associated with less tumor infiltrating immune cells. Methods: Cultured ACC cell lines NCI-H295R and JIL-2266 were treated with the selective GCR antagonist relacorilant and expression of GCR target genes analyzed by qPCR. Nanostring NCounter was used on FFPE extracted RNAs of hormonally active and inactive tumours. Cell viability was measured by CellTiter GloAssay and protein expression quantified by Western blotting for the glucocorticoid receptor (GR) and the cancer-testis antigen PRAME. Results: Hormonally active tumours showed a downregulation of immune-related genes and an upregulation of glucocorticoid related genes. Up to 1 µM relacorilant in combination with 400 nM hydrocortisone had no effect on ACC cell viability, but expression of GCR target genes was significantly altered in a cell line specific manner. In NCI-H295R cells, the expression of CYP17A1 was dose-dependently repressed by relacorilant, while genes encoding the IL1 receptor were consistently up-regulated. PRAME was expressed in NCI-H295R and repressed by relacorilant. Conclusion: Treatment with relacorilant leads to a downregulation of glucocorticoid related genes like CYP17A1 in NCIH295R and an upregulation in immune related genes like IL1R1. This might lead to lower steroid levels and higher immune cell infiltration and contribute to a better response to the immunotherapy.

PT4: ASSESSING MONOCYTE MIGRATION AND CO-CULTURE WITH ADRENOCORTICAL CANCER CELLS

Cong Hong [1], Constanze Hantel [2], Michael Conall Dennedy [1]

[1] *Discipline of Pharmacology and Therapeutics, School of Medicine, University of Galway, Ireland,* University of Galway, Ireland, Galway, Ireland
[2] *Endocrinology Research Laboratory Clinic for Endocrinology, Diabetology and Clinical Nutrition,* University of Zurich, Zurich, Switzerland

Adrenocortical carcinoma (ACC) is a lipid, typically steroidogenic cancer which carries a 5-year prognosis of <10%. Therapeutic options are limited including surgery, where possible and mitotane – a poorly tolerated and efficacious insecticide. Recent data have demonstrated that the immune environment of adrenocortical carcinoma is deficient in lymphocytes while demonstrating a relative rich monocyte/macrophage population in the tumour microenvironment. We have investigated the gradient for migration of circulating peripheral monocytes to ACC. ACC cells were seeded in the bottom chamber of the transwell system. Monocytes were isolated from human peripheral blood, by negative selection using magnetic beads with high purity (>95%) and quality. Migration of monocytes at 24/48h was evaluated by identifying those remaining in the top chamber, the floating fraction in the bottom chamber and those adherent to ACC cells in the bottom chamber. Analysis of monocyte/macrophages was undertaken by flow cytometry using the following markers: Sytox Blue, CD45, CD14, CD16 and HLA-DR. At 24h, when compared to control conditions, more monocytes had migrated to the ACC cells seeded lower chamber and attached to the bottom [HAC15(298.3; $p<0.0001$), followed by MUC1(190.5; $p<0.001$) and H295R (240.2; $p<0.0001$)]. Similar results were observed in migration at 48h. Predominantly Classical monocytes (CD14++CD16-HLA-DR+) migrated to metastatic cancer cells (MUC1), and Non-Classical monocytes (CD14+CD16+HLA-DR+) migrated to primary cancer cells (H295R/ HAC15) at 48h. We demonstrate monocytes migrate and associate with ACC cells in 24-48h which requires more detailed analysis of (i) monocyte polarization and (ii) ACC cells chemokine milieu following monocyte migration.

PT5: HIGH FILAMIN A EXPRESSION IN ADRENOCORTICAL CARCINOMAS IS ASSOCIATED WITH A FAVOURABLE TUMOR BEHAVIOUR: A EUROPEAN MULTICENTRIC STUDY

Erika Peverelli [1, 2], Rosa Catalano [1], Emanuela Esposito [1, 3], Emma Nozza [1, 3], Cristina Lucia Ronchi [4, 5], Barbara Altieri [5], Michaela Luconi [6], Anna Angelousi [7], Giorgio Croci [8], Francesca Bravi [1], Serena Palmieri [2], Sofia Frigerio [2], Emanuele Ferrante [2], Maura Arosio [1, 2], Giovanna Mantovani [1, 2]

[1] *Department of Clinical Sciences and Community Health,* University of Milan, Milan, Italy
[2] *Endocrinology Unit,* Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[3] *PhD Program in Experimental Medicine,* University of Milan, Milan, Italy
[4] *Institute of Metabolism and System Research,* University of Birmingham, Birmingham, United Kingdom
[5] *Division of Endocrinology and Diabetes, Department of Internal Medicine I,* University Hospital, University of Würzburg, Würzburg, Germany
[6] *Endocrinology Unit, Department of Experimental and Clinical Biomedical Sciences "Mario Serio",* University of Florence, Florence, Italy
[7] *Department of Internal Medicine,* Laikon General Hospital, Medical School, National and Kapodistrian University of Athens, Atene, Greece
[8] *Pathology Unit,* Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

The overexpression of insulin-like growth factor 2 (IGF2), frequently found in adrenocortical carcinomas (ACC), promotes cell growth by overactivating IGF system in an autocrine loop. The cytoskeleton protein filamin A (FLNA) acts as a repressor of IGF2 signalling in ACC cells and is involved in cell proliferation and response to insulin-like-growth factor 1 receptor (IGF1R) inhibitors. The aims of this study were to test the expression of FLNA in a large cohort of ACC and in adrenocortical adenomas (ACA) by immunohistochemistry and to evaluate the relationship between FLNA expression and clinicopathological features and outcome of patients with ACC (sex, age, hormone production, ENSAT stage, ki67, Weiss score, resection status, S-GRAS score, therapy, overall, progression-free and disease specific survival). We found that the majority of ACC (71.4%) did not express FLNA, whereas FLNA loss was a rare event in ACA (15.4%, $p<0.001$ vs ACC). In addition, the analyses showed a correlation between the expression of FLNA and a less aggressive tumor behaviour in ACC. Indeed, the subgroup of ACC with FLNA positive cells >5% ("high FLNA") showed lower ENSAT stage, Weiss score, resection status and S-GRAS score compared to those with FLNA positive cells ≤

5 called “low FLNA” ($p<0.05$). Moreover, patients belonging to high FLNA group had a longer overall survival and disease specific survival than those lacking FLNA ($p<0.05$). In conclusion, our data support a protective role for FLNA in ACC, suggesting that FLNA could be a potential molecular marker for the diagnosis and prognosis prediction in ACC.

PT6: INVESTIGATING THE ROLE OF DEUBIQUITINASES IN ADRENOCORTICAL CARCINOMA

Carmina Teresa Fuss [1, 2], Victoria Marnet [1, 2], Oliver Hartmann [1], Michaela Reissland [1], Nikolett Pahor [1], Laura-Sophie Landwehr [2], Martin Fassnacht [2], Markus Elmar Diefenbacher [1]

[1] *Department of Biochemistry and Molecular Biology,* Protein Stability and Cancer Group, Würzburg, Germany
[2] *Department of Internal Medicine I,* Division of Endocrinology and Diabetes, Würzburg, Germany

Adrenocortical carcinoma (ACC) is a rare endocrine malignancy presenting with an incidence of one per million per year and an overall 5-year survival rate under 35%. Currently, curative treatment is limited to full surgical resection, while the adrenolytic drug mitotane remains the only approved medical therapy option leaving a huge demand for innovative therapeutic strategies. Genetic alterations observed in ACC commonly lead to activation of Wnt/β-Catenin signaling, most frequently caused by mutations in the CTNNB1 gene encoding for the nuclear effector β-Catenin. The key players of Wnt/β-Catenin signaling are known to be under tight control of the ubiquitin-proteasome-system. However, the role of deubiquitinases (DUBs) regarding β-Catenin regulation in ACC remains unknown. Analyzing publicly available data, we preselected potentially relevant DUBs linked to patient survival in ACC and Wnt-Signaling. To confirm interesting targets, we performed quantitative PCR in six established ACC cell lines leading us to further investigation of USP10. We observed elevated protein abundance in primary tumor tissue compared to the normal adrenal gland as well as a high portion of USP10 in its active state across all ACC cell lines. Continuing the characterization of USP10 expression and localization, we performed immunohistochemical stainings on Tissue Micro Arrays (TMAs). Subsequential quantification of the TMAs incorporating a manually trained algorithm to distinguish tumor and surrounding tissue confirmed significantly higher USP10 expression in ACC as well as endocrine inactive adenoma compared to normal adrenal gland. Treatment with a Pan-DUB inhibitor, the proteasome inhibitor Bortezomib and a USP10-specific inhibitor led to a reduction of cell growth in ACC cell lines. In summary, we so far found strong indication for dysregulation of USP10 in adrenocortical tumors, therefore making it potential therapeutic target in ACC.

PT7: EXPERT CONSENSUS: RECOMMENDATION FOR MITOTANE TREAMENT IN PEDIATRIC ACC

Maria Riedmeier [1], Martin Fassnacht [2], Verena Wiegering [1]

[1] *Department of Pediatrics, Division of Pediatric Hematology, Oncology and Stem cell transplantation, ,* University Hospital, University of Wuerzburg, Würzburg, Germany
[2] *Department of Medicine, Division of Endocrinology and Diabetes,* University Hospital, University of Wuerzburg, Würzburg, Germany

On behalf of the pediatric working groups ENSAT-PACT and ICPACT: Mitotane plays an important role in the treatment of pediatric ACC, but experience with the drug is still limited in the pediatric age group. Adverse effects are common, wide-ranging, and potentially life-threatening. Treating guidelines for pediatric patients have not been established yet. Thus, an international panel of pediatric experts from all over the world was assembled, and the Delphi method was used to reach a consensus on over 40 statements regarding therapy indication, management and handling of side effects. The goal of this approach was to improve and standardize therapy of pediatirc ACC. Results of this recommendation will be presented on the conference.

Guided Poster Tour
(NAPACA)

PT8: HDAC2 REGULATED PATHWAYS IN HYPERCORTISOLIC ADRENAL PATHOLOGY

Sharmilee Vetrivel [1], Ru Zhang [1], Marleen Schobel [2], Ali Khan [2], Martin Reincke [1], Silviu Sbiera [2], Anna Riester [1]

[1] *LMU Klinikum, Ludwig-Maximilians-University,* Endocrinology Medizinische Klinik und Poliklinik IV , Munich, Germany
[2] *Department of Internal Medicine I,* Division of Endocrinology and Diabetes University Hospital, University of Würzburg, Würzburg, Germany

Genome wide analysis focused on epigenetic regulators has not been explored extensively in adrenal tumors. Among the histone deacetylases, HDAC2 showed differential expression in adrenal samples of patients with Cushing's syndrome. Therefore, HDAC2 role is extensively analysed in the study. HDAC2 based ChIP Seq was performed in frozen adrenal tissue samples of patients with cortisol producing adrenocortical carcinoma, ACC(n=10), Cortisol Producing Adenoma, CPA (n=11) and Primary Bilateral Macronodular Hyperplasia, PBMAH (n=9). Controls included normal adrenal tissue samples from patients who underwent kidney surgery (n=10[AR1]). Peak calling in comparison to the respective input controls (no antibody enrichment) were done. HDAC2 was found to be enriched primarily in the distal intergenic regions, in all the samples. Increased HDAC2 peaks were found in in ACC (average number of peaks, n=3673) in comparison to CPA (n=370), PBMAH (n=439) and controls (n=380). Pathway mapping based on the HDAC2-enriched genes identified neuronal system pathways to be enriched in both control and ACC samples. Interestingly, controls also showed enrichment of pathways related to NMDA signaling, which was lost in ACC. Comparatively, pathways related to potassium channels and cGMP were enriched in ACC. The sequencing data is being compared with transcriptomics from the same samples to identify the nature of the HDAC2 bound regions (activation or repression). In addition, bioinformatic analyses are being done to characterize the motifs of shared molecular targets between different biological samples. ChIP Seq analyses has enabled characterizing distinct genetic signatures of HDAC2 among the patient cohorts.

PT9: IN VITRO ANALYSIS OF MIRNA BASED REGULATION IN CUSHING'S SYNDROME

Xiaohui Xia [1], Sharmilee Vetrivel [1], Ru Zhang [1], Martin Reincke [1], Silviu Sbiera, [2], Anna Riester [1]

[1] *Medizinische Klinik und Poliklinik IV,* LMU Klinikum, Ludwig-Maximilians-University, Munich, Germany
[2] *Department of Internal Medicine I, Division of Endocrinology and Diabetes,* University Hospital, University of Wurzburg, Wurzburg, Germany

MicroRNAs (miRNAs) are non-coding RNA molecules with diverse cellular regulatory functions including adrenal steroidogenesis. Our preliminary work based on miRNA-next generation sequencing characterized the alterations of miRNA profiles in adrenal samples from differenr CS subtypes, then QPCR validation in other CS subtypes identified potential miRNA candidates with specific roles in controlling cortisol production. This study we focused on role of miRNAs with significant differences from our NGS and QPCR analyses (miR-1247-5p, miR-379-5p, miR-363-5p, miR-486-5p, miR-551-3p miR-144-5p, miR-144-3p, miR-132-3p, miR-486-5p). These miRNAs were tested for their effect on cortisol production via transfected in various adrenocortical cell lines (NCI-H295R, JILL-2266, CU-ACC1). Initial evaluation found that in the NCI-H295R cell line, cortisol productions significantly decreased when treated with miRNA-132-3p (15.24±1.11ng/ml, -35.8%), miR-486-5p (17.32±0.48ng/ml, -27.0%), miR-144-3p(17.93±2.76ng/ml, -24.4%) compared to the controls (23.73±1.18ng/ml, $p<0.05$). Consecutively, target mining of the candidate miRNAs using miRTarBase and TargetScan identified various target genes of the miRNAs, then assessed in CS adrenal samples from our previous studies. The analyses identified the target genes of miR-144-3p: MYCN (log2FC=3.897, pval=2.075E-05), ETS1(log2FC=1.457, pval=3.163E-06), miR-486-5p: ARHGAP5 (log2FC=0.610, pval=0.0283) and miR-132-3p: RPH3A (log2FC=0.796, pval=8.074E-05), SPRY (log2FC=1.399, pval=2.28E-04) to be significantly upregulated in CS samples compared to the controls. QPCR analyses of the identified target genes in the transfected cell lines are in progress. Positive miRNA candidates from the in vitro analyses will be further validated and explored via LC-MS/MS and QPCR of target genes. our study presents an extensive characterization of miRNA in cortisol production and helps to understand the fine-tuning of adrenal stress response.

PT10: ARMC5 REGULATES SIRT1 EXPRESSION IN ADRENOCORTICAL CELLS

Annabel Berthon [1, 2], Fabio Faucz [2], Benjamin Feldman [2], Ludivine Drougat [2], Stephanie Espiard [2], Isadora Cavalcante [1], Bruno Ragazzon [1], Jérome Bertherat [1], Constantine Stratakis [2, 3, 4]

[1] *Endocrinology, metabolism,* Institut Cochin , Paris, France
[2] *Section on endocrinology & genetics,* NICHD, NIH , Bethesda, United States
[3] *Pikermi,* Research Institute, ELPEN, Athens , Greece
[4] *Human Genetics & Precision Medicine,* IMBB, FORTH, Heraklion, Greece

Pathogenic ARMC5 variants are the main genetic cause of Primary Bilateral Macronodular Adrenal Hyperplasia (PBMAH) explaining roughly 20% of index cases. As little was known on ARMC5 function, we investigate the consequences of Armc5 up- and down-regulation in transient zebrafish models using an RNAseq analysis. We then identified alterations of several members of SIRT1 signaling and confirm that there is a significant increase of SIRT1 protein in PBMAH tissues mutated for ARMC5. As ARMC5 has been described as an adaptor for Cullin 3 ubiquitination complex in 2022, our data suggest that SIRT1 degradation could be dependent on its ubiquitination by the Cullin3-ARMC5 complex. Accordingly, we demonstrate that ARMC5 interacts with SIRT1 in the H295R human adrenocortical cells and that ARMC5 knockdown leads to a decrease of SIRT1 ubiquitination. Although there is an elevation of SIRT1 protein in ARMC5 mutated PBMAH, we observed a decrease in SIRT1 activity in these tissues as well as in adrenal cells of 18-month-old Armc5+/- mice presenting an elevation of plasma corticosterone level. This reduction of SIRT1 activity leads then, to an abnormal protein acetylated profile that could play a role in the development of PBMAH and in the cortisol hypersecretion. Altogether, these data support that ARMC5 could regulate SIRT1 protein accumulation (possibly through Cullin3 complex) but could also regulate its activity by a mechanism that remains to be determined.

PT11: ACTH RESPONSES DURING THE HUMAN CORTICOTROPIN-RELEASING HORMONE STIMULATION TEST CAN PREDICT BIOCHEMICAL REMISSION AFTER UNILATERAL ADRENALECTOMY IN PRIMARY BILATERAL MACRONODULAR ADRENAL HYPERPLASIA

Irene Tizianel [1, 2], Mario Detomas [3], Timo Deutschbein [3, 4], Martin Fassnacht [3], Nora Albiger [5, 6], Carla Scaroni [1, 2], Filippo Ceccato [1, 2]

[1] *Department of Medicine DIMED, Padova, Italy,* Endocrine Unit, Padova, Italy
[2] *University-Hospital of Padova, Italy,* Endocrine Unit, Padova, Italy
[3] *Department of Internal Medicine I, Division of Endocrinology and Diabetes, Würzburg, Germany,,* University Hospital, University of Würzburg, Würzburg, Germany,, Würzburg, Germany
[4] *Medicover Oldenburg MVZ,, Germany,* Medicover Oldenburg MVZ, Oldenbur, Germany, Oldenbur, Germany
[5] *Radiotheray Unit,* Istituto Oncologico Veneto;, Padova, Italy
[6] *ULSS6 Euganea, Padova, Italy,* Endocrinology, Padova, Italy

Purpose Primary bilateral adrenal hyperplasia (PBMAH) is associated with hypercortisolism and a heterogeneous clinical expression in terms of cortisol secretion and related comorbidities. Historically, treatment of choice was bilateral adrenalectomy (B-Adx), however, recent data suggest that unilateral adrenalectomy (U-Adx) may be an effective alternative. For the latter, factors predicting the postsurgical outcome (e.g. biochemical control) have not been identified yet. Methods PBMAH patients undergoing U-Adx for overt Cushing's Syndrome (CS) in two tertiary care centers were retrospectively analysed. Remission was defined as a normalization of urinary free cortisol (UFC) without the need for medical treatment. The potential of hCRH test as a predictor of U-Adx outcome was evaluated in a subgroup. Results 23 patients were evaluated (69 % females, mean age 55 years). Remission rate after U-Adx was 74% at last follow up (median 115 months from UAdx). Before U-Adx, a positive ACTH response to hCRH (Δ%ACTH increase > 50% from baseline) was associated with higher remission rates. Conclusion Three of four patients with PBMAH are surgically cured with U-Adx. Pre-operative hCRH testing can be useful to predict long-term remission rates.

PT12: HIDDEN CORTISOL EXCESS IN PATIENTS WITH TYPE 2 DIABETES AND OBESITY (DOCOR STUDY)

Valentina Morelli [1], Alberto Giacinto Ambrogio [1], Vittoria Favero [2], Chiara Parazzoli [2], Marina Croci [1], Antonio Conti [1], Alberta Dall'Antonia [2], Sabrina Corbetta [1, 3], Carmen Aresta [1], Iacopo Chiodini [2, 4]

[1] *Department of Endocrine and Metabolic Diseases,* IRCCS Istituto Auxologico Italiano, , Milan, Italy
[2] *Department of Medical Biotechnology and Translational Medicine,,* University of Milan,, Milan, Italy
[3] *Department of Biomedical, Surgical and Dental Sciences, ,* University of Milan, Milan, Italy
[4] *Unit of Endocrinology,* Ospedale Niguarda Cà Granda, Milan, Italy

Background: Mild hypercortisolism has been associated with an increased prevalence of osteoporosis, hypertension, type 2 diabetes (T2D), and obesity. In patients with the aforementioned diseases, hypercortisolism may remain occult (hidden hypercortisolism, HidHyCo). The HidHyCo prevalence is around 3% and 1% in diabetic and obese patients respectively. We report preliminary data of a study assessing the HidHyCo prevalence in T2D patients and in obese non-T2D patients and the clinical characteristics more frequently associated with the HidHyCo presence. Methods: HidHyCo was diagnosed if cortisol levels after 1mg-dexamethasone suppression test (F-1mgDST) were >50 nmol/L, confirmed by cortisol levels after 2+2mgDST. Results: So far 87 obese non-T2D patients (Group 1, age 51.7±13.5 yrs, BMI 37.9±6.4 kg/m2) and 40 T2D patients (Group 2, age 65.4±6.8 yrs, BMI 31.8±6.8 kg/m2) were enrolled. One patient in Group 1 and 10 patients in Group 2 showed increased F-1mgDST levels. HidHyCo prevalence was confirmed in 1.1 % and 7.5% in Group 1 and Group 2, respectively. All the HidHyCo patients (n=4) were taking multiple anti-hypertensive drugs. Subsequent evaluations are ongoing to define the origin of HidHyCo. After excluding HidHyCo patients, F-1mgDST was positively associated with age ($r= 0.527$, $p<0.001$), glucose levels ($r=0.313$, $p=0.01$) and indirectly associated with BMI ($r= -0.282$, $p<0.05$). F-1mgDST values were higher in Group 2 compared with Group 1 (Group1 0.85±0.28, Group2 1.37±0.49, $p<0.001$), even after adjusting for age. Conclusions: Preliminary data suggest a higher prevalence of HidHyCo in T2D patients than previously described and a correlation between cortisol secretion and glycometabolic control in eucortisolemic subjects.

PT13: ADRENAL INCIDENTALOMAS AND PSYCHOMETRIC ASPECTS

Chrysoula Mytareli [1], Evangelia Andreadaki [1], Foteini Thanasoula [1], Vasiliki Siampanopoulou [1], Konstantinos Toutouzas [2], Demosthenes Panagiotakos [3], Anna Angelousi [1]

[1] *Department of Endocrinology, First Department of Internal Medicine,Laikon General Hospital, Endo-ERN centre for adrenal disease,* Athens Medical School, NKUA, Athens, Greece
[2] *First Department of Cardiology, Hippokration General Hospital,* Athens Medical School, NKUA, Athens, Greece
[3] *Department of Nutrition and Dietetics,* School of Health Sciences and Education, Harokopio University, Athens, Greece

Introduction Neuropsychiatric disorders are prevalent in patients with hypercortisolism, whereas data are scarce in patients with adrenal incidentalomas (AIs). The aim of our study is to assess the psychological aspects of patients with AIs. Methods Fifty-six patients with AIs (n=28 with non-functioning AIs (NFAIs) and n=28 with autonomous cortisol secretion (ACS) based on the post-1mg overnight dexamethasone suppression test (ODST) cortisol levels (F)) and 51 age-and sex-matched controls (without AIs in computed tomography) followed in our center were enrolled. The clinical and hormonal data were associated with data from validated generic questionnaires; the European Quality of Life (EuroQoL-5D-5L) and the Depression, Anxiety, Stress scale (DASS-21). Results The prevalence of metabolic complications did not differ significantly between the groups. The median scores of the overall DASS, especially the depression and anxiety subscales, were higher in AI patients compared with controls (32 vs 22, 10 vs 4, 8 vs 4, p=0.01/0.01/0.02 respectively) whereas no significant difference was observed between NFAIs and ACS. ACS patients with post-1mg ODST F>3 μg/dL (n=14) presented higher total DASS than those with post-1mg ODST F:1.8-3 μg/dL (49.6 ± 28.4 vs 28.9 ± 22.7, p=0.04). The impaired anxiety-depression EuroQoL domain validated these results (64.7% vs 13.6% reported moderate-extreme anxiety/depression-related problems, p=0.05). The post-1mg ODST F was positively correlated with both scales in ACS group (rs=0.41, p=0.03). Conclusion Our study confirmed that AI patients

PT14: SAFETY OF ADRENALECTOMY IN ELDERLY PATIENTS: A RETROSPECTIVE MATCHED CASE-CONTROL STUDY

Charlotte L. Vietor [1, 2], Inge S. van Egmond [1, 2], Gaston J.H. Franssen [1], Richard A. Feelders [2], Tessa M. van Ginhoven [1]

[1] *Department of Surgical Oncology and Gastrointestinal Surgery,* Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, Netherlands
[2] *Department of Internal Medicine, division of Endocrinology,* Erasmus University Medical Center , Rotterdam, Netherlands

Background: Adrenal masses have become more prevalent over the years. Given the association of these masses with advancing age, the decision to perform surgery in older, sometimes asymptomatic patients presents a clinical dilemma. These patients are potentially more vulnerable to adverse postoperative outcomes due to increased frailty. Therefore, this study aimed to compare the postoperative course after adrenalectomy in patients aged 70 years and older to that of a younger cohort. Methods: This retrospective case-control study included patients aged ≥70 years who underwent adrenalectomy between 2000-2020, and propensity-score matched younger controls (<70 years). Patients were matched based on hormonal overproduction, malignant diagnosis, surgical approach and year of surgery. Results: The study included 77 elderly patients (median age 74 years) and 77 controls (median age 52 years; p<0.001). Serious complications (Clavien-Dindo ≥3) occurred in 9.1% of elderly patients and 6.5% of controls (p=0.773). The overall complication rate was 44.2% in both elderly and controls (p=1.000), with similar duration of hospital admission and mortality in both groups. Elderly patients experienced mostly infectious (32.4%) or cardiovascular complications (20.3%). Open surgery was associated with higher odds of developing complications (OR 7.65, 95%CI 2.26-30.96; p=0.002) in the elderly, whereas malignant diagnosis and American Society of Anesthesiologists (ASA) classification ≥3 were not. Conclusion: Patients aged 70 years and older who undergo adrenalectomy have a similar postoperative course and complication rate as younger patients, with most postoperative complications being minor, and mortality being minimal. Therefore, older age itself should not be a reason to avoid adrenalectomy.

Guided Poster Tour
(PPGL)

PT15: ACTIVATION OF THE INTEGRATED STRESS RESPONSE IN SDHX KNOCK-OUT CELLS

Grace Salsbury [1], Charlotte Hall [1], Eugenie. S Lim [2, 1], Scott. A Akker [2, 1], J. Paul Chapple [1]

[1] *Endocrinology,* WHRI, Queen Mary University of London, London, United Kingdom
[2] *Endocrinology, St Bartholomew's Hospital,* Barts Health NHS Trust, London, United Kingdom

Mutations in each of the 4 subunits of the succinate dehydrogenase (SDH) enzyme – SDHA, SDHB, SDHC and SDHD (collectively SDHx) – predispose carriers to developing phaeochromocytomas and paragangliomas (PPGLs). We used CRISPR/Cas9 to generate isogenic SDHx knock-out (KO) cell lines to investigate the cellular consequences of loss of specific subunits of SDH and potentially identify differences that may underlie the heterogeneous clinical presentations of SDHx disease. Transcriptomic and metabolomic characterisation of these models confirmed alterations in gene expression and metabolic rewiring consistent with predicted tumour phenotypes. The capacity of tumour cells to undergo alterations in their transcriptome and metabolome is in part dependent on adaptive stress responses. Key amongst these is the integrated stress response (ISR) which, in tumours, acts to promote translation of proteins for tumour cell survival, migration and immune escape. We analysed this pathway in our SDHx KO models and observed increased expression of both the ISR transcription factor - Activating Transcription Factor 4 (ATF4) – and its downstream target asparagine synthetase (ASNS) in Sdhb and Sdhd KO cells. ASNS allows cells to synthesise asparagine de novo and an increase in its expression in Sdhb and Sdhd KO cells was consistent with increased asparagine synthesis observed in these models by metabolomic tracer analysis. In summary, we show evidence that the ISR is activated in Sdhb and Sdhd KO cells; this is of interest as the ISR has been identified as a target for therapeutic intervention in cancer.

PT16: AN IMMUNOHISTOCHEMICAL TOOL TO DETECT OR VALIDATE VARIANTS IN THE NF1 GENE IN PHEOCHROMOCYTOMAS

Julie Sanceau [1], Robert Ntihinyuzwa [1], Maria Montegudo [2], Margo Dona [3], Nicole Bechmann [4], Mercedes Robledo [2], Henri Timmers [3], Anne-Paule Gimenez-Roqueplo [1, 5], Alexandre Buffet [1, 5], Nelly Burnichon [1, 5], Judith Favier [1]

[1] *PARCC,* Université Paris Cité, Inserm, Paris, France
[2] *Hereditary Endocrine Cancer Group,* Spanish National Cancer Research Centre (CNIO) , Madrid, Spain
[3] *Department of Internal Medicine ,* Radboud University Medical Center, Nijmegen, Netherlands
[4] *Institute of Clinical Chemistry and Laboratory Medicine,* Technische Universität Dresden, Dresden, Germany
[5] *Service de médecine génomique des tumeurs et des cancers,* AP-HP, Hôpital Européen Georges-Pompidou, Paris, France

About 70% of Pheochromocytoma and Paraganglioma (PPGL) are genetically determined, half being caused by germline mutations in a predisposing gene, and the other half by somatic defects. Immunohistochemistry (IHC) was previously reported as an easy and useful approach to detect patients harboring SDHx or VHL mutations. Nevertheless, functional tools to validate other genes variants in PPGLs are lacking. Here, we tested an antibody to neurofibromin 1 (NF1) proteins in an important cohort of PPGL tissues from 4 ENSAT centers (Paris, Madrid, Nijmengen and Dresden). This collection included 99 samples harboring different types of mutations: 56 NF1, 17 from Cluster 1, 20 from Cluster 2 and 6 with no mutation identified. We addressed whether NF1 immunostaining correlated with the mutational status in the respective genes, at somatic or germline levels. Preliminary results show that NF1 IHC has a good specificity and sensitivity to detect NF1 gene variants. Double-blinded validation by a second observer is ongoing. If validation, this new tool could be added to the current panel of antibodies used for genetic variants validation in PPGL.

PT17: IMMUNOHISTOCHEMICAL ANALYSIS OF SOMATOSTATIN RECEPTOR 2, HIF-2A AND THE TUMOR MICROENVIRONMENT IN METASTATIC PHEOCHROMOCYTOMA AND PARAGANGLIOMA

Masaki Uchihara [1], Akiyo Tanabe [1], Akiko Miyagi Maeshima [3], Yuki Kojima [2], Tasunori Shimoi [2], Kazuki Sudo [2], Yuto Yamazaki [4], Kan Yonemori [2], Hiroshi Kajio [1]

[1] *Diabetes, Endocrinology, and Metabolism,* National Center for Global Health and Medicine, Shinjuku-ku, Tokyo, Japan
[2] *Medical Oncology,* National Cancer Center, Chuo-ku, Japan
[3] *Pathology,* National Cancer Center, Chuo-ku, Japan
[4] *Pathology,* Tohoku University, Sendai, Japan

Pheochromocytoma and paraganglioma (PPGL) are rare endocrine tumors with few effective treatment options for malignant cases. Novel therapies, including Lu-DOTA-TATE, HIF-2α inhibitors, and immune checkpoint inhibitors (ICIs) for patients with PPGL, have been investigated in several clinical trials. Concerning the predictive marker of these treatments, emerging evidence shows that somatostatin receptor 2 (SSTR2) and HIF-2α in other cancer types correlate with the tumor microenvironment (TME) activation and could be prognosis biomarkers. However, the association between the expression of these markers and clinical features in PPGL has not yet been clarified. We studied the immunohistochemical expression of HIF-2α, SSTR2A, and the immune cells in 53 patients with PPGL in two national center hospitals in Japan. The cohort included 28 metastatic diseases with a mean follow-up duration of 81.1 ± 82.6 months. SSTR2A expression was strongly increased in 19 samples. The expression of SSTR2A and HIF-2α were significantly associated with SDHB-IHC negative ($p=0.0110$, $p<0.0001$, respectively). Regarding prognosis, high SSTR2A expression was observed in metastatic tumors than in non-metastatic tumors ($p=0.2357$). SSTR2A was expressed independently of TME status except for M2-polarized TAMs (ratio of CD163+/CD68+) in patients with metastatic disease. No correlation was found between HIF-2α expression and TME markers. In conclusion, SSTR2A could be a prognostic marker for PPGL and essential in further clinical investigations. Whereas, since SSTR2A and HIF-2α were expressed mostly independently of TME status, immunohistochemical analysis of each marker was considered helpful for treatment selection for Lu-DOTA-TATE, HIF-2α inhibitors, and ICIs.

PT18: CANINE ADRENOMEDULLARY AND PHEOCHROMOCYTOMA ORGANOIDS: AN IN VITRO MODEL TO EXPLORE NEW TREATMENT OPTIONS

Marit van den Berg [1], Elpetra Timmermans-Sprang [1], Monique van Wolferen [1], Hans Kooistra [1], Sara Galac [1]

[1] *Clinical Sciences,* Faculty of Veterinary Medicine, Utrecht, Netherlands

Background: Given the current lack of effective medical treatment for pheochromocytomas (PCCs), there is a clear need for an in vitro model to explore new treatment options. Organoids are self-organizing, self-renewing, three-dimensional cellular structures that closely resemble the organ or tumour they originate from. As such, organoid cultures can constitute a valuable disease modeling and drug screening platform. Aim: We aimed to establish and characterize organoid cultures of canine normal adrenal medullas and PCCs. Methods: Normal adrenal tissues and PCC tumor tissues were cultured in a 3D matrix (basement membrane extract) with medium containing specific growth factors. Organoids were characterized using histology, immunohistology, and qPCR analysis. Results: We established four adrenomedullary organoid lines, which could be passaged every 3-4 weeks for an extended period (up to passage (P) 2 to 5). Two PCC tissue samples were used to develop PCC organoid cultures, which could be passaged after 4-6 weeks and are at P1 at the time of writing. Organoids had increased mRNA expression of adrenomedullary progenitor markers SOX10, nestin, and vimentin, while the mRNA expression of tyrosine hydroxylase and chromogranin A was low. Conclusions: This study has demonstrated the feasibility of establishing canine adrenomedullary and PCC organoid lines. Currently, the organoids are in a progenitor state and research efforts towards differentiation are ongoing. Canine adrenomedullary and PCC organoid lines have great potential as an in vitro research tool, paving the way towards identification of new treatment modalities for this difficult-to-treat tumor type.

PT19: DETECTION TUMOUR AND PLASMA CELL-FREE DNA EXOME SEQUENCING IN ADVANCED METASTATIC PARAGANGLIOMA

Ana Belén Moreno Cárdenas [1], Carlota Arenillas [1], Anna Casteràs [1], Jaume Capdevila [1], Jorge Hernando [1], Rodrigo Toledo [1]

[1] *Vall d'Hebron Barcelona Hospital Campus,* Vall d'Hebron, BARCELONA, Spain

Background: The study of plasma cell-free DNA (cfDNA) has been extensively applied in oncology to non-invasively identify and track circulating tumour mutations. Currently, the knowledge of the genomic landscape of metastatic pheochromocytomas and paragangliomas (mPPGLs) is still limited. Aim: To elucidate the utility of liquid biopsy to determine the genomic profiling of the advance aggressive mPPGLs. Results: Whole-exome sequencing (WES) was performed using germline, primary tumour, metastatic biopsy, and plasma cfDNA samples obtained from different patients with an aggressive mPPGLs. Patient PGL2 carried c.423+1G>A known germline SDHB mutation, evidencing loss-of-heterozygosity in the tumour. Somatic mutation results from both tumour and cfDNA WES revealed high concordance rates. There was an identification of pathogenic mutations exclusively in the metastasis and cfDNA sample (e.g. TP53 Y205C), evidencing tumor evolution. Patient PGL31 harbouring the c.338G>A germline SDHB mutation also presented the somatic driver mutation in PIK3CA H1047R in cfDNA plasma sample, which was also confirmed in the autopsy tumor sample. Conclusion: The comprehensive genomic profiling results from a biopsy of metastasis and plasma cfDNA uncovered the genomic landscape of a killing mPPGL. Liquid biopsy can be a complementary approach to tumour sequencing and help in the treatment of mPPGL patients.

PT20: RELATIONSHIP BETWEEN METANEPHRINE LEVELS AND INFLAMMATION-BASED SCORES IN PATIENTS WITH PHEOCHROMOCYTOMA

Chiara Parazzoli [1, 2], Alessandro Prete [2, 3], Vittoria Favero [1], Miriam Asia [3], Yasir S. Elhassan [2, 3], Iacopo Chiodini [1, 4], Cristina L. Ronchi [2, 3]

[1] *Department of Medical Biotechnology and Translational Medicine,* University of Milan, Milan, Italy
[2] *Institute of Metabolism and Systems Research,* University of Birmigham, Birmingham , United Kingdom
[3] *Department of Endocrinology,* Queen Elizabeth Hospital Birmingham, Birmingham , United Kingdom
[4] *Unit of Endocrinology,* Ospedale Niguarda Cà Granda, Milan, Italy

Introduction: Previous studies demonstrated that pheochromocytoma is associated with a systemic inflammatory state, as evidenced by changes in serum inflammation-based scores (IBS). However, it's still unclear whether this is directly related to the catecholamine hypersecretion. We investigated the relationship between metanephrines and IBS in patients with pheochromocytoma. Methods: A cohort of 68 patients with pheochromocytoma who underwent adrenalectomy (median age 53 years, 64.7%female) was retrospectively analysed. Plasma metanephrine and multiple IBS [(Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), Lymphocyte-to-Monocyte Ratio (LMR), Systemic-Immune-Inflammation Index (SII), Prognostic-Nutrition Index (PNI)] were assessed at baseline, early postoperatively (median 3 days, interquartile range, IQR, 2-5), and at the last follow-up (n=26, median time 15.6 months, IQR 5.7-42.3). Results: Metanephrine levels significantly correlated with all evaluated IBS; positively with NLR (r=0.4631,p-value=0.0002), PLR (r=0.3174,p-value=0.01), SII (r=0.3709,p-value=0.04) and negatively with LMR and PNI (r=-0.4368,p-value=0.0005 and r=-0.3741,p-value=0.004, respectively), even after adjustment for age, sex, ethnicity and BMI, except for PLR. There was no significant difference between IBS and mode of detection, tumour size, or history of hypertension, diabetes, or cardiovascular events. Early postoperatively, NLR (p=<0.0001) and SII (p=0.01) increased, whereas LMR and PNI decreased (each p=<0.0001) as compared to baseline. However, at the last follow-up, the lymphocyte count was higher than preoperatively (p=0.01) and consequently a significant decrease in NLR, PLR, SII and an increase in LMR were found. Conclusion: Metanephrine levels are associated with preoperative systemic inflammatory status and normalize after the

long-term follow-up. This suggests that the pro-inflammatory changes in pheochromocytomas are likely related to the metanephrine levels.

PT21: VALIDATION OF PASS AND GAPP OVERALL SCORE FOR MALIGNANCY PREDICTION IN PHEOCHROMOCYTOMA AND PARAGANGLIOMA

Lorenzo Tucci [1], Antonio De Leo [2], Kimberly Coscia [1], Giacomo Colombin [1], Saverio Selva [3], Laura Alberici [3], Caterina Balacchi [5], Donatella Santini [7], Daniela Turchetti [8], Cristina Nanni [9], Maria Abbondanza Pantaleo [10], Eugenio Roberto Cosentino [11], Giovanni Tallini [2], Cristina Mosconi [6], Valentina Vicennati [1], Uberto Pagotto [1], Guido Di Dalmazi [1]

[1] *Medical and Surgical Sciences Department, Endocrinology Unit,* Alma Mater Studiorum, IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy
[2] *Medical and Surgical Sciences Department, Anatomopathological Unit,* Alma Mater Studiorum, IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy
[3] *Abdominal and Endocrine Surgery Unit,* IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy
[4] *Medical and Surgical Sciences Department, Abdominal and Endocrine Surgery Unit,* Alma Mater Studiorum, IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy
[5] *Radiology Unit,* IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy
[6] *Medical and Surgical Sciences Department, Radiology Unit,* Alma Mater Studiorum, IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy
[7] *Anatomopathological Unit,* IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy
[8] *Medical and Surgical Sciences Department, Genetic Medicine Unit,* Alma Mater Studiorum, IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy
[9] *Nuclear Medicine Unit,* IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy
[10] *Medical and Surgical Sciences Department, Oncology Unit,* Alma Mater Studiorum, IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy
[11] *Cardiovascular Internal Medicine Unit,* IRCCS Policlinico Sant'Orsola-Malpighi, Bologna, Italy

Background: Pheochromocytomas and Paraganglioma(PPGL) follow up is based on Grading system for Adrenal Pheochromocytoma and Paraganglioma(GAPP) and Pheochromocytoma of the Adrenal gland Scaled Score (PASS), although widely debated for poor predictive efficacy. Aim: To validate PASS and GAPP malignancy prediction of PPGL. Methods: We retrospectively enrolled 84 PPGL(72 pheochromocytoma and 12 paraganglioma) who underwent surgery at Sant'Orsola-Malpighi Polyclinic since 1999 to 2022. Aggressive disease(AD) was defined by history of metastasis, recurrence, or persistence at last visit. Before surgery, patients underwent computerized tomography(CT) scan, symptoms evaluation, hormonal assessment, estimation of Upper Limit of Normal grading(ULN). After surgery, we conducted estimation of Ki67, PASS, GAPP, and genetic tests(on 54 patients). PASS and GAPP ability to predict AD was evaluated through univariate logistic regression with estimation of

sensitivity, specificity, negative(NPV) and positive predictive value(PPV). Results: 11 patients(8 pheochromocytomas, 3 paragangliomas; p=0.117) had AD, mean follow-up was 7.2±4.9 years. AD and non-AD shared similar unenhanced CT attenuation(p=0.422), equal ULN grading(p=0.888), equal proportion of patients with positive genetic test(p=0.156), non-functioning PPGL(p=0.745) and symptoms(p=0.146). Compared to non-AD, AD showed increased mass diameter(p=0.005), Ki67(p=0.017), PASS(p=0.009), GAPP(p=0.01), and more moderate-to-poorly differentiated tumours (p=0.016). GAPP positively predicted AD(Odds-Ratio(OR)=1.718; 95%Confidence Interval(CI)=1.106-2.669; p=0.016). GAPP≥5 showed 80% sensitivity, 61.3% specificity, 24.4% PPV, 95% NPV in predicting AD. PASS positively predicted AD (OR=1.257; 95%CI=1.037-1.524; p=0.020). PASS≥7 showed 77.8% sensitivity, 82% specificity, 38.9% PPV, 96.2% NPV in predicting AD. Conclusion: Increased cut-offs of PASS and GAPP may be useful for prediction of PPGL aggressivity, although scores with improved malignancy prediction are still needed.

Guided Poster Tour
(APA)

PT22: DIFFERENCES IN CIRCULATING MIRNA EXPRESSION IN PLASMA SAMPLES TAKEN DIRECTLY FROM ADRENAL VEINS IN UNI- AND BILATERAL PRIMARY ALDOSTERONISM

Bálint Vékony [1, 2], Gábor Nyirő [3, 2, 1], Zoltán Herold [2], János Fekete [4], Filippo Ceccato [5], Sven Gruber [6], Lydia Kürzinger [7], Mirko Parasiliti-Caprino [8], Siti Khadijah Syed Mohammed Nazri [9], Elena AB Aisha Azizan [9], Bálint Szeredás [1, 2], Felix Beuschlein [6], Péter Igaz [1, 2]

[1] *Department of Endocrinology,* Semmelweis University, Budapest, Hungary
[2] *Department of Internal Medicine and Oncology,* Semmelweis University, Budapest, Hungary
[3] *Department of Laboratory Medicine,* Semmelweis University, Budapest, Hungary
[4] *Department of Bioinformatics,* Semmelweis University, Budapest, Hungary
[5] *Endocrinology Unit, Department of Medicine DIMED,* University of Padova, Padova, Italy
[6] *Department of Endocrinology, Diabetology and Clinical Nutrition,* University Hospital Zurich (USZ) and University of Zurich (UZH), Zurich, Switzerland
[7] *Department of Internal Medicine I, Division of Endocrinology and Diabetes,* University Hospital, University of Würzburg, Würzburg, Germany
[8] *Endocrinology, Diabetes and Metabolism, Department of Medical Sciences,* University of Turin, Turin, Italy
[9] *Endocrine Unit, Department of Medicine,* The National University of Malaysia (UKM) Medical Centre, Kuala Lumpur, Malaysia

Background: Primary aldosteronism (PA) is the most common cause of secondary hypertension, causing around 10% of cases. The two main types of PA are unilateral aldosterone producing adenoma (APA) and bilateral adrenal hyperplasia (BAH). Whereas the treatment of adenoma is surgical, hyperplasia is treated with drugs. The gold standard differential diagnosis is adrenal vein sampling (AVS). We aimed to analyse the miRNA expression profile of adrenal vein samples to identify potential markers capable of differentiating uni- and bilateral PA. Methods: The miRNA expression profiles of 10 APA-s, and 8 BAH-s were examined in 36 AVS samples (both sides), with next generation sequencing. Qiagen miRNeasy Serum/Plasma and Qiaseq miRNA Library kits were used for sample preparation. Sequencing was performed on Illumina MiSeq. Alignments and miRNA counts were called by Qiagen GeneGlobe. Differential expression levels were analysed with the DeSeq2 algorithm and subsequently, with a neural-network based artificial intelligence, that tested the diagnostic performance of all differentially expressed miRNA-s, in combinations of 1-8. Results: Our results show that 5 of our trained AI models, consisting of 9 miRNAs in total have a specificity of above 90% and sensitivity above 70% for differentiating uni- and bilateral

PA. Conclusions: Markers that could differentiate the forms of PA would be of major clinical relevance. Our models could be improved with increasing the sample size. We are also working on validating our results by real-time PCR and retooling the artificial intelligence models to utilise qPCR data, to increase the ease-of-use and clinical viability of this method.

PT23: IMPROVEMENT OF SCREENING FOR PRIMARY ALDOSTERONISM USING SEX- AND RENIN-ADJUSTED CUT-OFFS FOR ALDOSTERONE

Sybille Fuld [1], Christina Pamporaki [1], Georgiana Constantinescu [1], Francesco Alessi [1], Mirko Peitzsch [2], Manuel Schulze [3], Jun Yang [4], Hanna Remde [5], Lydia Kürzinger [5], Lisa Müller [6], Martin Reincke [6], Sven Gruber [7], Felix Beuschlein [6, 7], Jacques Lenders [1, 8], Andrzej Januszewicz [9], Graeme Eisenhofer [1]

[1] *Department of Medicine III,* University Hospital Carl Gustav Carus, Technische Universität Dresden , Dresden, Germany
[2] *Institute of Clinical Chemistry and Laboratory Medicine,* University Hospital Carl Gustav Carus, University Hospital Carl Gustav Carus, Technische Universität Dresden , Dresden, Germany
[3] *Center for Information Services and High Performance Computing,* Technische Universität Dresden, Dresden, Germany
[4] *Centre for Endocrinology and Metabolism,* Hudson Institute of Medical Research, Clayton, Australia
[5] *Department of Internal Medicine I, Divison of Endocrinology and Diabetes,* University Hospital and University of Würzburg, Würzburg, Germany
[6] *Department of Medicine IV,* University Hospital and Ludwig Maximilian University Munich, Munich, Germany
[7] *Department of Endocrinology, Diabetology and Clinical Nutrition,* University Hospital and University of Zurich, Zurich, Switzerland
[8] *Department of Internal Medicine,* Radboud University Medical Center, Nijmegen, Netherlands
[9] *Institute of Cardiology,* Cardinal Stefan Wyszynski University, Warsaw, Poland

The aldosterone-renin-ratio (ARR) is a widely accepted screening test for primary aldosteronism (PA). However, the ARR lacks specificity, necessitating a high number of confirmatory tests. The objective of this study was to develop new cut-offs to improve specificity of aldosterone and renin measurements during screening for PA. In this multicenter study, logistic regression (LR) models and corresponding ROC-curves were developed based on plasma aldosterone, renin and sex. Diagnosis of PA was confirmed based on post-surgical outcomes after adrenalectomy or results of saline-infusion tests performed following positive screening determined either by a positive ARR or high probability based on machine learning or both. Negative screening or negative confirmatory test results were used to exclude PA. PA was confirmed in 165 (24.5%) of 674 patients referred for evaluation of secondary causes of hypertension. In females without PA, plasma concentrations of aldosterone were higher ($P=0.0210$), renin lower ($P<0.0001$) and the ARR higher ($P<0.0001$) than in male patients.

Areas under ROC-curves were larger for the LR model than for the ARR for both males (0.959vs0.895; P<0.0001) and females (0.944vs0.898; P<0.0053). At a diagnostic sensitivity of 95%, specificity could be improved from 60.1% for the ARR to 70.7% for females and from 61.3% to 83.6% for males using sex specific LR models based on aldosterone and renin. An equation for sex- and renin-adjusted aldosterone cut-offs was derived that facilitates application in clinical practice. Use of sex- and renin-specific cut-offs for plasma aldosterone improves diagnostic screening of patients with suspected PA by reducing false positive results.

PT24: VASCULAR AND HORMONAL INTERACTIONS IN PRIMARY ALDOSTERONISM

Nicolo' Faedda [1, 2], Alaa B Abdellatif [1], Isabelle Giscos-Douriez [2], Sheerazed Boulkroun [2], Maria-Christina Zennaro [1, 2, 3]

[1] *BioSPC,* Université Paris Cité, Paris, France
[2] *PARCC,* INSERM U970, Paris, France
[3] *Assistance Publique-Hôpitaux de Paris,* Hôpital Européen Georges Pompidou, Paris, France

Introduction: Primary Aldosteronism (PA) is the most frequent form of secondary hypertension. We make the hypothesis that adrenal vascular changes may modify adrenal cortex structure and function, creating a propitious environment for developing somatic mutations and Aldosterone Producing Adenoma (APA). Methods: To study the interaction between the vasculature and cell proliferation, zonation and aldosterone production in the adrenal cortex, we crossed a new mouse model expressing the Cre recombinase controlled by the Cyp11b2 promoter with mTmG mice (Cyp11b2-Cre::mTmG) allowing to track the zona glomerulosa (ZG) to zona fasciculata (ZF) transdifferentiation. Adrenal structure and vascularization were characterized under basal conditions, high or low salt diets (HSD/LSD) as well as dexamethasone (DEX) treatment, both followed by a recovery period. Results: In Cyp11b2-Cre::mTmG mice at day 1, only a few GFP+ cells are present in the ZG, while at day 14 all ZG-cells are GFP+. Subsequently, GFP+ cells transdifferentiate and colonize the ZF. Under HSD, Cyp11b2 expression was reduced, whereas under LSD, the ZG was expanded and Cyp11b2 expression increased and vessels area is expanded in the whole adrenal cortex. Additionally, salt diets do not affect ZG-to-ZF transdifferentiation. After 2 weeks of DEX treatment, complete disorganization of the ZF was observed; after three weeks of recovery ZF and vessel regeneration was complete. Conclusion: Evaluation of the hormonal influence on the steroidogenic cell lineage and the adrenal vascular network, as well as of vascular changes on hormonal parameters, provides new insight in the mechanisms of APA development.

PT25: COMPARISON OF CARDIOVASCULAR EVENTS AND TARGET ORGAN DAMAGE BETWEEN PRIMARY ALDOSTERONISM SUBTYPES: A MULTICENTER ENSAT STUDY

Fabio Bioletto [1], Marta Araujo-Castro [2], Chiara Lopez [1], Martina Bollati [1], Jorge Gabriel Ruiz-Sanchez [3], Angel Rebollo Román [4], Cristina Lamas Oliveira [5], Mauro Maccario [1], Mirko Parasiliti-Caprino [1]

[1] *Department of Medical Sciences,* University of Turin: Universita degli Studi di Torino, Turin, Italy
[2] *Endocrinology & Nutrition Department,* Hospital Universitario Ramón y Cajal, Madrid, Spain
[3] *Endocrinology & Nutrition Department,* Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain
[4] *Endocrinology & Nutrition Department,* Hospital Reina Sofía, Cordoba, Spain
[5] *Endocrinology & Nutrition Department,* Hospital Universitario De Albacete, Albacete, Spain

Background: Primary aldosteronism (PA) is associated with an increased rate of cardiovascular events and target organ damage compared to essential hypertension. However, there is conflicting evidence as to whether this cardiovascular risk excess might differ between PA subtypes. Methods: This is a multicenter retrospective cross-sectional study involving ENSAT centers within the APA working group. Any patient with PA subtyped by adrenal vein sampling (AVS) was eligible for inclusion; unilateral and bilateral subtypes were differentiated using a lateralization index ≥ 4 as the cut-off criterion. The following endpoints were evaluated at the time of PA diagnosis and compared between subtypes: left ventricular hypertrophy, chronic kidney disease, microalbuminuria, coronary artery disease, cerebrovascular disease, atrial fibrillation, heart failure. Results: Currently, data from 21 Spanish centers (134 patients) and 1 Italian referral center (128 patients) have been collected. At this interim analysis, no significant differences were detected in any endpoint taken individually (left ventricular hypertrophy: p=0.216; chronic kidney disease: p=0.280; microalbuminuria: p=0.509; coronary artery disease: p=0.493; cerebrovascular disease: p=0.508; atrial fibrillation: p=0.876; heart failure: p=0.094). However, when grouping these outcomes in a composite endpoint, patients with unilateral PA were more likely to present at least one complication than those with bilateral PA (55.6% vs 40.2%, OR=1.86 [95%CI: 1.14-3.04], p=0.013). Conclusions: At this interim analysis, patients with unilateral PA showed a higher overall complication burden than those

with bilateral PA. Data collection is still in progress, and further analyses on a larger sample will be needed to consistently evaluate finer differences in individual endpoints.

PT26: LEFT VENTRICULAR PRESSURE STRAIN; NOVEL AND EARLY PROGNOSTIC FACTOR FOR THE PRIMARY ALDOSTERONISM

Gamze Akkus [1], Ferhatcan Piskin [2], Ulcaz Aksoydan [1], Oğuz Akkus [3], Mehtap Evran [1], Murat Sert [1], Tamer Tetiker [1]

[1] E*ndocrinology,* Cukurova University Faculty of Medicine: Cukurova Universitesi Tip Fakultesi, Adana, Turkey
[2] R*adiology,* Cukurova University Faculty of Medicine: Cukurova Universitesi Tip Fakultesi, Adana, Turkey
[3] C*ardiology, M*ustafa Kemal University, Hatay, Turkey

Introduction: Primary aldosteronism (PA) is the most common cause of the secondary hypertension which is related with significantly increased cardiovascular mortality. Since hypertensive patients with PA have higher cardiometabolic and kidney diseases' risk factors than the patients with primary hypertension, treatment of PA should be done immediately. Although cascade the diagnosis of PA is very complex and confused many clinicians; elevated aldosterone/renin ratio and spontaneous hypokalemia commonly has been supported diagnosis of PA. Elevated aldosterone levels in patients with PA has detrimental effects on cardiovascular structure (the left ventricular (LV) remodelling, function etc.) In our study we aimed to compare the cardiac structure of patients with PA ı patients with primary hypertension (PHT) without PA. Material and methods: A total of 41 patients (PA/PHT;21/20) were enrolled between 2020-2022 years. After the baseline hormonal and biochemical evaluation, patients were assessed by the cardiac magnetic resonance imaging (CMR). CMR imaging was performed using a 1.5-T scanner (Arthitect, General Electrics, USA) with a five-element phased array cardiac coil. Cine-CMR images were analyzed semi-automatically using commercial software (Medis Suite version 3.1, Leiden, the Netherlands) by an experienced expert reader in our core lab, who was blinded to clinical information (Fig1). Results: Among two groups (PA vs PHT) mean gender and age (55.8±8.5 vs 53.1±8.5, p=0.465) were similar. Disease duration (p=0.34), existing of comorbidity (diabetes mellitus, thyroid disorder, dyslipidemia etc.) (p=0.368), medication of hypertension drug (p=0.478) and ambulatory blood pressure (p=354) were also similar. Groups left ventricular (LV) EF, LV end systolic volume (ESV) (p=0.371), end diastolic volume (EDV) (p=0.563) and mass index (p=0.617) were comparable between two group, LV circumferential strain measurements (-14.6±7.8 vs -5.7±11.0, p=0.005) were higher in patients with PA.

Conclusion: Although in daily clinical practice ejection fraction (EF) is most frequently used parameter of LV systolic function, myocardial strain is more sensitive marker which provide detailed information before the development of global and regional active deformation and apparent heart failure. In our study, we demonstrated increased LV strain may be the early prognostic factor to evaluate diastolic disfunction in patients with PA.

PT27: POSTOPERATIVE DYSLIPIDEMIA WITH LIPOPROTEIN (A) ELEVATION IN PATIENTS WITH PRIMARY HYPERALDOSTERONISM COMPARED TO OTHER ADRENAL DISORDERS

Jimmy Masjkur [1, 2, 3], Christopher Strey [2], Matthias Gruber [1], Georgiana Constantinescu [1], Stefan Bornstein [1], Graeme Eisenhofer [1]

[1] *Endocrinology MK3,* Uniklinikum Carl Gustav Carus Dresden, Dresden, Germany
[2] *Endocrinology,* eSwiss Surgical & Medical Center, St.Gallen, Switzerland
[3] *Endocrinology,* Universitas Hasanuddin, Makassar, Indonesia

Context: Adrenal disorders have been associated with an increased risk of metabolic syndrome and dyslipidemia. Changes in serum lipid levels after adrenalectomy of different type of hormonal imbalances will provide insight to improve patient care outcomes. Objective: This study assessed whether postoperative dyslipidemia is more pronounced for primary hyperaldosteronism than for other adrenal disorders. Design: Prospective cohort study with a retrospective component. Setting: Tertiary care referral center. Patients: Serum lipid profile was measured in two hundred and twenty two patients with an adrenal mass underwent hormonal evaluation. Adrenal hormone excess was excluded in 83 patients (defined as non-functional adenoma (NF)) and confirmed in 139. Of these 139 patients, 50 patients were diagnosed with primary hyperaldosteronism (PA), 42 with mild autonomous cortisol secretion (MACS) and 47 with pheochromocytoma (PC). Main Outcome and Measures: Statistical tests were applied to a panel of lipid including lipoprotein (a), total cholesterol, Low density lipoprotein cholesterol (LDL-C), High density lipoprotein cholesterol (HDL-C) and Triglyceride (TG) as well as LDL-C to HDL-C ratio (RLH). The average was measured in mean and median. Multivariate analysis was performed to measure the interactions of different variables. Results: Patients with PA who underwent surgery showed higher changes on plasma concentrations of lipoprotein (a) (P=0,0036), LDL-C (P=0,0067), Trigylceride (P=0,0222) and total cholesterol (P=0,0145) than PA patients without surgery. PA patients with surgical approach showed significantly higher changes on plasma concentrations of lipoprotein (a) (P<0,05-0,0001), LDL-C (P<0,05-0,0001) and total cholesterol (P<0,05-0,0001) than patients with any other type of adrenal disorders after surgery. Changes on TG level in PA patients underwent adrenalectomy were higher compared to that of PC (P<0,05) and NF (P<0,001). Post

operative changes on HDL-C level in MACS patients were significantly different to PA (P<0,05) and PC (P<0,05) groups. Multivariate analyses showed a significant influence of Aldosterone-to-renin-ratio (ARQ) on post- operative changes in Lp(a) (P=0.0019), LDL-C (P=0.025) and TC (P=0.0491). Changes in lp(a) after surgery was also associated with pre-operative level of Aldosterone (P=0,00958) and post-operative changes of anti-diabetic medication (0,0017). Aldosterone was associated with post-operative change in LDLC-C (p=0.0089) and TC (P=0.011). After disease stratification, pre-operative ARQ and Aldosterone were significantly associated with changes in LDL-C (P=0.0201 and 0.0098) and lp(a) (P=0.0042 and P= 0.0098) only in patients with PA and not with that of other adrenal disorders. Conclusions: PA patients showed higher progress of dyslipidemia with significant elevation of lipoprotein (a) level after adrenalectomy compared to patients with other adrenal disorders. This effect was significantly associated with aldosterone levels themselves. Emphasis should be placed on post-operative lipid profile monitoring to prevent cardiovascular complication.

PT28: THE IMPACT OF SURGICAL VERSUS MEDICAL THERAPY ON RENIN PLASMA ACTIVITY, RENAL FUNCTION, AND HYPERTENSION CONTROL IN PATIENTS WITH PRIMARY ALDOSTERONISM

Stacey Rolak [2], Nanditha Venkatesan [2], Raul Gregg Garcia [2], Irina Bancos [1]

[1] *Endocrinology,* Mayo Clinic, Rochester, United States
[2] *Internal Medicine,* Mayo Clinic, Rochester, United States

Bilateral primary hyperaldosteronism (PA) is treated with mineralocorticoid receptor antagonist (MRA) therapy. The aim of this study was to compare the impact of curative adrenalectomy versus MRAs in patients with PA on plasma renin activity (PRA), renal function, and hypertension control. This was a single center retrospective study of patients with unilateral PA treated with adrenalectomy or bilateral PA treated with MRAs, 2017-2021. Of 73 patients, 53 underwent unilateral adrenalectomy for unilateral PA and 20 had bilateral PA treated with MRA. At baseline, when compared to bilateral PA, patients with unilateral PA had higher plasma aldosterone concentrations (median 31 ng/dL vs 19 ng/dL, P= 0.001), similarly suppressed PRA, and similar estimated glomerular filtration rate (eGFR). Uncontrolled hypertension (>130/90 mmHg) was seen in 40 (75%) of patients with unilateral PA and 14 (70%) patients with bilateral PA, despite a more intensive antihypertensive regimen in unilateral PA. At a median follow up of 108 days, 24 (45%) patients treated with adrenalectomy and 16 (80%) patients treated with MRAs demonstrated PRA >1.5 ng/mL/hr, P = 0.008. eGFR decreased by at least 10 points in 15 (30%) patients treated with adrenalectomy vs in 11 (59%) patients treated with MRAs (P=0.033). Hypertension control improved, with a mean decrease in systolic blood pressure of -11 mmHg (95%CI -16.5-(-5.5)) without between group differences. Despite less severe PA at baseline, patients treated with MRAs demonstrate a higher prevalence of non-suppressed PRA, lower eGFR, similar hypertension control, and a more intense antihypertensive regimen during follow-up.

Poster Presentations

PP1: BONE FRAGILITY IN PATIENTS WITH ADRENOCORTICAL CARCINOMA UNDERGOING MITOTANE THERAPY

Deborah Cosentini [1], Salvatore Grisanti [1], Marta Laganà [1], Nunzia Di Meo [2], Valentina Cremaschi [1], Antonella Turla [1], Carlo Cappelli [3], Paola Loli [4], Davide Farina [2], Alfredo Berruti [1]

[1] *Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health,* Medical Oncology Unit, University of Brescia, Brescia, Italy
[2] *Department of Medical and Surgical Specialties, Radiological Sciences and Public Health,* Radiology 2, University of Brescia, Brescia, Italy
[3] *Department of Experimental Sciences,* Unit of Endocrinology and Metabolism, University of Brescia, Brescia, Italy
[4] *Clinica Polispecialistica San Carlo,* Endocrinology, Paderno Dugnano, Milan, Italy

Background: A recent study (PMID 34905056) showed that mitotane treatment in patients with adrenocortical carcinoma (ACC) is associated with a great proportion of vertebral fractures (VFs) independently from cortisol supplementation. Methods: A consecutive series of ACC patients who performed a DXA (Dual Energy X-ray Absorptiometry) scan has been collected. The primary aim was to evaluate the prevalence of osteoporosis in patients treated with mitotane. Secondary aims were: 1) differences in densitometric parameters on the basis of the presence of VFs; 2) changes in these parameters during at least 6 months of mitotane treatment. Results: Eighty-four ACC patients were included. Osteoporosis was observed in 31 patients (37%) and osteopenia in 30 patients (36%) after a median mitotane treatment of 11 months (range 6-1454). VFs were found in 16 patients (19%), which were moderate-severe in 8 of them. Age and the presence of previous fractures were found to be associated with a higher risk of VFs during mitotane (p 0.04 and 0.01, respectively). Neither BMD nor body composition differed on the basis of VFs. FRAX score corrected for trabecular bone score (TBS) was found to be related to a higher risk of VFs (p 0.03 and 0.04 respectively). In the 26 patients in whom 2 DEXA scans were available before and after mitotane therapy, an increase in fat body mass was found (p 0.01). Conclusion: ACC patients receiving mitotane therapy are at high risk of osteoporosis and VFs. FRAX corrected for TBS could be a useful predictive factor for VF risk.

PP2: EVOLUTIONARY PROFILING OF ADVANCED ADRENOCORTICAL CARCINOMA

Samuel Backman [1], Fredrik Axling [1], Johan Botling [5], Christofer Juhlin [3], Jan Zedenius [4], Britt Skogseid [2], Per Hellman [1], Olov Norlén [1], Peter Stålberg [1], Joakim Crona [2], Tobias Åkerström [1]

[1] *Department of Surgical Sciences,* Uppsala University, Uppsala, Sweden
[2] *Department of Medical Sciences,* Uppsala University, Uppsala, Sweden
[3] *Department of Oncology-Pathology,* Karolinska Institutet, Stockholm, Sweden
[4] *Department of Molecular Medicine and Surgery,* Karolinska Institutet, Stockholm, Sweden
[5] *Department of Immunology, Genetics, and Pathology,* Uppsala University, Uppsala, Sweden

Background: The genetic landscape of primary adrenocortical carcinoma has been well characterized in several large-scale studies. However, the genetic landscape of advanced metastatic ACC is less well understood. Aim: To characterize the genetic landscape of metastatic adrenocortical carcinoma and to delineate the pathways to metastatic disease. Methods: Nine patients from whom blood/normal tissue, fresh-frozen primary tumor tissue and metastatic tumor tissue was available were included. In total 28 tumor samples were included and subjected to whole genome sequencing, RNA Sequencing, and DNA methylation analysis using the Illumina EPIC array. Results: We find mutations and CNV events affecting known ACC genes. Comparison of primary tumors and paired metastases reveals heterogeneity even with regard to canonical ACC driver genes. A majority of our samples are affected by wide-spread LOH followed by genome doubling events. Mutation timing analyses suggests that the LOH events usually occur early in tumor development. Analyses of DNA methylation and gene expression patterns reveals that paired primary and metastatic tumors are transcriptomically and epigenetically similar. Finally, we perform subclonal deconvolution using PyClone-VI. Conclusion: Our study advances the understanding of the biology of advanced adrenocortical carcinoma and will help inform future studies.

PP3: INFLAMMATION-BASED SCORES PREDICT TOXICITY AND OUTCOME IN ADVANCED ADRENOCORTICAL CARCINOMA PATIENTS TREATED WITH EDP-M CHEMOTHERAPY

Valentina Cremaschi [1], Marta Laganà [1], Deborah Cosentini [1], Antonella Turla [1], Salvatore Grisanti [1], Alfredo Berruti [1]

[1] *Medical Oncology Unit,* ASST Spedali Civili, Brescia, Italy

EDP-M chemotherapy is the first-line standard of care in advanced adrenocortical carcinoma (ACC). Pre-treatment inflammation scores have been tested in several neoplasms and have shown to correlate with patients' outcomes. The prognostic effect of these parameters was also confirmed in ACC patients treated with EDP-M. It is not known whether these scores may also have a predictive role on EDP-M toxicity. We retrospectively reviewed data of patients treated in our center with EDP-M and collected clinical and biochemical data assessed before the EDP-M start. Primary objective was to confirm the prognostic role of NLR (calculated as neutrophil/lymphocyte count) in terms of overall survival (OS). Secondary objectives were to explore a prognostic or predictive role in terms of treatment response or G3-G4 treatment toxicities of many other immune-inflammation indexes. Sixty-one patients entered the study, 74% were dead at the time of the analysis, 41% had cortisol hypersecretion, 56% presented a GRAS pejorative status at diagnosis. Higher NLR correlated with a shorter OS (HR=2.117; CI=1.131-3.961; p=0.019) while higher absolute neutrophil count correlated with both shorter OS (HR=2.706; CI=1.455-5.033; p=0.002) and shorter PFS (HR=1.793; CI=1.037-3.101; p=0.037). In addition, higher level of PNI (Prognostic Nutritional Index) correlated with a lower risk for developing G3-G4 toxicities (HR=0.222; CI=0.054-0.912, P=0.037). In conclusion, inflammation-based scores assessed at baseline are not only predictors of patients' outcomes but may help in identifying patients destined to exhibit severe toxicity. The latter finding is new and interesting as it may have potential implications in the management of ACC patients given EDP-M.

PP4: HIGH DOSE RATE BRACHYTHERAPY COMBINED WITH PD-1 BLOCKADE AS A TREATMENT FOR METASTATIC ADRENOCORTICAL CARCINOMA

Paul Schwarzlmueller [1], Stefanie Corradini [2], Max Seidensticker [3], Petra Zimmermann [4], Jochen Schreiner [1], Tanja Maier1 [1], Alexandra Triebig [1], Thomas Knösel [5], Montserrat Pazos [2], Thomas Pfluger [6], Isabel Weigand [1], Claus Belka [2], Jens Ricke [3], Martin Reincke [1], Ralf Schmidmaier [1], Matthias Kroiss [1]

[1] *Department of Internal Medicine IV ,* LMU Hospital, Munich, Germany
[2] *Department of Radiotherapy and Radiation Oncology ,* LMU Hospital, Munich, Germany
[3] *Department of Diagnostic and Interventional Radiology ,* LMU Hospital, Munich, Germany
[4] *Department of General, Visceral and Transplant Surgery ,* LMU Hospital, Munich, Germany
[5] *Institute of Pathology,* LMU Hospital, Munich, Germany
[6] *Department of Nuclear Medicine ,* LMU Hospital, Munich, Germany

The response rate of advanced adrenocortical carcinoma (ACC) to standard chemotherapy with mitotane and etoposide/doxorubicin/cisplatin (EDP-M) is unsatisfactory and benefit is frequently short lived. Immune checkpoint inhibitors (CPI) have been examined in patients after failure of EDP-M, but objective response rates are only approximately 15 %. High dose rate (HDR) brachytherapy is a catheter-based internal radiotherapy and expected to favorably combine with current immunotherapies. Here we describe three cases of patients with advanced ACC who were treated with HDR brachytherapy and the CPI pembrolizumab. None of the tumors were positive for established response markers to CPI. All patients were female, had progressed on EDP-M and received external beam radiation therapy for metastatic ACC. Pembrolizumab was initiated 7 or 23 months after brachytherapy in two of the cases and prior to brachytherapy in one case. Best response of lesions treated with brachytherapy was complete (n=2) or partial response (n=1) that was ongoing at last follow up after 23, 45 and 4 months, respectively. Overall treatment response was complete remission and partial remission in the two patients with brachytherapy prior to pembrolizumab. The third patient developed progressive disease with severe Cushing's syndrome and died due to COVID-19. Immune-related adverse events of colitis (3°), gastroduodenitis (3°), pneumonitis (2°) and thyroiditis (1°) occurred in the two patients with systemic response. HDR brachytherapy was effective in locally controlling metastases. Sequential combination with CPI may increase the efficacy of

immunotherapy in ACC. Systematic studies are required to confirm this preliminary experience and to understand underlying mechanisms.

PP5: SIAH1, A REGULATOR OF THE WNT/B-CATENIN PATHWAY IN ADRENOCORTICAL CELLS

Pierre Puygrenier [1], Karine Perlemoine [1], Bruno Ragazzon [1], Anne Jouinot [1], Constantine Stratakis [2, 3], Jérôme Bertherat [1], Annabel Berthon [1]

[1] *Endocrinologie et métabolisme,* Institut Cochin, Paris, France
[2] *ELPEN,* Research Institute, Athens, Greece
[3] *IMBB,* Human Genetics & Precision Medicine, Heraklion, Greece

The most frequent molecular alteration in adrenocortical tumors (ACT) is the activation of the Wnt/β-catenin pathway that is associated with poor prognosis in adrenocortical carcinoma. An activating β-catenin mutation (p.S45) inhibiting its proteosomal degradation by the canonical destruction complex is responsible for this abnormal activation in roughly 30% of ACT. However, the E3 ubiquitin protein ligase 1, SIAH1 is able to ubiquitinate β-catenin even while mutated in intestinal cells, we then, investigate if SIAH1 regulates both wild-type and mutant β-catenin in MUC-1 and H295R human adrenocortical cells, respectively. We demonstrate that SIAH1 interacts with β-catenin and increases both wild-type and mutant β-catenin ubiquitination through its catalytic RING finger domain. Overexpression of SIAH1 then, results in a decrease in β-catenin half-life (25%) that is sufficient to reduce β-catenin transcriptional activity (50%) measured with the TOPFlash reporter even in presence of β-catenin mutation (p.S45) in H295R cells. However, the overexpression of SIAH1 deleted from its catalytic domain does not affect neither β-catenin activity nor its ubiquitination suggesting that the effect of SIAH1 on β-catenin activity depends on its catalytic domain. Although SIAH1 is also able to ubiquitinate β-catenin in MUC1 cells, its overexpression rather promotes β-catenin activation in response to 6h treatment with a β-catenin activator, BIO (bromoidirubin-3-oxime). Altogether, our results suggest that SIAH1 indirectly regulates β-catenin transcriptional activity and that promoting SIAH1 activity could limit the activation of this pro-oncogenic pathway while Wnt/β -catenin signaling is constitutively activated.

PP6: ESTROGEN-MIMETIC EFFECT OF MITOTANE IN PATIENTS AFFECTED BY ADRENOCORTICAL CARCINOMA: A PROSPECTIVE OBSERVATIONAL COHORT STUDY

Antonella Turla [1], Marta Laganà [1], Valentina Cremaschi [1], Andrea Abate [2], Mariangela Tamburello [2], Sandra Sigala [2], Salvatore Grisanti [1], Angela Gambino [3], Deborah Cosentini [1], Alfredo Berruti [1]

[1] *Medical Oncology Unit, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health,* University of Brescia, ASST Spedali Civili, Brescia, Italy
[2] *Section of Pharmacology, Department of Molecular and Translational Medicine,* University of Brescia, Brescia, Italy
[3] *Department of Gynecology and Obstetrics,* University of Brescia, ASST Spedali Civili, Brescia, Italy

Background: Mitotane is the mainstay of medical treatment of adrenocortical carcinoma (ACC), both in the adjuvant and metastatic settings. Among the side effects of the drug are those related to its mimetic estrogenic effect, the frequency of which has not been investigated. Methods: We present a prospective, case-control, observational study evaluating the prevalence of menorrhagia, endometrial thickening, and ovarian cysts in female ACC patients (pts) exposed to mitotane, compared with a control group balanced for age and menopausal status. Secondary objectives were the correlation between gynecological alterations and mitotane treatment duration (TTR), menopausal status, and hormonal profile. Results: 44 female pts, 22 pts treated with mitotane (F-ACC pts) and 22 in the control group (FC pts), were enrolled. A higher prevalence of menometrorrhagia (45.4% vs 4.5%, p 0.004) and ovarian cysts (59.1% vs 4.5%, p <0.001) was observed in F-ACC pts. No significant difference was found in the prevalence of endometrial thickening (31.8% vs 27.3%, respectively, p 0.526). The occurrence of these side effects due to the mimetic estrogenic action of the mitotane showed no correlation with mitotane serum levels, menopausal state, circulating estradiol, and SHBG levels, with the sole exception of ovarian cysts, which were more frequent in patients with estradiol levels >23 ng/ml than those with lower levels: 40.9% vs 4.5%, respectively (p 0.024). Conclusions: Estrogen-related side effects are frequent in ACC patients treated with mitotane and require proper clinical management.

PP7: IGF2R: A NEW PLAYER IN THE INSULIN-LIKE GROWTH FACTOR 2 (IGF2) PATHWAY SUSTAINING ADRENOCORTICAL CARCINOMA CELLS GROWTH

Emma Nozza [1, 2], Rosa Catalano [1], Emanuela Esposito [1, 2], Anna Maria Barbieri [1], Giusy Marra [1], Donatella Treppiedi [3], Genesio Di Muro [1, 4], Federica Mangili [3], Sonia Di Bari [1], Constanze Hantel [6, 5], Serena Palmieri [3], Sofia Frigerio [3], Emanuele Ferrante [3], Maura Arosio [3, 1], Giovanna Mantovani [3, 1], Erika Peverelli [3, 1]

[1] *Department of Clinical Sciences and Community Health,* University of Milan, Milan, Italy
[2] *PhD Program in Experimental Medicine,* University of Milan, Milan, Italy
[3] *Endocrinology Unit,* Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[4] *PhD Program in Endocrinological Sciences,* University Sapienza of Rome, Rome, Italy
[5] *Department of Endocrinology, Diabetology and Clinical Nutrition,* University Hospital Zurich (USZ) and University of Zurich (UZH), Zurich, Switzerland
[6] *Medizinische Klinik und Poliklinik III,* University Hospital Carl Gustav Carus Dresden, Dresden, Germany

Adrenocortical carcinomas (ACC) are rare endocrine tumors that originate in the cortex of the adrenal gland. They are characterized by the overexpression of insulin-like growth factor 2 (IGF2), whose bond with two tyrosine-kinase receptors, IGF1R and IR, activates a cancer-promoting signalling cascade. Another component of the IGF system is mannose 6-phosphate/insulin-like growth factor 2 receptor (IGF2R), a scavenger receptor able to bind specifically IGF2. Its main role is to internalise IGF2 and direct it to lysosomal degradation, suggesting an anti-oncogenic role. However, the study of its involvement in different tumors has highlighted a diverse role, even making it a marker of poor prognosis. Aim of the present study was to deepen the knowledge on IGF2R role in ACC investigating its expression levels, involvement in the proliferative mechanism and interaction with the other receptors of the IGF2 pathway. IGF2R expression was evaluated in two human ACC cell lines (MUC-1 and H295R), 7 ACC tissue samples, and 7 normal adrenal tissues (NA). Our results showed that ACC tissues and cell lines had increased IGF2R protein levels compared to NA ($p<0.01$). To investigate IGF2R role in cell proliferation we altered its expression in MUC-1 and H295R cell lines. We demonstrated that IGF2R genetic silencing reduced cell proliferation (-11.65±8.73% $p<0.01$ in MUC-1; -14.02±12.35% $p<0.01$ in H295R). Similarly, IGF2R-neutralizing antibody incubation exerted anti-mitotic effects (-16.85±2.57% $p<0.05$ in MUC-1). On the opposite, IGF2R

transient transfection promoted cell growth (+14.84±12.54% $p<0.01$ in MUC-1; +24.49±15.62% $p<0.001$ in H295R). To investigate the molecular mechanism involved, we tested the effect of IGF2R manipulations on the expression of the other receptors of the IGF2 pathway. MUC-1 silenced for IGF2R showed a significant decrease in IGF1R protein expression (-35 ±37% $p<0.05$); accordingly, after IGF2R transfection, IGF1R protein level was increased (+20±6% $p<0.001$). In conclusion, IGF2R is involved in the mechanisms driving cell proliferation in ACC, possibly relating also to the alteration of IGF1R expression. Since its upregulation in ACC and its pro-mitotic action, IGF2R may represent a promising therapeutic target for the treatment of ACC.

PP8: THERAPEUTIC POTENTIAL OF TARGETING THE FLNA-BINDING PARTNER WEE1 IN ADRENOCORTICAL CARCINOMAS

Emanuela Esposito [1, 2], Rosa Catalano [1], Emma Nozza [1, 2], Annamaria Barbieri [1], Sara Maioli [1], Jens Geginat [1], Giusy Marra [1], Donatella Treppiedi [1], Genesio Di Muro [1, 3], Federica Mangili [1], Sonia Di Bari [1], Serena Palmieri [4], Sofia Frigerio [4], Costanze Hantel [5, 6], Emanuele Ferrante [4], Maura Arosio [1, 4], Giovanna Mantovani [1, 4], Erika Peverelli [1, 4]

[1] *Department of Clinical Sciences and Community Health,* University of Milan, Milan, Italy
[2] *PhD Program in Experimental Medicine,* University of Milan, Milan, Italy
[3] *PhD program in Endocrinological Sciences,* University Sapienza of Rome, Rome, Italy
[4] *Endocrinology Unit,* Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[5] *Department of Endocrinology, Diabetology and Clinical Nutrition,* University Hospital Zurich (USZ) and University of Zurich (UZH), Zurich, Switzerland
[6] *Medizinische Klinik und Poliklinik III,* University Hospital Carl Gustav Carus Dresden, Dresden, Germany

The cytoskeletal actin-binding protein filamin A (FLNA) is poorly expressed in adrenocortical carcinomas (ACC) compared to adenomas (ACA), and this might contribute to sustain the increased cell proliferation by upregulating IGF1R expression and its downstream signaling. In mouse neural progenitor cells, increased protein expression levels of the CDK1 kinase Wee1 have been found after loss of FLNA. This protein has a leading role in regulating the G2-M checkpoint and functions as a mitotic inhibitor. Wee1 is overexpressed in several cancer types and its pharmacological inhibitor Adavosertib (AZD1775) is currently undergoing clinical trials. Aims of the present project are to investigate the role of FLNA in regulating Wee1, the effects of Wee1 inhibition on cell proliferation and apoptosis, as well as the effects of FLNA levels on AZD efficacy in human H295R and MUC-1 cell lines. The analysis of protein expression levels of FLNA and Wee1 in 6 ACC and 8 normal adrenal tissues revealed that ACC express lower levels of FLNA (0.4±0.7 and 2.9±0.9, respectively, $p<0.05$), while significantly increased Wee1 (0.26±0.1 and 0.01±0.06, respectively, $p<0.001$). In MUC-1 cells a correlation between Wee1 and FLNA expression was shown. Indeed, FLNA silencing induced an increased expression of Wee1, phosphorylated CDK1 and cyclin B1 (+1.6±0.2, +1.7±0.4 and +1.4±0.2 fold, $p<0.001$ vs negative control, respectively). On the contrary, FLNA overexpression resulted into a reduced expression of Wee1, phosphorylated CDK1 and cyclin B1 (-51±5%, $p<0.001$, -60±5%, $p<0.001$, -67±20%, $p<0.01$ vs mock). Treatment with AZD1775 induced a

dose-dependent reduction of cell proliferation (-32±4.6%, $p<0.001$; -78±4.8%, $p<0.001$ vs bas at 250 nM) and an increase of apoptosis (+6±1.5-fold, $p<0.001$; +4±0.5-fold, $p<0.001$ vs bas at 1 mM) in H295R and MUC-1 cell lines, respectively. Flow cytometric analysis of apoptotic subpopulations showed that, in MUC-1, Wee1 inhibition specifically stimulated an increase of the early apoptotic cells (+5-fold, $p<0.001$ vs bas at 1 mM). Moreover, AZD1775 induced a time-dependent reduction of CDK1 phosphorylation, with a maximum after 2 hours (-67±9.5%, $p<0.01$; -90.5±1.7%, $p<0.001$ vs bas at 250 nM in H295R and MUC-1, respectively). Interestingly, FLNA knockdown potentiated AZD1775 effects on cell proliferation (-51±6.4%, $p<0.01$ vs negative control at 250 nM) in MUC-1 cells. In conclusion, this work demonstrates that low FLNA levels in ACC correlate to a high Wee1 expression, contributing to an increased cell growth. Moreover, it proposes Wee1 inhibition as a new potential therapeutic approach for ACC, particularly for those lacking FLNA.

PP9: CHEMOTHERAPEUTIC RESISTANCE IN ADRENOCORTICAL CARCINOMA

Sarah Feely [1], Nathan Mullen [1], Cong Hong [1], Abhay Pandit [2], Constanze Hantel [3], Michael Dennedy [1]

[1] *School of Medicine,* Discipline of Pharmacology and Therapeutics, University of Galway, Galway, Ireland
[2] *Biomedical Science Building ,* Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CURAM), Galway, Ireland
[3] *Department of Medicine IV ,* Ludwig Maximilian University of Munich , Munich, Germany

Adrenocortical carcinoma (ACC) is a rare aggressive cancer with low overall survival. Adjuvant mitotane is the only drug specifically improved for treatment of ACC. It improves survival but is limited by poor response rates and the devlopment of resistance. It can be combined with etoposide, doxorubicin, and cisplatin for metastatic ACC. However, this scheme presents limited efficacy. The exact mechanisms of these agents in ACC is not fully understood. Understanding these effects could improve their therapeutic potential. To investigate the underlying mechanisms in vitro, we treated ACC cells- MUC-1, HAC15 and H295R in monolayer and 3D cell culture models with increasing doses of mitotane, cisplatin and gemcitabine. Annexin V and sytox blue staining was used for cell death analysis with The Click-iT™ Plus EdU Flow Cytometry Assay Kits analysing cell cycle changes. All cells were resistant to gemcitabine up to doses of 1000uM. Cisplatin and mitotane induced apoptotic cell death in H295R and HAC15 cells. In MUC-1 cells, cisplatin induced cell death via apoptosis whereas mitotane induced necrosis. All cells were more resistant to mitotane in 3D cell culture compared to monolayer. MUC-1 cells were more resistant to cisplatin at the highest dose only. HAC15 and H295R cells were more resistant to cisplatin at all doses in 3D compared to monolayer. We highlight the mechanisms of cell death of chemotherapeutic agents in ACC and their effects on cell cycle highlighting important pathways and markers involved. Understanding these mechanisms offers potential novel therapeutic exploitation, particularly in chemotherapeutic resistant disease.

PP10: CIRCULATING TUMOR CELLS: A NOVEL TECHNIQUE FOR SINGLE CELL ISOLATION AND ANALYSIS IN ADRENOCORTICAL CARCINOMA

Giulia Cantini [1], Francesca Salvianti [1], Roberta Armignacco [3], Letizia Canu [1], Laura Fei [1], Arianna Pia Propato [1], Luca Galtarossa [1], Tonino Ercolino [4], Gabriella Nesi [2], Massimo Mannelli [1], Pamela Pinzani [1], Michaela Luconi [1]

[1] *Experimental and Clinical Biomedical Sciences,* University of Florence, Florence, Italy
[2] *Department of Health Sciences,* University of Florence, Florence, Italy
[3] *Institut Cochin, INSERM ,* Paris Descartes University, Paris, France
[4] *Endocrinology,* University Hospital of Careggi, Florence, Italy

Specific markers for early diagnosis and for studying tumour heterogenicity are urgently needed for adrenocortical carcinoma (ACC), a rare and aggressive tumor, characterized by a poor prognosis, in particular when metastatic at diagnosis. As previously demonstrated, circulating tumor cells (CTCs) have been detected in ACC patients and their number seems to have prognostic value. The non-standardize detection approach with cell filters used so far in ACC for CTC isolation can affect the clinical value of this marker and limits the analysis of the genetic variance at single-cell level. In this study, we aimed to develop a new and more specific method for single CTC isolation in ACC patients. Blood samples collected in Streck tubes from 5 ACC patients were subjected to size-based enrichment with the Parsortix Technology (Angle PLC, UK). Recovered cells were immunostained for CD45 and with antibodies against the Steroidogenic factor 1, for leucocytes (WBC) and CTCs of adrenocortical origin, respectively. The immunostained cells were imaged and sorted with the DEPArray system (Silicon Biosystems). Our study allowed the set-up of a standardized method to isolate single CTCs in ACC patients. For the first time, we applied to ACC a combination of Parsortix/DEParray technologies with a specific nuclear immunostaining to isolate CTCs and demonstrate their adrenocortical origin in order to evaluate their mutational profile at single cell level. In conclusion, this new procedure allows the genetic analysis of individual CTCs for the detection of chromosomal alterations/copy number variants by using a minimally-invasive blood drawing. This approach will be useful to drive personalized post-surgery therapies in ACC patients.

PP11: INSULIN-LIKE GROWTH FACTOR 1 RECEPTOR (IGF1R) AND INSULIN RECEPTOR (IR) DIFFERENT ROLES IN PRIMARY AND METASTATIC ADRENOCORTICAL CARCINOMA CELLS

Rosa Catalano [1], Emma Nozza [1, 2], Emanuela Esposito [1, 2], Anna Maria Barbieri [1], Giusy Marra [1], Donatella Treppiedi [8], Genesio Di Muro [1, 3], Federica Mangili [8], Costanze Hantel [4, 5], Sandra Sigala [6], Elisa Cassinotti [7], Ludovica Baldari [7], Valentina Morelli [9], Serena Palmieri [8], Sofia Frigerio [8], Emanuele Ferrante [8], Maura Arosio [1, 8], Giovanna Mantovani [1, 8], Erika Peverelli [1, 8]

[1] *Department of Clinical Sciences and Community Health,* University of Milan, Milan, Italy
[2] *PhD Program in Experimental Medicine,* University of Milan, Milan, Italy
[3] *PhD Program in Endocrinological Sciences,* University Sapienza of Rome, Rome, Italy
[4] *Department of Endocrinology, Diabetology and Clinical Nutrition,* University Hospital Zurich (USZ) and University of Zurich (UZH), Zurich, Switzerland
[5] *Medizinische Klinik und Poliklinik III,* University Hospital Carl Gustav Carus Dresden, Dresden, Germany
[6] *Section of Pharmacology, Department of Molecular and Translational Medicine,* University of Brescia, Brescia, Italy
[7] *Department of Surgery,* Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[8] *Endocrinology Unit,* Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[9] *Unit for Bone Metabolism Diseases and Diabetes,* Istituto Auxologico Italiano, IRCCS, Milan, Italy

Adrenocortical carcinoma (ACC) is a rare endocrine tumor characterized by a poor prognosis and molecular heterogeneity. Although the insulin-like growth factor 2 (IGF2), overexpressed in the majority of ACC, drives a proliferative autocrine loop by binding IGF1R and the isoform A of the insulin receptor (IRA), most studies focused on IGF1R. Recently, a higher expression of IRA was observed in ACC vs normal adrenal tissues (NA), suggesting its potential involvement in modulating IGF2 effects in adrenocortical tumorigenesis. Aim of this study was to investigate the expression and different contribution of IGF1R and IR in mediating IGF2 tumorigenic effects in ACC cell lines and primary cultures. First, we demonstrated an IGF1R higher expression in ACC than NA and a greater IRA/IRB ratio in ACC than adrenocortical adenomas and NA. MUC-1 and TVBF-7, derived from ACC metastatic tissues, presented a lower expression of IGF1R, IRA and IRB but a greater amount of IGF2, sign of an active pathway, than H295R derived from primary tumor. IGF1R, IR and IGF1R+IR silencing did not

impact on H295R proliferation. On the contrary, in metastatic ACC cells, IGF1R+IR knockdown decreased proliferation, suggesting an involvement of both receptors. At last, in ACC primary cultures, expressing higher levels of IRA than IRB, proliferation was reduced in IR silenced cells, but not after IGF1R depletion. In conclusion, we found a tumor-specific role of IGF1R and IR. In ACC metastases both receptors seem to play as main actors in promoting cell growth but only IR might mediate proliferation in primary tumor.

PP12: SOMATIC MUTATIONS IN ADRENOCORTICAL CARCINOMA: INVESTIGATING NEW PROGNOSTIC BIOMARKERS

Liang Zhang [1], Samuel Backman [2], Joakim Crona [1]

[1] *Department of Medical Sciences,* Uppsala University, Uppsala, Sweden
[2] *Department of Surgical Sciences,* Uppsala University, Uppsala, Sweden

Background: Tumor proliferation and stage is currently used to identify adrenocortical carcinoma (ACC) patients with a high risk of recurrence after surgery. Patterns of chromosomal aneuploidy pattern, drive gene mutation status and CpG island methylator phenotype have also been associated with prognosis. Aim: To collect a machine learning training dataset to enable search of new prognostic biomarkers. Methods: A literature search identified ACCs with paired clinical data and exome or whole genome sequencing. Information on prognostic markers (ENSAT stage, Ki67, resection status) as well as outcome data (Recurrence free and overall survival, metastasis) were collected. Results: 214 patients among which 67.8% were female with a median (interquartile range, IQR) age at diagnosis of 45 (27-59) years were included. 71.7% (142/198) of the tumor was classified as functional. Tumor stage was available for 201 patients: stage I (10%), II (43.3%), III (24.4%), and IV (22.4%). Tumor proliferation was available in 15 patients. Survival data was available in 197 patients: 39.3% of patients had died and the median follow-up time of 37 months (range: 18.4-67 months). Out of 80 patients, 66.3% experienced a relapse. Metastatic disease was observed in 24.6% (51/207). All patients had paired genetic data with a total of 16,286 coding and non-coding somatic variants were identified in 9,298 genes. Median number of somatic mutations was 28 (IQR 14-50). Conclusion: We have obtained a dataset to apply machine learning to identify novel patterns of somatic mutations associated with prognosis. Further results will be supplemented in the updated version.

PP13: GENE REGULATION BY BETA-CATENIN AND NR5A1 IN ADRENOCORTICAL CARCINOMA: ARE WE GETTING IT RIGHT ?

Enzo Lalli [1, 2, 3]

[1] *IPMC,* CNRS, Valbonne, France
[2] *IPMC,* Inserm, Valbonne, France
[3] *IPMC,* Université Côte d'Azur, Valbonne, France

CTNNB1 (encoding beta-catenin) activating mutations and overexpression of transcription factor NR5A1 are associated with poor outcome in adult patients with ACC. In a recent paper (Mohan et al., 2023) the authors concluded that beta-catenin plays an important role in driving adrenocortical differentiation in H295R ACC cells by associating with NR5A1 on chromatin and coregulating gene expression. Reanalysis of the data generated by the authors showed that the majority (437) of NR5A1/beta-catenin overlapping ChIP peaks contained one or more TCF (the canonical transcription factor partner for beta-catenin) binding motifs, associated or not to an NR5A1/NR5A2 motif, while only 155 NR5A1/beta-catenin intersect ChIP peaks contained exclusively an NR5A1/NR5A2 motif without an associated TCF binding site. These data suggest that beta-catenin predominantly interacts with cognate TCF binding sites on DNA even when beta-catenin peaks contain NR5A1/NR5A2 motifs. To investigate the crosstalk of NR5A1 and beta-catenin on gene expression programs in H295R cells, I compared the published datasets of both NR5A1–regulated and beta-catenin–regulated genes in this cell line. Out of 44 genes positively regulated by beta-catenin, only 5 (CADPS, GRPR, ISM1, ITGA9 and SHOC1) were in common with genes positively regulated by NR5A1 in at least one of those datasets while only 4/29 negative beta-catenin target genes (ITGA8, JAG1, FAM105A and LXN) were commonly downregulated by NR5A1 and beta-catenin. Overall, in contrast to the conclusions by Mohan et al., these data represent strong evidence that in H295R ACC cells NR5A1 and beta-catenin regulate mostly distinct gene expression programs.

PP14: THE INTRATUMORAL MICROBIOTA COMPOSITION MODULATES ADRENOCORTICAL CANCER RESPONSIVENESS TO MITOTANE

Laura Fei [1, 2], Giulia Cantini [1, 2], Elena Niccolai [3], Arianna Pia Propato [1, 2], Letizia Canu [1, 2], Luca Galtarossa [1], Soraya Puglisi [4], Gabriella Nesi [5], Amedeo Amedei [3], Michaela Luconi [1, 2]

[1] *Experimental and Clinical Biomedical Sciences,* University of Florence, Florence, Italy
[2] *Florence ENS@T Center of Excellence,* ENS@T , Florence, Italy
[3] *Experimental and Clinical Medicine,* University of Florence, Florence, Italy
[4] *Internal Medicine, Department of Clinical and Biological Sciences,* University of Turin, Orbassano, Italy
[5] *Department of Health Sciences,* University of Florence, Florence, Italy

The infiltrating microbiota represents a novel cellular component of solid tumor microenvironment affecting tumor progression and response to therapy. Mitotane (MTT) efficacy as the first line therapy of adrenocortical carcinoma (ACC) is limited to a therapeutic window level (14-20 mg/L). Novel markers able to predict those patients who would reach this window would improve patient's management. The aim of our study was to evaluate the presence of intratumoral bacterial microbiota in 26 human ACC tissues versus 9 healthy adrenals; moreover, the association between the relative bacterial composition profile, the tumor mass characteristics and MTT ability to reach high circulating levels in the early phase of treatment, was explored. Bacterial DNA was found in all adrenal samples from both tumors and healthy cortex with significant differences in the microbial composition between malignancy and normal adrenals: ACC specimens were characterized by a higher abundance of the Proteobacteria phylum (Pseudomonas and Serratia genera). Proteobacteria low abundance negatively associated with tumor size, Ki67 and cortisol-secretion. Mitotane levels reached higher levels at 9 months in ACC patients with high abundance of Proteobacteria and low abundance of Bacteroidota, Firmicutes and Streptococcus. In conclusion, this is the first indication that human ACCs are characterized by infiltrating bacteria and their specific abundance profile seems to influence the rapid increase in circulating MTT levels.

PP15: THE IMPACT OF SURGERY ON SURVIVAL OF PATIENTS PRESENTING WITH METASTASIZED ADRENOCORTICAL CARCINOMA: A SYSTEMATIC REVIEW AND META-ANALYSIS

Charlotte L. Vietor [1, 2], Ivo J. Schurink [1], Dirk J. Grunhagen [1], Cornelis Verhoef [1], Gaston J.H. Franssen [1], Richard A. Feelders [2], Tessa M. van Ginhoven [1]

[1] *Department of Surgical Oncology and Gastrointestinal Surgery,* Erasmus MC Cancer Institute, Erasmus University Medical Center, Rotterdam, Netherlands
[2] *Department of Internal Medicine, division of Endocrinology,* Erasmus University Medical Center, Rotterdam, Netherlands

Background: Up to 30% of adrenocortical carcinoma (ACC) patients have metastasized disease upon initial presentation. Palliative surgery can be considered to relieve symptoms of mass effect or hormonal overproduction. Since systemic treatments currently fail to sufficiently improve survival, the potential survival benefit of surgery in asymptomatic metastatic ACC patients remains a topic of debate. Therefore, this study aims to assess the survival benefit of surgical strategies for patients with metastatic ACC. Methods: Relevant databases were searched from January 2000 until June 2023 for studies on surgery for metastatic ACC. Overall survival data after surgical options were analysed. Results: Twelve studies were included, with nine articles focusing on primary site surgery, two articles on metastasectomy, and four articles on complete versus incomplete debulking surgery. Baseline patient characteristics were described in five articles (47%), and indication for surgery was lacking in all included articles (unclear if surgery was performed for invalidating symptoms). Therefore, there is a considerable possibility of selection bias. Whereas meta-analysis suggests improved 2-year survival after primary site surgery (31% versus 8%, $p<0.01$) and a trend towards improved 2-year survival after complete debulking surgery (54% versus 33%, $p=0.05$), these data are not applicable to decide on surgery for asymptomatic metastatic ACC patients specifically due to inadequate description of patient characteristics and indication for surgery in the available literature. Conclusion: Based on the available literature, there is currently no evidence to support routine surgical treatment of asymptomatic metastatic ACC patients in order to improve survival. Future studies should provide adequate patient characteristics in order to enable interpretation of their results for clinical use.

PP16: TIME TRENDS IN ADRENOCORTICAL CARCINOMA BETWEEN 1993 AND 2020

Rebecca Steenaard [1], *Harm Haak* [1]

[1] *Internal Medicine,* Máxima MC, Eindhoven, Netherlands

We aimed to determine development in adrenocortical carcinoma incidence, survival and treatment using the nation-wide prospective Netherlands Cancer registry. All 697 adult patients with adrenocortical carcinoma diagnosed between 1993 and 2020 were included. The median age-adjusted incidence rate based on the European Standard Population was 1.62 per million person-years [0.8-2.11] and was stable over time. We saw a gradual increase in stage III on diagnosis (15 to 25%) with a stable proportion of stage IV (40%). Even with this increase, the one-year survival (46%) and the five-year survival (29%) of the total group remained stable over time. Since the founding of the Dutch Adrenal Network in 2004, more patients were referred to an expertise center which was associated with a survival benefit (adjusted HR 0.70, 95%CI[0.57-0.85]). Multivariate cox-regression also showed a survival benefit from adrenalectomy (HR 0.53, 95%CI[0.43-0.65]) and mitotane therapy (HR 0.73, 95%CI[0.55-0.98]) in the total group. When looking at stage IV disease, adrenalectomy, surgical control of disease, chemotherapy and mitotane therapy were all associated with a survival benefit.

PP17: OUTCOMES OF PATIENTS WITH RECURRENT ADRENOCORTICAL CARCINOMA

Ivana Dora Vodanović [1], Karin Zibar Tomšić [1], Anja Barač Nekić [1], Ivana Kraljević [1, 2], Darko Kaštelan [1, 2]

[1] *Endocrinology and Diabetology Department,* University Hospital Centre Zagreb, Zagreb, Croatia
[2] *School of Medicine,* University of Zagreb, Zagreb, Croatia

Background: The objective of this study was to evaluate the outcomes of patients with adrenocortical carcinoma (ACC) who had disease recurrence after complete surgical resection of the primary tumor. Methods: This retrospective study included 18 patients, 16 of whom had R0 resection and two Rx resection of the primary tumor. The main endpoints of the study included assessment of progression-free survival and overall survival after disease recurrence. Results: Out of 52 patients who had undergone radical resection of ACC, 18 (12 (66.7%) female; age 49 (18-71) years) experienced disease recurrence after median time of 29 (18-50 months). Five patients had local recurrence, nine had distant metastases, while other four had both local recurrence and distant metastases. Various treatment modalities of ACC recurrence were applied; eight patients (44.4%) underwent surgical removal of the recurrent lesion(s), one patient (5.6%) received surgery along with chemotherapy, one (5.6%) received surgery combined with locoregional therapy, one patient (5.6%) received locoregional therapy along with chemotherapy, two patients (11.1%) underwent only locoregional therapy, whereas four patients (22.2%) received only chemotherapy. Mitotane treatment was initiated/continued in 13 (72.2%) patients. Following ACC recurrence, 11 (61.1%) patients experienced further disease progression after 15 (8-24) months. Five (27.8%) patients died after 8, 27, 29, 29 and 81 months of disease recurrence. Mean progression-free survival and overall survival after ACC recurrence were 23 and 119 months, respectively. Shorter time to ACC recurrence negatively impacted patient survival (p=0.048). Conclusion: By combining different treatment modalities, prolonged survival of patients with recurrent ACC can be achieved. However, a shorter time to ACC recurrence is associated with worse survival outcomes.

PP18: ADRENOCORTICAL CARCINOMA CELLS REWIRE THEIR METABOLISM TO OVERCOME CURCUMIN ANTITUMORAL EFFECTS OPENING A WINDOW OF OPPORTUNITY TO IMPROVE TREATMENT

Marta Claudia Nocito [1], Ivan Casaburi [1], Paola Avena [1], Adele Chimento [1], Arianna De luca [1], Rosa Sirianni [1], Vincenzo Pezzi [1]

[1] *Pharmacy and Health and Nutrition Sciences,* University of Calabria, Rende, Italy

Extensive research suggests that curcumin interferes with multiple cell signaling pathways involved in cancer development and progression. This study aimed to evaluate curcumin effects on adrenocortical carcinoma (ACC), a rare but very aggressive tumor. Curcumin reduced growth, migration and activated apoptosis in three different ACC cell lines, H295R, SW13, MUC-1. This event was related to a decrease in estrogen-related receptor-α (ERRα) expression and cholesterol synthesis. More importantly, curcumin changed ACC cell metabolism, increasing glycolytic gene expression. However, pyruvate from glycolysis was only minimally used for lactate production and the Krebs cycle (TCA). In fact, lactate dehydrogenase, extracellular acidification rate (ECAR), TCA genes and oxygen consumption rate (OCR) were reduced. We instead found an increase in Glutamic-Pyruvic Transaminase (GPT), glutamine antiport transporter SLC1A5 and glutaminase (GLS1), supporting a metabolic rewiring toward glutamine metabolism. Targeting this mechanism, curcumin effects were improved. In fact, in a low glutamine-containing medium, the growth inhibitory effects elicited by curcumin were observed at a concentration ineffective in default growth medium. Data from this study prove the efficacy of curcumin against ACC growth and progression and point to the concomitant use of inhibitors for glutamine metabolism to improve its effects.

PP19: MSI STATUS AS A PROGNOSTIC MARKER OF RESPONSE TO IMMUNOTHERAPY, IN PATIENTS WITH METASTATIC ADRENOCORTICAL CARCINOMA (ACC)

Maria Boudina [1], Pavlos Papakotoulas [2], Chrisanthi Zouli [1], Eleana Zisimopoulou [1], Aimilia Fotiadou [1], Mariana Stamati [1], George Christantoniou [1], Chrysanthi Balodimou [1], Elpiniki Argiropoulou [1], Alexandra Chrisoulidou [1]

[1] *Endocrinology,* Theagenio Cancer Hospital, Thessaloniki, Greece
[2] *Oncology,* Theagenio Cancer Hospital, Thessaloniki, Greece

Background: Adrenocortical carcinoma (ACC) is a very rare and aggressive tumor with poor prognosis that may develop at any age. Chemotherapy and radiotherapy plus mitotane have been administered in progressive, metastatic ACC patients, with poor results. The past few years, immunotherapy has been tried in such patients with disease progression after first line chemotherapy. Case presentation: We studied 5 patients with ACC (3 females- 2males), with median age 45.6 years. 40% of patients were Stage II at diagnosis, whereas 60% were Stage IV. All patients were treated with mitotane from the time of diagnosis. Three patients were treated with synchronous chemotherapy with EDP and mitotane and two patients, who were Stage II at diagnosis, received chemotherapy during follow up, due to local recurrence and new metastases development. All patients appeared disease progression, though they received cytotoxic chemotherapy. Immunotherapy with Pembrolizumab was delivered to all patients. Three of them had Microsatellite Instability (MSI) Status evaluation prior to immunotherapy whereas two did not have. One out of 3 had MSI High Status. She has been receiving Pemprolizumab for 22 months and her Abdomen CT is indicative of great decrease in her liver metastases (>60%), according to RECIST Criteria. On the other hand, the rest four patients, 2 with MSI stable Status and 2 without MSI evaluation, showed a PFS of 3-4 months. Conclusions: Immunotherapy with checkpoint inhibitors, in association with MSI Status of the tumor, seems to be a very promising in the treatment of ACC.

PP20: ADRENAL CORTICAL CARCINOMA INITIALLY MISTAKEN FOR AN ONCOCYTIC TUMOR- CASE STUDY

Karolina Wegrzyn [1], Grzegorz Sokołowski [1], Alicja Hubalewska- Dydejczyk [1]

[1] *Department of Endocrinology,* University Hospital of Jagiellonian, University Medical College, Cracow, Poland

We present a case report of a patient diagnosed with adrenal cortical carcinoma initially mistaken for an oncocytic tumor. Adrenal cortical carcinoma is a rare and highly aggressive cancer and its diagnosis can be challenging due to its nonspecific clinical presentation and radiological characteristics. In this case, the patient had an incidentally detected left adrenal gland mass during a CT scan. The imaging was initially suggestive of an adenoma. Hormonal tests showed disrupted cortisol circadian rhythm, and dexamethasone suppression tests failed to suppress cortisol secretion, indicating an endogenous hypercortisolism of adrenal origin. The patient underwent laparoscopic adrenalectomy. Histopathological examination initially revealed an oncocytic cortical tumor of uncertain malignant potential with no large malignant features but a small focus of necrosis present, with variable Ki 67 proliferative activity mostly below 2% and focally up to 4.7%. Despite the surgery, the patient continued to experience hypercortisolism. Further imaging studies were performed, Ga 68 scintigraphy did not show any lesions. Abdominal MRI revealed suspected clear cell renal carcinoma and angiomyolipoma. Additionally, there were scattered focal liver lesions suspected to be metastases. 18F-FDG PET show an active metabolic area in the right iliac bone, suggesting differentiation between solitary plasmacytoma and metastasis from clear cell renal carcinoma. A biopsy of the iliac bone lesion confirmed adrenal tumor metastasis, and the initial diagnosis was reclassified as an oncocytic variant of adrenal cortical carcinoma. In conclusion, this case highlights the diagnostic challenges associated with adrenal cortical carcinoma and emphasizes the importance of close monitoring and follow-up.

PP21: LONG-TERM SURVIVAL OF A PATIENT WITH METASTATIC ADRENOCORTICAL CARCINOMA

Barbara Ziółkowska [1]

[1] *2nd Clinic of Radiotherapy and Chemotherapy,* Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice Branch, Gliwice, Poland

In August 2014, a 36-year-old woman presented with left flank pain. Imaging revealed a mass of the left adrenal gland without metastases. After adrenalectomy, histology revealed adrenocortical carcinoma, with a 7 points Weiss score, Ki67 5%, resection R1. Hormonal investigations were normal. Regarding the non-radical resection status, adjuvant therapy with mitotane was initiated. The treatment had been continued for 30 months until February 2017, when the patient got pregnant. Abdominal MRI re-examination performed during pregnancy revealed multiple enlarged retroperitoneal lymph nodes, local recurrence and peritoneal dissemination. In November 2017, the patient gave birth to a healthy newborn boy via vaginal delivery at 38 weeks of gestation. In December 2017, systemic therapy was started (mitotane and eight cycles of chemotherapy EDP) with the best response of the stable disease. After 14 months, further progression occured, and in September 2019, the reinduction of EDP (Doxotubicine administered until cumulative total lifetime dose) was started, with best response of the stable disease. Twelve months later, the carcinoma recurred. In March 2021, the second reinduction with platinum-based therapy was started (five cycles of Carboplatin plus Etoposide). Due to the further progression, the growing tumour in the adrenal bed was irradiated dfx 1,8 Gy to the total dose of 45Gy, and then in June 2022, the fourth line of chemotherapy with Capecitabine and Temozolomide was initiated. Until recently, the patient remains on treatment, and no disease progression has been observed. The patient has been alive with metastatic disease for six years and four months.

PP22: THE 3D IN VITRO ADRENOID MODEL RECAPITULATES THE COMPLEXITY OF THE ADRENAL GLAND IN CANCER CONDITION

Arianna Pia Propato [4, 1], Serena Martinelli [2], Giulia Cantini [4, 1], Daniele Guasti [2], Laura Fei [4, 1], Letizia Canu [4, 1], Luca Galtarossa [1], Susan Richter [3], Nicole Bechmann [3], Giovanna Danza [1], Mario Maggi [4, 1], Massimo Mannelli [1, 4], Elena Rapizzi [2, 4], Michaela Luconi [1, 4]

[1] *Experimental and Clinical Biomedical Sciences,* University of Florence, Florence, Italy
[2] *Experimental and Clinical Medicine,* University of Florence, Florence, Italy
[3] *Institute of Clinical Chemistry and Laboratory Medicine,* Technische Universität Dresden, Dresden, Germany
[4] *Florence ENS@T Center of Excellence,* ENS@T , Florence, Italy

The adrenal gland is an endocrine organ consisting of the cortex of mesenchymal and the medulla of neuroectodermal origin. Since adrenal organogenesis, a correct differentiation and developing program of the gland is ensured by the crosstalk between the chromaffin and adrenocortical steroidogenic cells, essential for the functionality of the adult organ, including hormone synthesis and stress-response. The interaction between the two endocrine components is also likely to be pivotal in the development and progression of adrenocortical cancer and pheochromocytoma. The aim of this work was to develop an in-vitro 3D functional model of the whole adrenal gland, overcoming the limitations associated with adrenal cell single population research approaches, to study the role of such interactions in adrenal cancer. We set up a 3D-model, called adrenoid (ADR), established by mixing the human adrenocortical carcinoma cell line, H295R, with the mouse pheochromocytoma cell line, MTT. The ADR mass showed a round morphology and clear margins starting from 48 hours of induction, which were maintained until day 10, with a growth rate significantly higher compared to spheroids composed only by MTT cells (MTT-S). Confocal analysis of differential fluorescence staining of the two cell lines demonstrated that ADR organized from the very first hours in small clusters of H295R dispersed in a core of MTT cells. Electron transmission microscopy confirmed the strict interaction occurring between H295R and MTT cells, identified by functional ultrastructure features such as liposomes associated with mitochondria and expanded SER, typical of steroidogenic cells, while chromogranin granules associated with RER mark catecholaminergic cells. Interestingly, these specialized features are more evident in ADRs compared with single cell type monolayers, suggesting that coculture in 3D can potentiate endocrine differentiation. The maintenance of the dual endocrine activity in ADRs was further

demonstrated by tyrosine hydroxylase and chromogranin expression for the adrenal medulla, and by the steroidogenic factor-1 11b-HSD for the cortex components. Finally, mass spectrometry analysis demonstrated DOPA, dopamine, and both catecholamine production in ADRs, with a significant increase in noradrenaline at 10 vs 5 days of cultures. Steroid hormone secretion (androstenedione, testosterone, progesterone and 17OH progesterone, DHEA/DHEAS and 11betadeossicortisol) was also demonstrated by mass spectrometry, though cortisol in ADR conditioned media was undetectable. Interestingly, the ratio between neuroendocrine and steroidogenic cells tended to decrease between 5 and 10 days, corresponding to a decrease in steroidogenic activity of the ADR. In conclusion, ADR represents an innovative in vitro 3D model that maintains the spatial and functional complexity of the adrenal gland of origin, thus being useful to extend the repertoire of preclinical models of endocrine tumours for investigating the cellular mechanisms of adrenal cancer and testing of novel drugs.

PP23: EFFECT OF COMMONLY USED DRUGS ON FRACTIONATED METHANEPHRINE CONCENTRATIONS IN 24-HOUR URINE COLLECTION IN PATIENTS WITH INCINDENTALOMA

Marta Opalińska [1], Joanna Kokoszka [2], Magdalena Kolasa [2], Monika Szewczyk [2], Katarzyna Sitarz [3], Jolanta Bugajska [4], Joanna Berska [4], Krystyna Sztefko [4], Alicja Hubalewska-Dydejczyk [1]

[1] *Departament of Endocrinology,* Jagiellonian University, Medical College, Kraków, Poland
[2] *Department of Endocrinology, Oncological Endocrinology and Nuclear Medicine,* University Hospital in Krakow, Kraków, Poland
[3] *.,* University Hospital in Krakow, Kraków, Poland
[4] *Clinical Biochemistry Department of Pediatric Institute,* Jagiellonian University Medical College, Kraków, Poland

Introduction: Due to the frequent occurrence of adrenal incidentalomas, diagnosis of their hormonal activity is a significant clinical concern. Approximately 7-10% of adrenal tumors are pheochromocytomas, which even in silent form can lead to hemodynamic instability during surgery. According to the ESE and ENSAT guidelines in any case of adrenal tumors greater than 1 cm pheochromocytoma should be excluded based on plasma metanephrines level and urinary metanephrines excretion. Such approach can be challenging in patients with significant comorbidities or when patients are on drug which interfere with analytical measurement. Aim of the study: The aim of this study was to evaluate the effect of selected drug on metanephrines excretion in urine in patients with incidentaloma. Materials: 1102 patients with incidentalomas confirmed by CT or MR were included in the study. Patients with a clinical or biochemical diagnosis of Cushing's or Conn syndrome as well as patients likely having pheochromocytoma were excluded. In all patients, 24-hour urinary excretion of normetanephrine, metanephrine and 3-methoxytyramine were measured by high performance liquid chromatography (HPLC) with electrochemical detection. The information on concomitant medication (β-blockers, Ca-blockers, loop diuretics, thiazide diuretics, potassium-sparing diuretics, α-blockers, ACE inhibitors/aniotensin II receptor antagonists, metformin, other antidiabetic drugs, lipid lowering drugs, proton pump inhibitors, levothyroxines, thyreostatics, antidepressants, neuroleptics, benzodiazepines, glucocorticosteroids and B receptor agonists) were collected in all analyzed cases. Results: Urine excretion of normetanephrine was significantly higher in patients (without

medication vs. medication) using Ca-blockers (282.6 vs 333.8µg/24h, p<0.0001), β-blockers (288.9 vs 332.3µg/24h, p<0.0001), loop diuretics (299.4 vs 374.5µg/24h, p<0.0001), α-blockers (299.3 vs 352.7µg/24h, p=0.0001), non-metformin antidiabetic drugs (301.0 vs 366.15µg/24h, p=0.0001) and neuroleptics (305.4 vs 384.3 µg/24h, p=0.0307). Urinary metanephrine excretion was significantly higher in patients taking α-blockers (109.2 vs 136,9µg/24h, p<0.0001) and was lower in patients taking non-metformin antidiabetic drugs (114.3 vs. 99.3µg/24h, p=0.0247), antidepressants (114.3 vs 97.7=8µg/24h, p=0.0207) and glucocorticosteroids (114.6 vs 94.8µg/24h, p=0.0043). Urinary 3-methoxytyramine concentration was significantly higher in patients taking thiazide diuretics (191.8 vs. 264.5µg/24h, p=0.0031) and antidepressants (195.2 vs 317.9µg/24h, p=0.0005). The other drugs had no significant effect on the results of urinary excretion of catecholamines. Conclusion: Many of the commonly used drug groups significantly affect the results of 24-hour urine collection of fractionated methanephrines. Interpretation of these results should be adjusted and take into account the effect of the drug groups used on the results obtained and individualized in the clinical context.

PP24: LARGE INTERNATIONAL COLLABORATIVE STUDY ON THE ROLE(S) OF SUCCINATE DEHYDROGENASE (SDH) GENE VARIANTS IN PARAGANGLIOMA-PHEOCHROMOCYTOMA PHENOTYPES

Jean-Pierre Bayley [1], Peter Devilee [1]

[1] *Human Genetics,* Leiden University Medical Center, Leiden, Netherlands

We recently found that the category of succinate dehydrogenase (SDH) gene variant (missense/truncating) significantly impacts paraganglioma-pheochromocytoma phenotypes (Bayley et al. PLoS One, 2022;17(9).), a discovery that will open a path to personalized genetic counselling and clinical surveillance for individuals carrying succinate dehydrogenase subunit B (SDHB) and subunit D (SDHD) gene variants. In a new international collaborative study, a total of 57 centres located in Europe, North America, Asia and Australia have joined together to provide genetic and phenotypic data on >2700 paraganglioma-pheochromocytoma patients carrying a total of 393 SDHB, SDHC and SDHD gene variants. In the current study we will use the largest genetic dataset on paraganglioma-pheochromocytoma patients assembled to date to first summarise basic tumour phenotypes in this very large patient group. We will then externally validate our previous results and extend the detailed understanding of the role of specific SDHB/SDHD missense variants in paraganglioma-pheochromocytoma. Although analysis is ongoing, this study has already produced exciting new findings regarding age of disease onset, parameters of malignancy, as well the specific role of SDH gene variants. In addition to immediate clinical benefits in terms of risk prediction and improved surveillance, this effort will facilitate future international collaborations aimed at predicting risk in unaffected carriers of SDH variants, as well as opening a path to a better understanding of the functional (biological) underpinnings of these associations.

PP25: MI RNAS EXPRESSION PATTERN IN PHEOCHROMOCYTOMAS AND PARAGANGLIOMAS: A PROGNOSTIC TOOL?

Foteini Thanasoula [1], Maria Yavropoulou [3], Georgios Kyriakopoulos [2], Chrysoula Mytareli [1], Eva Kassi [4], Gregory Kaltsas [5], Anna Angelousi [1]

[1] *Endocrinology Unit, 1st Dpt of Internal Medicine,* National and Kapodistrian University,Laikon Hospital, Athens, Greece
[2] *Department of Pathology,* Evangelismos Hospital, Athens, Greece
[3] *Unit of Endocrinology, 1st Department of Propaedeutic and Internal Medicine,* National and Kapodistrian University of Athens, Laikon Hospital, Athens, Greece
[4] *Department of Biochemistry, 1st Department of Propaedeutic and Internal Medicine,* National and Kapodistrian University of Athens,, Athens, Greece
[5] *Unit of Endocrinology, 1st Department of Propaedeutic and Internal Medicine,* National and Kapodistrian University of Athens, Laikon Hospital, Athens, Greece

Introduction: Pheochromocytomas (PCs) and paragangliomas (PGLs) are rare neuroendocrine tumors, exhibiting 5-26% incidence of metastatic disease. No reliable histomorphological markers exist to distinguish benign from metastatic PCs/PGLs. Recent data have shown altered expression of specific microRNAs between metastatic and benign PCs/PGLs, suggesting their potential prognostic role. Aim: The analysis of specific microRNA's expression pattern in PC/PGLs tissues samples and normal ones. Methods: MiRNA15, miRNA16, miRNA101 and miRNA183 expression was analysed through polymerase chain reaction (RT-qPCR) in 48 formalin-fixed paraffin-embedded tissue samples (FFPE) of patients with PCs/PGLs (n=36) and in 12 FFPE tissue samples of normal adrenal medulla. We included 16 patients diagnosed with PCs (n=7/16 metastatic) and 20 with PGLs (n=4/20 metastatic). Six patients had germline mutations (n=4 with SDHX, n=1 NF1 and n=1 RET). Results: Our analysis showed a statistical significant overexpression of the miRNA101 in PCs tissue samples comparing with the normal adrenal medulla samples (p=0.01) as well as a in patients' tissues with metastatic PCs/PGLs comparing with the non-metastatic ones (p=0.05). No statistical significant difference was found regarding the expression of mi RNA15, miRNA16 and miRNA183 in PC/PGLs tissues samples and the normal ones. Conclusion: The miRNA101 was overexpressed in all PCs tissues compared to the normal as well as in all metastatic PCs in comparison with non-metastatic ones. SDHD mutation may enhance the overexpression of miR-101 in malignant tumors. Further analysis should be conducted to clarify it's prognostic role in PC/PGLs tumours.

PP26: CHARACTERISATION OF ADULT SDHB ZEBRAFISH MUTANTS TO STUDY SDHB-ASSOCIATED PARAGANGLIOMAS

Margo Dona [1], Jasmijn Miltenburg [1], Iris Versteeg [1], Niek Strijker [1], Inge Outersterp [1], Alex Nowak [1], Bibian Verstegen [1], Denise Mallander [1], Femke ten Seldam [1], Ana Merthens [1], Andor Veltien [2], Ine van Raaij [3], Andre Olthaar [4], Benno Kusters [3], Teun van Herwaarden [4], Tom W.J. Scheenen [2], Aswin Menke [5], Mirko Peitzsch [7], Catleen Conrad [7], Susan Richter [7], Graeme Eisenhofer [7], Arend Heerschap [2], Marnix Gorissen [6], Henri J.L.M. Timmers [1]

[1] *Internal Medicine,* Radboud University Medical Center , Nijmegen, Netherlands
[2] *Medical Imaging,* Preclinical Imaging Center, RadboudUMC, Nijmegen, Netherlands
[3] *Pathological Anatomy,* Radboud University Medical Center , Nijmegen, Netherlands
[4] *Laboratory Medicine,* Radboud University Medical Center , Nijmegen, Netherlands
[5] *Metabolic Health Research,* TNO quality of Life, Leiden, Netherlands
[6] *Animal Ecology and Physiology,* Institute for Water and Wetland Research,Radboud University Nijmegen, Nijmegen, Netherlands
[7] *Medicine III,* Clinical Chemistry and laboratory medicine, Dresden, Germany

Introduction and aim: Patients with SDHB mutations have the highest risk to develop incurable metastatic phaeochromocytomas and paragangliomas (PPGLs). In order to be able to study the pathways behind tumorigenesis and identify and test new therapeutic targets model systems are required. We generated a sdhbrmc200 zebrafish mutant and investigated the potential of heterozygous adult zebrafish as PPGL tumour model. Methods: To detect and monitor tumour development we designed multiple functional read-outs, e.g. histology, behavioural analysis, catecholamine measurements and anatomical imaging (Magnetic Resonance Imaging) including succinate detection via Magnetic Resonance Spectroscopy. Results: So far, no changes in gross morphology, the amount or morphology of chromaffin cells were detected in heterozygous sdhb mutant fish. Behavioural tracking of adult fish showed an higher activity in heterozygous sdhb mutant fish. Normetanephrine and 3-methoxytyramine levels in the urine of heterozygous sdhb mutant fish were normal compared to wild-type siblings. However, an increase in succinate level in muscle tissue was observed in a subpopulation of heterozygous sdhb adult fish compared to wild-type fish. Conclusion: Until now, sdhb heterozygous adult zebrafish did not develop an obvious tumour phenotype, despite the observation of elevated systemic succinate levels. Human heterozygous carries of germline SDHB gene mutation require a second somatic mutation for tumorigenesis. In order to attempt the induction of tumour formation in adult sdhb heterozygous mutant fish, we are currently exploring different

aggravation strategies: including radiation to induce a second hit, hypoxia to induce HIF-1α stabilisation and a diet to increase the level of reactive oxygen species.

PP27: GENOTYPE CHARACTERISATION OF ROMANIAN PATIENTS WITH PGLS

Sofia Lider [2, 3], Monica Gheorghiu [1, 3], Attila Patocs [4, 5], Corin Badiu [1, 3]

[1] *Thyroid,* National Institute of Endocrinology CI Parhon, Bucharest, Romania
[2] *Endocrinology,* National Institute of Geriatric and Gerontology Ana Aslan, Bucharest, Romania
[3] *Endocrinology,* University of Medicine and Pharmacy Carol Davila, Bucharest, Romania
[4] *Genetics,* Semmelweis University, Budapest, Hungary
[5] *Genetics,* Hungarian Academy of Sciences, Budapest, Hungary

Introduction: Paragangliomas (PGLs) are rare tumors arising of the chromaffin tissue. Approximately 50% of patients with PGLs harbour a germinal mutation. Nowadays more than 21 genes are known to be involved in PGLs development. Aim: To describe the genotype aspects of a cohort with patients from a Tertiary Centre of Endocrinology from Romania. Material and methods: We retrospectively retrieved DNA probes from the peripheral blood cells of the patients with PGLs from 1976-2022 and were analysed using NGS gene panel in a Genetic Lab from Hungary. Results: From 113 patients diagnosed with PGLs, in 80 we could proceed genetic test. For the rest of 40, the DNA probes were not available. From those 80 patients, 47 (58.5 %) had germinal mutation either in a PGLs related/non-related gene, while 33(41.5%) had any mutation. The most prevalent mutated gene was RET (20), followed by NF1 MEN1 (3), (2), VHL (2), SDHB (2), SDHD (1), MET (1). Other genes identified in these patients were: MSH2 (in a patients with clinical features of neurofibromatosis), MSH6 (in a patients with thoracic PGL), BRIP1 (in 2 patients with metastatic PHEO), MSH3, FANCA, KIF1B, BARD1, ATM, CASR, BMPR1A, PALB2, RAD51B, RB1. Conclusion: RET mutation is the most prevalent in Romanian patients with PGLs. We identified new genes involved in PGLs physiopathology. A special attention should be allocated to mutation involved in metastatic PGLs. Our study reinforces the high variability of germinal mutation in PGLs and opens a new direction in the diagnosis and research of these tumors.

PP28: IDENTIFICATION OF NOVEL SUSCEPTIBILITY GENES IN HEAD AND NECK PARAGANGLIOMAS

Sara Mellid [1], Ángel Mario Martínez-Montes [1], María Monteagudo [1], Alberto Díaz-Talavera [1, 2], Rocío Letón [1], Emiliano Honrado [3], Miguel Soria-Tristán [4], Mercedes Robledo [1, 2], Alberto Cascón [1, 2]

[1] *Hereditary Endocrine Cancer Group,* Spanish National Cancer Research Centre (CNIO), Madrid, Spain
[2] *Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER),* Ministry of Science and Innovation, Health Institute Carlos III, Centro de Investigación Biomédica en Red (CIBER), Madrid, Spain
[3] *Anatomical Pathology Service,* Hospital of León, León, Spain
[4] *Oncology Department,* Hospital Universitario de Getafe, Getafe, Spain

Pheochromocytomas (PCCs) and paragangliomas (PGLs) are rare neuroendocrine tumors with a high heritability rate. Nearly 40% of patients carry germline mutations in one of the 18 known susceptibility genes, and an additional 30% develop sporadic tumors with known driver mutations. Among patients without mutations (germline or somatic), PGLs located in the head and neck (H&N) are frequent. In 2018, our group identified for the first time the presence of mutations in the DNMT3A gene in patients with H&N PGLs. Differential expression analysis derived from this study allowed us to select a group of six H&N PGLs with similar molecular and transcriptional characteristics to DNMT3A-mutated tumors but without any alteration in known susceptibility genes, including DNMT3A. To note, these tumors did not present 1p or 11q chromosomal losses by SNParray, and SDHB immunohistochemistry was positive in all cases, ruling out SDH genes (the most frequently mutated genes in H&N PGLs) as being involved in their development. To identify the molecular alteration responsible for the development of these tumors, we first aim to increase the number of candidate tumors harboring a common alteration. 30 H&N PGLs without mutations in known susceptibility genes have been selected to perform a gene expression study by RNAseq in hopes of identifying additional tumors with the same expression profile as the previously identified cases. Next, whole genome sequencing of the selected cases (blood and tumor DNA samples) will be performed in order to identify shared alterations (point mutations or rearrangements), and functional studies will be carried out to demonstrate the pathogenicity of the identified alterations.

PP29: PROTEINS AND PEPTIDES FROM THE GRANIN FAMILY AND INSM-1 IN THE BIOCHEMICAL DIAGNOSTICS OF PHEOCHROMOCYTOMA

Piotr Glinicki [1], Alicja Szatko [1], Magdalena Ostrowska [1], Lucyna Papierska [1], Wojciech Zgliczyński [1]

[1] *Department of Endocrinology,* Centre of Postgraduate Medical Education, Warsaw, Poland

Introduction: Pheochromocytoma is a rare, usually benign tumor composed of neuroendocrine (chromaffin) cells of the adrenal medulla. It is the cause of secondary arterial hypertension. The biochemical diagnosis of a pheochromocytoma is based on the determination of concentration/excretion of catecholamine metabolites in blood or urine. The most sensitive biomarkers in the biochemical diagnosis of pheochromocytoma are plasma free methanephrines (metanephrine, normetanephrine and 3-methoxytyramine) assayed with chromatography tandem mass spectrometry (LC-MS/MS). Neuroendocrine cells have the ability to produce various proteins and neuropeptides, which, together with catecholamines, are released into the blood and can be designated as so-called circulating tumor markers. Purpose: The aim of the study was to assess the usefulness of the determination of levels of selected Granin family proteins and INSM-1 (Insulinoma-associated protein 1) in the diagnosis of patients with pheochromocytoma. Material and methods: Patients were divided into 4 groups: patients with pheochromocytoma (n=39), patients with incidentaloma (n=20), patients with primary arterial hypertension (n=20), control group – healthy volunteers (n=40). The following biochemical determinations were performed in all patients: plasma levels of metanephrine, normetanephrine and 3-methoxytyramine, concentration of chromogranin B (CgB), proSAAS, INSM-1, chromogranin A (CgA) and derivates peptides: Pancreastatin/chromogranin A (250-301), Serpinin/prepro-chromogranin A (429-454), WE-14/prepro-chromogranin A (342-355) and Catestatin. Biochemical determinations were made using the LC-MS/MS technique with various immunochemical techniques (RIA, IRMA, ELISA). Results: In patients with adrenal pheochromocytoma levels of: CgA, WE-14 and Catestanin were significantly different ($p<0.001$) compared to control groups (adenoma, hypertension and healthy subjects). The concentration of INSM-1 was significantly higher ($p <0.001$) in patients with pheochromocytoma compared to the group of healthy people. In the group of patients with pheochromocytoma, the following indicators of the diagnostic value of the analyzed biomarkers

were obtained: CgA: 82% sensitivity and 100% specificity (AUC 0,930); CgB: 87% sensitivity and 77% specificity (AUC 0,885); WE-14: 90% sensitivity and 95% specificity (AUC 0,959); Catestatin: 80% sensitivity and 92% specificity (AUC 0,903); Pancreastatin: 80% sensitivity and 95 specificity (AUC 0,913); proSAAS: 82% sensitivity and 67% specificity (AUC 0,760); INSM-1: 97% sensitivity and 100% specificity (AUC 0,976). Conclusion: Determination of biomarkers: CgA, WE-14, Catestatin and INSM-1 had the highest diagnostic value in patients with pheochromocytoma.

PP30: ROLE OF SUCCINATE AND SUCNR1 IN HUMAN PHEOCHROMOCYTOMA CELLS

Francesca Amore [1, 2], *Daniele Guasti* [3], *Letizia Canu* [1, 2], *Daniele Bani* [3], *Mario Maggi* [1, 2], *Elena Rapizzi* [2, 4]

[1] *Department of Experimental and Clinical Biomedical Sciences,* University of Florence, Florence, Italy
[2] *ENS@T Center of Excellence,* University of Florence, Florence, Italy
[3] *Imaging Platform, Dept. of Experimental and Clinical Medicine,* University of Florence, Florence, Italy
[4] *Department of Experimental and Clinical Medicine,* University of Florence, Florence, Italy

Mutations of the succinate dehydrogenase (SDH) subunits impair SDH activity leading to the intracellular accumulation of succinate, which is transported to the extracellular space where it activates its specific G-coupled protein receptor 1 (SUCNR1). A growing body of evidence indicates that both cytosolic and extracellular succinate promote cancer growth, but the signalling pathways involved are still largely unclear. Interestingly, along with a histone hyper-methylation, we found for the first time a hyper-succinylation of the proteins in hPheo1 SDHB deficient cells compared with hPheo1 parental. Moreover, SDHB deficient cells showed an upregulation of the SUCNR1. Noteworthy, the addition of succinate to the culture medium induced an increase of succinylation in both hPheo1 cell populations, but an increase in SUCNR1 expression level only in parental cells. Besides, we found a rise in ERK1/2 phosphorylation in SDHB deficient cells, that intriguingly, was not coupled with an increase in cell proliferation. Indeed, SDHB deficient cells proliferate significantly less than parental ones. Recently, Kuo and colleagues (doi:10.1186/s12929-022-00878-z) reported that extracellular succinate induces tumour cell migration through ERK1/2 activation and mitochondrial fission. In line with these results, we not only observed that SDHB deficient cells migrated significantly more than parental ones, but also, that in these cells, the mitochondrial fission protein (Drp1) and its active form (phospho-Drp1) are upregulated. Our results shed some light on the role SUCNR1 in cancer growth and metastasis. In this scenario, unravelling the specific pathways activated by this receptor might lead to the identification of effective therapeutic targets.

PP31: COMPOSITE PHEOCHROMOCYTOMA AS A COMMON NEOPLASM IN NF1

Ewelina Rzepka [1], Anna Bogusławska [1], Anna Kurzyńska [1], Marta Opalińska [1], Elwira Przybylik-Mazurek [1], Aleksandra Gilis-Januszewska [1], Alicja Hubalewska-Dydejczyk [1]

[1] *Chair and Department of Endocrinology,* Jagiellonian University Medical College, Cracow, Poland

Up to 40% of pheochromocytoma cases have a genetic background. The prevalence of pheochromocytoma in neurofibromatosis type 1 (NF1) was reported to be from 0.1 to 5.7%. Our aim was to analyse clinicopathological characteristics of pheochromocytoma cases in the course of NF1 syndrome. We performed a database search for pheochromocytoma patients, diagnosed and treated in Endocrinology Department, University Hospital in Cracow from 2005 to 2021. In the group of 183 patients with histologically confirmed pheochromocytoma, 8 cases with NF1 were identified (4.4%). The group of patients comprise 4 men and 4 women. Median patient's age was 44 years (range: 29-70 years). Most cases were diagnosed incidentally (6/7). The most common manifestation of the disease was hypertension (5/8). In majority of patients (6/8), NF1 diagnosis was stated based on clinical picture, in two cases genetic testing was performed. One patient had aggressive, metastatic tumour, which subsequently led to patient's death, the rest of patients remained disease-free during follow-up (median time: 44.5 months). One patient had synchronous bilateral disease. In 4 subjects, histopathological examination revealed composite pheochromocytoma with ganglioneuroma component. There were no significant differences between NF1 and non NF1 pheochromocytomas regarding sex, age, tumour size, PASS score, levels of metanephrines. In patients with NF1, composite pheochromocytoma was diagnosed much more often than in non-NF1 cases (with p value = 0.003). Pheochromocytoma in the course of NF1 is very often diagnosed incidentally. We propose to exclude NF1 in cases with composite pheochromocytoma, since it seems to be more common in NF1 patients.

PP32: NEUROPEPTIDE Y (NPY) AND HUMAN COCAINE- AND AMPHETAMINE-REGULATED TRANSCRIPT (CART) IN PATIENTS WITH ADRENAL PHEOCHROMOCYTOMA

Alicja Szatko [1], Magdalena Ostrowska [1], Lucyna Papierska [1], Wojciech Zgliczyński [1], Piotr Glinicki [1]

[1] *Department of Endocrinology,* Centre of Postgraduate Medical Education, Warsaw, Poland

Introduction: Pheochromocytoma is a rare tumor that develops from chromaffin cells of the adrenal medulla. Along with catecholamines, chromaffin cells have the ability to secrete various proteins and neuropeptides into the blood. The aim of the study: The assessment of the utility of neuropeptide Y and CART in the diagnosis of pheochromocytoma. Material and methods: Patients were divided into 4 groups: 1. Pheochromocytoma (n=51), 2. Adrenal incidentaloma (n=23), 3. Primary arterial hypertension (n=20), 4. Control group (n=52). The biochemical determinations were performed in all patients: plasma levels of metanephrine and normetanephrine, concentration of chromogranin A (CgA), neuropeptide Y (NPY) and human cocaine- and amphetamine-regulated transcript (CART). Biochemical determinations were made using the LC-MS/MS technique and immunochemical techniques (IRMA, ELISA) were used. Results: Pheochromocytoma vs. adenoma: CgA: 84% sensitivity and 96% specificity (AUC 0,932); NPY: 80% sensitivity and 78% specificity (AUC 0,808) and CART: 43% sensitivity and 100% specificity (AUC 0,768). Pheochromocytoma vs. primary hypertension: CgA: 78% sensitivity and 100% specificity (AUC 0,945); NPY: 47% sensitivity and 100 specificity (AUC 0,615) and CART: 72% sensitivity and 85% specificity (AUC 0,797). Pheochromocytoma vs. healthy subjects (blood donors): CgA: 84% sensitivity and 98% specificity (AUC 0,923); NPY: 90% sensitivity and 86% specificity (AUC 0,897) and CART: 60% sensitivity and 49% specificity (AUC 0,403). Conclusions: Among the analyzed biomarkers, CgA concentration determination presented the highest discriminant value between patients with pheochromocytoma and other study groups.Neuropeptide Y showed high specificity between the analyzed groups, especially in the differential diagnosis of adenoma and primary hypertension.

PP33: A CLINICOPATHOLOGIC ANALYSIS OF 5 KINDREDS WITH MEN2A IN GREECE CARRYING THE UNCOMMON MUTATION OF (GLY533CYS) IN EXON 8 OF RET PROTOONCOGENE

Chrysoula Koukoula [1], Olga Papalou [1], Stylianos Tsagarakis [1], Dimitra- Argyro Vassiliadi [1]

[1] *Department of Endocrinology Diabetes and Metabolism, National Expertise Center for Rare Endocrine Diseases ,* Evangelismos Hospital , Athens, Greece

Background: The G533C mutation on exon 8 of the RET gene is a rare genetic alteration linked to MEN2A (Multiple Endocrine Neoplasia type 2A) worldwide. However, it is clustering in South America and Mediterranean countries being the most prevalent mutation in Greece. There is limited data on the clinical course of these patients while it has been suggested that Greek patients with this mutation are at greater risk of pheochromocytoma compared to their South America counterparts (reported prevalence of 0,8%). Objective: We report 5 MEN2A families, a total of 16 patients, affected by a mutation (Gly533Cys) in exon 8, aiming to analyze the clinicopathologic characteristics. Results: Among 16 patients from 5 different families with the G533C mutation, 12/16 had myeloid thyroid cancer (MTC), 6/16 pheochromocytoma and 2/16 hyperparathyroidism. Among the ones with pheochromocytoma, 4/6 had bilateral adrenal involvement. The most common first manifestation of the syndrome was MTC and the median age at diagnosis was 43 years. Pheochromocytoma was the first manifestation in 3/16 of patients (median age of diagnosis 48 years), while in 1 of them there was a simultaneous diagnosis of both pheochromocytoma and MTC. After a median follow-up of 15 years, the majority of patients are disease-free. Conclusion: Patients with Gly533Cys mutation can develop the entire clinical spectrum of MEN2A. Compared to reports of South American patients with the same mutation, pheochromocytoma is more prevalent in Greek patients as it was encountered in almost half of our patients, quite commonly with bilateral involvement.

PP34: INCIDENCE AND GEOGRAPHIC DISTRIBUTION OF PHEOCHROMOCYTOMA AND PARAGANGLIOMA IN SLOVAK POPULATION

Ivana Jochmanova [1], *Michal Ircha* [1]

[1] *Faculty of Medicine,* Pavol Jozef Šafárik University in Košice, Košice, Slovakia

Pheochromocytoma (PHEO) and Paraganglioma (PGL) are neuroendocrine tumors originating from chromaffin tissues of adrenal medulla or extraadrenal paraganglia and are characterized by catecholamine overproduction. Excess of catecholamines and the risk of metastatic disease lead to increased morbidity and mortality as well as decreased quality of life of patients. While there is number of studies focused on the ethiopathogenesis, diagnosis, and treatment of these tumors, their epidemiology is not well known in many, especially small countries. In our present study we assessed the incidence of PHEO/PGL in Slovakia and compared it to other countries. Based on the aquired data, the incidence of PHEO/PGL in Slovakia is 0.13 cases per 100,000 inhabitants, while PHEO prevails (0.12 cases per 100,000 inhabitants). Within Slovak Republic we noted differences between the districts of the country. Compared to other countries, the overall incidence of PHEO/PGL in Slovakia seems to be slightly lower, however, this can be also the result of lower reporting reporting of the cases into the national database.

PP35: PEPTIDE RECEPTOR RADIONUCLIDE THERAPY (PRRT) IN INOPERABLE/METASTATIC PPGLS – TIME TO EXPAND PRRT INDICATIONS?

Pedro Souteiro [1], Raquel Calheiros [1], Sara Santos [1], Isabel Inacio [1], Joana Oliveira [1], Ana Paula Santos [1], Isabel Torres [1]

[1] *Endocrinology Department,* Instituto Portugues de Oncologia Francisco Gentil do Porto, Porto, Portugal

Introduction: Inoperable/metastatic pheochromocytomas and paragangliomas (PPGLs) can be a challenge since there are only few therapeutic weapons to deal with progressive tumour load and uncontrolled catecholamine hypersecretion when surgery is not possible. Case 1: 67-year-old male, diagnosed with a single inoperable non-secreting neck paraganglioma with 28x35 mm. Surgery was not a viable option considering carotid artery encasement. 68Ga-DOTANOC-PET/CT revealed intense radiopharmaceutical fixation in the lesion and the genetic study identified an ATM gene mutation. The patient was then proposed to 177Lu-DOTATE treatment and completed 4 cycles. Both post-therapeutic 68Ga-DOTANOC-PET/CT and neck MRI showed considerable tumour reduction (29x19mm). Case 2: 45-year-old male, SDHB-mutation positive, with a stage IV functioning abdominal paraganglioma with diffuse pulmonary, ganglionic, hepatic and bone metastasis. Strong 68Ga-DOTANOC-PET/CT positivity lead to the decision of treating the patient with 4 cycles 177Lu-DOTATE instead of chemotherapy. Serial post-therapeutic scintigraphies showed a significant decrease in 68Ga-DOTANOC fixation and in urinary normetanephrine levels. Case 3: 45-year-old female presented with weight loss, thoracic pain, severe dyspnoea, cough and haemoptysis. The CT revealed a giant thoracic paraganglioma with 13cm and bronchopulmonary and cardiac compression. Surgery was attempted but an early intraoperative massive tumour bleeding with cardiac arrest precluded further dissection. An endobronchial prosthesis was then inserted and chemotherapy with CAPTEM scheme was initiated. The patient presented with a G4 thrombocytopenia after the first cycle and the treatment was definitely interrupted. After considering PRRT vs. sunitinib therapy, the 68Ga-DOTANOC-PET/CT results led to the decision of initiating 177Lu-DOTATE that the patient is now awaiting. Conclusions: PRRT with 177Lu-DOTATE takes advantage of somatostatin receptors expression by endocrine cells but it's only officially approved for gastroenteropancreatic neuroendocrine tumours (GEP-NETs). However, its off-

label use in PPGL has been increasingly reported with g in patients with PPGLs. Future studies and guidelines will probably address the use of PRRT in this new setting.

PP36: ADRENAL HEMORRHAGE IN PHEOCHROMOCYTOMA – A RARE AND LIFE-THREATENING CONDITION

Kristina Saravinovska [1], Ljiljana Marina [1], Antoan Stefan Šojat [1], Tijana Petkovic [1], Natalija Antic [1], Dunja Simona Petkovic [1], Bogdan Dugic [2], Miomira Ivovic [1], Zorana Arizanovic [1], Svetlana Vujovic [1]

[1] *National Centre for Infertility and Endocrinology of Gender,* Clinic for Endocrinology, Diabetes and Metabolic diseases, University Clinical Centre of Serbia, Belgrade, Serbia
[2] *School of Electrical Engineering,* University of Belgrade, Serbia, Belgrade, Serbia

Case report: A 48-year-old female was referred to our outpatient clinic for functional testing of an adrenal incidentaloma visualized for the first time by abdominal ultrasound (US) as part of systematic check-up. A follow-up CT described a heterogeneous right adrenal mass (26x23 mm, poor lipid content, parenchymatous calcifications, described as atypical adrenal adenoma). She also reported poorly controlled hypertension, diagnosed two years prior, and was on a two-step antihypertensive therapy. She described hypertensive episodes at night-time (210/130 mmHg) with headache, malaise, occasional diarrhea, and a sudden onset of irregular menstrual cycle. Upon initial examination, we scheduled her for full functional testing. Two days after the testing, she came to our Clinic due to a sudden onset of sharp abdominal pain, predominantly localized in the right lumbar and iliac region with severe malaise. She was hypotensive with tachycardia with a drop in hemoglobin, so she was hospitalized. The US showed an increase in the adrenal mass (50x35 mm) with a suspected zone of hemorrhage. The following CT described the right adrenal gland with a dominant cystic lesion and irregular septal wall thickness, 58x56x50 mm, with a nodular enlargement (22x20x13 mm), and a non-determinable absolute and relative washout. Catecholamines and metanephrines in three 24h urine samples were elevated. Autonomous cortisol secretion and hyperaldosteronism were excluded. Glucocorticoid therapy was introduced, and urgent laparotomy and right adrenalectomy were performed. Intraoperatively, there were severe fluctuations in blood pressure and further blood loss. She was discharged a few days later, hemodynamically stable. The pathohistological finding confirmed pheochromocytoma with, a PASS score of 4. Conclusion: Adrenal hemorrhage has been described in patients with sepsis, trauma, pregnancy, Covid-19, and hematological conditions causing significant morbidity and mortality. Spontaneous adrenal

hemorrhage is a rare but life-threatening complication in patients with pheochromocytoma and represents a significant clinical challenge.

PP37: WALKING THE TIGHTROPE: PREGNANCY AND PHEOCHROMOCYTOMA MANAGEMENT

Mia Manojlović [1,2], Ivana Bajkin [1,2], Dragana Tomić Naglić [1,2], Tijana Ičin [1,2], Kristina Stepanović [1,2], Milica Lekin [1]

[1] *Clinic for Endocrinology, Diabetes and Metabolic Disorders,* Clinical Center of Vojvodina, Novi Sad, Serbia
[2] *Faculty of Medicine,* University of Novi Sad, Novi Sad, Serbia

Introduction: Pheochromocytoma is a tumor that arises from chromaffin cells of the adrenal medulla and secretes one or more catecholamines. The prevalence of pheochromocytoma in pregnant women is approximately 0.002%. Case report: Herein, we present the case of a 28-year-old woman who was admitted to the Department of Obstetrics and Gynecology at 31 weeks of gestation during her third pregnancy, complicated by uncontrolled hypertension. She had a history of preeclampsia in her second pregnancy, which unfortunately resulted in newborn death. During hospitalization, elevated levels of normetanephrine were detected, and an abdominal MRI revealed a soft tissue mass (32 x 30 x 35 mm) on the left adrenal gland, that was consistent with a diagnosis of pheochromocytoma. Subsequently, the patient was treated with an alpha-blocker, followed by a beta-blocker and calcium channel blocker, effectively controlling her blood pressure and heart rate. Pregnancy was terminated by Caesarean section at 34 weeks of gestation, resulting in the birth of a live female infant. The patient was discharged from the hospital in good general condition. Afterward, chromaffin tissue scintigraphy (MIBG) revealed minimal radioisotope accumulation in the right adrenal gland, which could potentially be attributed to physiological accumulation. MEN2 syndrome was ruled out. The patient was recommended for a left adrenalectomy, which was successfully performed, and she was discharged in good general condition for further outpatient treatment. Conclusion: Timely diagnosis of pheochromocytoma is crucial for successful treatment and favorable pregnancy outcomes.

PP38: PRRT TREATMENT OF METASTATIC PARAGANGLIOMA WITH SDHB MUTATION

Katarina Mlekus Kozamernik [1]

[1]*Endocrinology,* UMC Ljubljana, Ljubljana, Slovenia

In 2012, a woman aged 60 underwent surgery for paraganglioma. The pathohistological report indicated a low proliferation index (Ki67<1%) and no lymph node metastases. In early 2018, she experienced symptoms similar to those in 2012, including sweating, hypertension, and abdominal pain. Urinary norepinephrine and plasma normetanephrine were above ULN. FDG PET/CT imaging showed a 4 cm tumor in front of the aorta and inferior vena cava at the L4-L5 level, along with a small (< 1 cm) metabolically active lesion in the sacrum. A 123I-MIBG SPECT was negative. The patient underwent repeated surgery in 2018 to remove the tumor above the aortic bifurcation, which was confirmed as recurrent paraganglioma with focal necrosis and regressive changes. Genetic testing revealed a pathogenic variant in the SDHB gene. In late 2019, the patient's urinary norepinephrine level increased, and PET/CT with Ga DOTATATE confirmed a relapse of the disease with two lesions with pronounced SSTRs - one in the L3/4 vertebrae and the other in the sacrum. She then received radiotherapy for both lesions. However, in May 2021, her plasma normetanephrine level was high again, and PET/CT with Ga DOTATATE detected a new lesion in the pedicle of L3. From September 2021 to September 2022, the patient underwent four peptide receptor radionuclide therapies (PRRT), and the last Ga DOTATATE scan in March 2023 indicated regression of the aortocaval tumor and the two skeletal tumors (in the sacrum and the pedicle of the L3 vertebra). There were no new lesions with expressed SSTRs.

PP39: HYPERTENSIVE CRISIS, ACUTE CEREBROVASCULAR EVENT AND POLYCYSTIC KIDNEY DISEASE IN PATIENT WITH AN ADRENAL TUMOR

Tijana Petkovic [1], Ljiljana Marina [1], Miomira Ivovic [1], Antoan Stefan Sojat [1], Natalija Antic [1], Dunja-Simona Petkovic [1], Kristina Saravinovska [1], Zorana Arizanovic [1], Svetlana Vujovic [1]

[1] *National Centre for Infertility and Endocrinology of Gender,* Clinic for Endocirnology, Diabetes and Metabolic Diseases, University Clinical Centre of Serbia, Belgrade, Serbia

Introduction: Autosomal dominant polycystic kidney disease (ADPKD) carries a 4 times higher risk of intracranial aneurysm occurrence compared to the general population. In the presence of hypertension (HTN) in ADPKD, there is a high mortality rate due to aneurysm rupture and a morbidity rate due to neurological sequelae. Case report: A 41-year-old patient was referred to a tertiary institution due to hypertension and hypokalemia. One year earlier, he underwent transcranial surgery due to aneurysm rupture during a hypertensive crisis (220/120 mmHg). He had mild HTN for two years prior, without therapy. During neurosurgical treatment, hypokalemia of 2.3 mmol/l occurred. Abdominal CT revealed tumor-like lesion of the left adrenal gland (12x11 mm), 16 HU. Also, polycystic kidney disease was diagnosed with numerous septated, calcified and hemorrhagic cysts (Bosniak 2/2f). Mineralocorticoid receptor antagonist (MRA) therapy was initiated, up to 100 mg daily. HTN was well-controlled with a three-step therapy (ACE inhibitor, diuretic, MRA). Due to the significant cerebrovascular event, functional testing of the adrenal glands was conducted on the aforementioned therapy. Considering inconclusive findings, the patient was readmitted for a "wash-out" in hospital conditions. Upon discontinuation of MRA, clear hypokalemia occurred, and potassium chloride was administered, with good blood pressure control achieved with calcium antagonist and alpha-blocker. The retesting results indicated primary hyperaldosteronism (PA) (ALD 313/908 ng/l, PRA <0.5 ng/ml/h). Subsequently, the patient was referred to left adrenalectomy, confirming an adenoma in the adrenal cortex. Regular check-ups showed normal potassium levels without therapy and excellent HTN control with monotherapy. Due to a strong positive family history of ADPKD, the patient's brother was asked for testing, but he refused. One year later, he died due to cerebrovascular bleeding. Conclusion: the association between PA and

ADPKD is more often than previously thought, so PA screening in these patients might be plausible.

PP40: RARE THROMBOEMBOLIC COMPLICATIONS AFTER PHEOCHROMOCYTOMA SURGERY

Natalija Antić [1], Ljiljana Marina [1, 2], Miomira Ivović [1, 2], Antoan Stefan Šojat [1], Tijana Petković [1], Kristina Saravinovska [1], Dunja Simonona Petković [1], Bogdan Dugić [3], Zorana Arizanović [1], Svetlana Vujović [1, 2]

[1] *National Centre for Infertility and Endocrinology of Gender,* Clinic for Endocrinology, Diabetes and Metabolic Disease, University Clinical Centre of Serbia, Belgrade, Serbia
[2] *Faculty of Medicine,* University of Belgrade, Belgrade, Serbia
[3] *School of Engineering,* University of Belgrade, Belgrade, Serbia

Case report: A 48-year-old female was admitted to our Clinic for a functional re-evaluation of a prior non-functional left adrenal incidentaloma due to a significant increase in size after 8 years (22 x 27 x 24 vs 30 x 35 x 35 mm). The adenoma was initially visualized on a CT scan in 2015, performed as a part of an evaluation for non-Hodgkin lymphoma, and the follow-up did not show any changes in size and morphology until the CT and MRI performed in 2023. The subssequent hormonal assessment had shown both a catecholamine excess and autonomous cortisol secretion, so she was referred to an endocrine surgeon and treated preoperatively with doxazosine. Due to residual fibrosis and scaring, the endocrine surgeons opted for a left open adrenalectomy with laparotomy. Glucocorticoid therapy was initiated immediately after surgery, however, five days later, she reported a sudden onset of abdominal pain and malaise accompanied by vomiting and nausea. The native abdominal X-ray described a partial ileus, and the emergency abdominal and pelvic CT scans described left renal artery thrombosis with kidney infarction. She was treated conservatively, with a multidisciplinary approach with LWMH, corticosteroids, parenteral nutrition and antibiotics until she was fully stabilized, and she was discharged home. The pathohistological finding confirmed Pheochromocytoma PASS score 2, Ki67% 0.85. Conclusion: A few cases of acute renal arthery thrombosis with renal infarction have been described after adrenalectomy for pheochromocytoma. A prolonged catecholamine exces can cause blood hypercoagulability, hemodynamic changes, and endothelial dysfunction.

PP41: ADENOMATOID TUMOUR OF ADRENAL GLAND, PRIMARLY THOUGHT TO BE A PHEOCHROMOCYTOMA

Mari Minasyan [1], Ewelina Rzepka [1], Aleksandra Gilis-Januszewska [1], Alicja Hubalewska-Dydejczyk [1]

[1] *Endocrinology Department ,* Jagiellonian University-Hospital, Cracow, Poland

Adenomatoid tumor (AT) is a rare benign tumour, which usually occure in the genital system. Other localisation such as the adrenal gland is very uncommon. We present a case of 56 year old man with many comorbidities who presented with a right adrenal gland tumour (MRI: 35x23 mm non homogenous tumour) and unspecifically elevated metoxycatechilamines in the urine. Patient didn't present spells of high blood pressure, tachykardia, anxiety or other signs characteristic in pheochromocytoma. Hormonal assesment exclude either Cushing (correct supression if cortisol on 1 mg dexamethasone supression test) or Conn syndrome (normal range of aldosterone, renine). Due to elevation of metoxycatechilamines in the urine, we implemented doxazosine before the right adrenalectomy. Patient underwent the surgery without any complications. Histopathological evaluation showed adenomatoid tumour. The case showed extremely rare bening tumour of adrenal gland- adenomatoid tumour- which primarly was thought to be a pheochromocytoma. Due to the rarity of the disease, there are no guidelines regarding follow up manner in patients after surgical treatment of adrenal adenomatoid tumour. Further multicenter observations are needed in order to create such guidelines.

PP42: IS IT A REAL ADRENAL MASS? A CASE OF A GIANT RETROPERITONEAL TUMOR IN THE COURSE OF PROBABLE TYPE I NEUROFIBROMATOSIS

Łukasz Kluczyński [1], Edyta Tkacz [2], Aleksandra Gilis-Januszewska [1], Alicja Hubalewska-Dydejczyk [1]

[1] *Chair and Department of Endocrinology,* Jagiellonian University Medical College, Kraków, Poland
[2] *Department of Endocrinology,* University Hospital, Kraków, Poland

A 27-year-old woman with a yearly history of weight loss and amenorrhea was admitted to the regional hospital. Neurofibromatosis type I (NF1) was confirmed in her sister and brother and the patient presented features of the disease. Physical examination revealed multiple neurofibromas, café-au-lait spots and tumor in the left subcostal region. In the USG and CT of the abdomen a giant mass 18x14x12 cm, with areas of necrosis and decay, most likely coming out of the adrenal gland, was visualized. Thickening of the urine bladder wall was found as well. The patients was referred to the Department of Endocrinology, where the biochemical assessment confirmed excluded hormonal activity of the mass. No exophytic lesions in the urinary bladder were found during cystoscopy. The patient was sent for surgery – partial resection was performed. The histopathological examination revealed giant mass (17 cm, 1450 g) containing malignant peripheral nerve sheat tumor with Ki-67 55%, preserved tissue of a normal adrenal gland and neurofibroma multiforme. Follow-up PET-CT showed residual tumor mass and probable dissemination to the chest and abdominal structures. Unfortunately, despite introduced chemotherapy, the patient died. Retroperitoneal sarcomas are rare tumors that may mimic adrenal masses. The report proves they should be considered in the differential diagnosis, particularly in patients with (probable) NF1. The prognosis is unfavorable, especially in the case of advanced lesions.

PP43: FROM ROUTINE PSA CHECK TO MEN 2A DIAGNOSIS

Rok Vrčkovnik [1], *Tomaž Kocjan* [1, 2]

[1] *Department of Endocrinology, Diabetes and Metabolic Diseases,* University Medical Centre Ljubljana, Ljubljana, Slovenia
[2] *Departement of Internal Medicine,* Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

A 73-year-old male was found to have progressively increasing PSA levels during routine check-ups. Prostate biopsy confirmed a prostatic adenocarcinoma (Gleason 3+4 and 4+4). 18F-Choline PET/CT was done next; however, this imaging did not only exclude metastatic disease, but also showed a highly metabolically active thyroid nodule. An ultrasound-guided fine needle biopsy of the nodule confirmed MTC (Bethesda 6). Calcitonin level was high at 1290 ng/L (ref. 8.3 – 14.3 ng/L). Catecholamines and nor/metanephrines were routinely measured in a 24h urine sample before thyroidectomy and were positive. Abdominal CT scan showed a 44 mm partially cystic formation of the left and an 11 mm nodule of the right adrenal gland, both with CT characteristics of PHEO. Laboratory testing for PHPT was negative. After a course of phenoxybenzamine, the patient was planned for laparoscopic total left and partial right adrenalectomy, however, bilateral total operation was finally done due to technical issues. Histopathological report confirmed bilateral PHEO with a PASS score of 8/20 on the left, and 3/20 on the right side. Total thyroidectomy and laparoscopic radical prostatectomy followed in the next weeks. Genetic testing was consistent with MEN 2A (NM_020975.6(RET):c.1900T>G (p.Cys634Gly)). Currently, the patient is doing well on hydrocortisone, fludrocortisone, and levothyroxine supplementation. The family history for MEN2A-related manifestations was negative. Genetic testing results of patient's close relatives are pending.

PP44: AN OVERVIEW OF ADRENAL VEIN SAMPLING FOR PRIMARY ALDOSTERONISM IN CROATIA (2016-2023)

Karin Zibar Tomsic [1], Anamarija Alduk [2], Darko Kastelan [1,3]

[1] *Department of Endocrinology,* University Hospital Center Zagreb, Zagreb, Croatia
[2] *Department of Radiology,* University Hospital Center Zagreb, Zagreb, Croatia
[3] *School of Medicine,* University of Zagreb, Zagreb, Croatia

Background: Although adrenal vein sampling (AVS) is a technically demanding procedure, it is still considered the gold standard for subtyping primary aldosteronism (PA). The aim of the study was to analyse the success rate of AVS in patients with PA. Methods: In all patients AVS was performed sequentially during continuous stimulation with adrenocorticotrophic hormone by one interventional radiologist. Adrenal vein cannulation was considered successful if the selectivity ratio was ≥ 5:1. Results: Over the 7-year period, 179 procedures were performed in 166 patients (101 men; age 44-60, median 52 years). In the first two years (2016-2017) 30 AVS procedures were performed (group 1) with the success rate of 60% (18/30) whereas in the period from 2018 to 2020 (group 2) and from 2021 to May 2023 (group 3), the success rate of AVS procedures was 79% (57/72 and 61/77), ($p=0.019$, $\chi 2=21.294$). Out of 43 unsuccessful AVS, cannulation of the right and left adrenal veins failed in 31 and 7 cases, respectively, whereas in five patients catheterisation failed on both sides. In 13 patients, AVS was repeated after an unsuccessful first procedure, and the success rate of the second AVS was 85% (group 1 50%; group 2 87%; group 3 100%). Conclusion: The AVS success rate has increased over time from an initial 60% to 79% at the end of the observed period. In selected cases, repeated AVS might be justified after an unsuccessful first procedure.

PP45: NEW DATA ON THE DIAGNOSIS PREVALENCE AND TREATMENT OF PRIMARY ALDOSTERONISM: THE EXPERIENCE OF A SINGLE ENDOCRINOLOGY CENTER

Athina Markou [1], Gouli Aggeliki [1], Nikos Voulgaris [1], Vaios Tsiavos [1], Christos Gravanis [1], Ernestini Tyfoxilou [1], Labrinh Papanastasiou [1], Sophia Vlachou [1], Chara Kapsali [1], Irene Giagourta [1], Theodora Kounadi [1], George Zografos [1], Gregory Kaltsas [3], George Chrousos [4], George Piaditis [1]

[1] *Endocrinology,* Gna 'G. Gennimatas', Athens, Greece
[2] *3rd Department of Surgery,* Gna 'G. Gennimatas', Athens, Greece
[3] *1st Department of Propaedeutic Medicine,* Laiko Hospital, Athens, Greece
[4] *Endocrinology,* University Research Institute of Maternal and Child Health and Precision Medicine, Athens, Greece

Aim: To present the results of 14-year duration of prospective studies on the diagnosis, treatment and prevalence of PA, of a single Endocrinology Center. Patients and Methods: We studied 992 hypertensive patients and 278 controls matched for age, sex and BMI. All participants underwent the currently used diagnostic tests for PA, modified by the addition of dexamethasone. Among the hyperternsive patients, 67.8% underwent a Fludrocortisnone-Dexamethasone-Suppression-Test (FDST), 58.4% a Dexamethasone-Captopril-Valsartan-Test (DCVT) and 65.4% of the FDST group underwent a Dexamethasone-Saline-Infusion-Test (DSIT). Among the 194/278 normotensive controls, 68.2% underwent an FDST and 68.6% a DSIT. The 104/278 hypertensive controls underwent a DCVT. The normal cut-offs for aldosterone suppression were obtained from the controls. All controls had normal adrenal morphology on computerized tomography. Results: Hypertensive participants had significantly higher SBP/DBP and lower potassium compared with controls. The prevalence of PA was 17.8% using the basal ARR as screening test and 33.4% (332/992) without it. 65/332 (19.5%) PA patients had a single and 15/332 (4.5%) bilateral adrenal adenomas. 23 patients did not suppress cortisol. 252/332 patients with PA received treatment with MRAs. Complete biochemical (K+>3.9 mmol/L, REN> 7.5 mU/L) and clinical (SBP/DBP <140/90 mmHg) control was achieved in 188/252 (74.6%). 48/332 patients with PA and a single adrenal adenoma, received treatment with adrenalectomy, achieving a biochemical recession of 94%. Conclusion: Our modified methodology on PA diagnosis, allows the detection of milder forms

of the disease, increasing thus its prevalence. Early diagnosis and targeted treatment permits effective prevention of tissue damage to the target organs.

PP46: BENIGN ADRENAL ADENOMAS ARE ASSOCIATED WITH REDUCED PREVALENCE OF COVID-19

Alan Kelsall [1], Chris Johns [1], Eleanor Hills [1], Jenny Zhao [1], John Newell-Price [1]

[1] *Oncology and Metabolism,* University of Sheffield, Sheffield, United Kingdom

Introduction: Adrenal incidentalomas (AI) are commonly found on imaging done for indications other than to assess the adrenal glands. Prevalence increases with age and is around 10% in people over 70y.The majority of AI are benign adenomas, with 30-50% exhibiting mild autonomous cortisol secretion (MACS). Clinical guidelines recommend use of dexamethasone to improve outcomes of COVID-19. Hypothesis: Benign adrenal adenomas protect against COVID-19. Methods: Reports for all computed tomography pulmonary angiogram (CTPA) scans at Sheffield Teaching Hospitals between 11/03/2020 and 10/11/2021 were assessed for details of adrenal incidentaloma. Scan requests mandated recording COVID-19 status. Patients with a positive COVID test within 2 weeks prior to the CTPA were classed as COVID positive for the analyses.Duplicate scans were removed. Results: A total of 4347 CTPA scans were included. The median age was 65 (IQR 49-77) and the majority of patients were female (55.1%). 76 (1.75%) patients had a benign adenoma. COVID19 positivity was found in 897 (20.63%). The presence of a benign adenoma was associated with a 70% reduced odds of being COVID positive (aOR 0.30, 95% CI 0.12-0.75, p = 0.01), after adjusting for age and sex. Conclusion: Prevalence of adrenal adenoma was associated with significantly reduced odds of being SARS-CoV2 positive. Secretion of mild excess cortisol (MACS) may be protective against developing severe COVID-19.

PP47: PRESENTATION AND MANAGEMENT OF ADRENAL TUMOURS OVER TIME: A REAL-LIFE EXPERIENCE FROM A UK TERTIARY CARE CENTRE

Onnicha Suntornlohanakul [1, 2, 3, 4], Sumedha Mandal [3], Pratyusha Saha [3], Shadman Ahmed [3], Jessica Parkin-Crawshaw [3], Miriam Asia [2], Wiebke Arlt [5, 3, 2, 1], Yasir Elhassan [1, 2, 3], Alessandro Prete [1, 2, 3], Cristina Ronchi [1, 2, 3]

[1] *University of Birmingham,* Institute of Metabolism and Systems Research, Birmingham, United Kingdom
[2] *Queen Elizabeth Hospital Birmingham,* Department of Endocrinology, Birmingham, United Kingdom
[3] *Birmingham Health Partners,* Centre for Endocrinology, Diabetes and Metabolism, Birmingham, United Kingdom
[4] *Faculty of Medicine, Prince of Songkla University,* Endocrinology and Metabolism Unit, Division of Internal Medicine, Songkhla , Thailand
[5] *Imperial College London,* Medical Research Council London Institute of Medical Sciences, London , United Kingdom

Background: Adrenal tumours are found in 3-7% of adults and current guidelines have standardised the approach to these patients, but evidence of their impact on clinical care is lacking. Methods: Review of adrenal tumour patients referred to the Queen Elizabeth Hospital Birmingham, UK, between 1998-2022, with a sub-analysis based on the publication of the 2016 ESE-ENSAT guidelines on adrenal incidentalomas. Results: 1398 patients were included (55.7% women; 14.6% bilateral tumours; median age at diagnosis 60 years [IQR 49-70]; n=407 evaluated before and n=991 after implementation of the guidelines). Incidental discovery was the most frequent presentation (63.7%). The median tumour diameter was 2.9 cm (IQR 1.9-4.7) and 30.7% were ≥4cm. Unenhanced CT Hounsfield units (HU) were available for 763 patients (54.6%); of these, 54.3% had heterogeneous tumours or HU ≥10. After standardised work-up, the three most common diagnoses were adrenocortical adenoma (ACA, 55.9%), phaeochromocytoma (13.3%), and adrenocortical carcinoma (10.6%). The presumed diagnosis at initial referral changed after work-up in 374 patients (36.5%), especially those referred for indeterminate lesions (n=318). In this group, 47.5% of patients were re-classified as having ACAs. Following the publication of the guidelines, the proportion of adrenalectomies and follow-up visits in non-functioning ACAs decreased from 6.1% to 4.8% and 89.6% to 69.9%, respectively ($p<0.05$ by Fisher test). Conclusion: ACAs were the most common aetiology in a large cohort of adrenal tumours. Reassuringly, almost half of the indeterminate lesions were

eventually diagnosed as ACAs. The ESE-ENSAT 2016 guidelines positively impacted practice, reducing the number of unnecessary investigations and surgeries.

PP48: URINARY STEROID PROFILING BY LIQUID-CHROMATOGRAPHY TANDEM MASS SPECTROMETRY: METHOD VALIDATION AND COMPARISON TO GC-MS

Mariwan H Sayda [1], Lorna C Gilligan [1], Fozia Shaheen [1], Alessandro Prete [1, 2], Wiebke Arlt [1, 2, 3], Angela E Taylor [1, 2]

[1] *Institute of Metabolism and Systems Research,* University of Birmingham, Birmingham, United Kingdom
[2] *Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners,* University of Birmingham, Birmingham, United Kingdom
[3] *MRC London Institute of Medical Sciences,* Imperial College London, London, United Kingdom

Gas chromatography-mass spectrometry (GC-MS) is the gold standard method for urinary steroid profiling. GC-MS quantifies steroids from mineralocorticoid, glucocorticoid, and androgen classes and is a valuable discovery tool. However, GC-MS requires chemical derivatisation, long run times, is labour intensive, and unsuitable for rapid multi-sample analysis. To improve sample throughout we developed a urinary steroid profiling method using ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-LC-MS/MS). We optimised mass spectrometric and chromatographic parameters for twenty-nine steroids, and determined the quantification range and reproducibility of the assay. Sample preparation involved addition of isotopically labelled internal standards followed by hydrolysis to remove the sulphate and glucuronide conjugates. The subsequent unconjugated steroids were extracted using C18 96-well solid-phase extraction. Separation of steroids was achieved using a Waters HSS T3 column (1.8 μm 1.2 x 50 mm) and a water and methanol (both 0.1 % formic acid) gradient on a Waters Acquity UPLC system coupled to a Waters TQ-XS mass spectrometer. The method was clinically validated, and quantification compared to GC-MS. All steroids were separated in a run time of 22 minutes. The method quantified steroids across the large concentration ranges observed in urine (0.5-3000ng/mL). Lower limits of quantification ranged from 0.5 to 10 ng/ml. Method had acceptable recovery and matrix effects. GC-MS and UPLC-MS/MS showed significantly similar quantitation for all steroids. We have developed a powerful tool for comprehensive profiling of the urinary steroid metabolome using UPLC-MS/MS. Compared to established GC-MS methods, the current assay reduces sample preparation and run time, allowing greater sample throughput.

PP49: SALIVARY STEROID PROFILE: THE SIMULTANEOUS QUANTIFICATION ANDROGENS, GLUCOCORTICOIDS AND MINERALOCORTICOIDS IN HUMAN SALIVA

Joshua Bain [1], Angela Taylor [1], James Hawley [1], Lorna Gilligan [1]

[1] *The Institute of Metabolism & Systems Research,* University of Birmingham, Birmingham, United Kingdom

Steroid profiling of biological fluids has been used for clinical and diagnostic research since the 1950's. Most methods focus on blood and urine, however more recently saliva analysis has been utilised as a non-invasive, simple to collect sample for diagnosis of conditions such as Cushings and androgen excess. Despite its ease of collection saliva remains an underutilised biofluid. To investigate the utility salivary steroid profiling for endocrine research, we aimed to develop and validate a liquid chromatography tandem-mass spectrometry method to quantify salivary androgens, glucocorticoids, and mineralocorticoids. Furthermore, we aimed to investigate the correlations between urine, serum and saliva steroids. 20 steroids were included in the assay. Calibrants and samples were spiked with isotopically labelled internal standards and extracted using supported liquid extraction with methyl tert-butyl-ether. Analysis of steroids was performed on an Acquity UPLC chromatography system coupled with Waters TQ-XS mass spectrometer. The method was clinically validated. The lower limit of quantification of the assay was ≤0.2 ng/mL for all steroids. Assay precision of low, medium, and high (0.2, 0.5 and 1 ng/mL) spiked QCs resulted in variation of ≤20%. Calibrations were linear from 0.02-10ng/mL with correlation coefficients (R2) of ≥0.98. Matrix effects, analyte recovery, reproducibility and carryover demonstrated acceptable validation outcomes. Ten healthy participants provided matched serum, urine and saliva to investigate correlations between the biofluids. 10 of the 20 steroids were detectable in saliva from healthy volunteers. In future this method will be utilised to obtain a healthy control reference cohort and investigate endocrine conditions.

PP50: OUTCOME OF UNILATERAL ADRENALECTOMY IN BILATERAL ADRENAL ADENOMAS/HYPERPLASIA, ASSOCIATED WITH MILD AUTONOMOUS CORTISOL EXCESS: A SYSTEMATIC REVIEW AND META-ANALYSIS

Olga Papalou [1], Charalampos Tsentidis Tsentidis [2], Chrysoula Koukoula [1], Efstratios Kardalas [1], Panagiotis Mourelatos [1], Aristidis Diamantopoulos [1], Eirini Sampanioti [1], Kyriaki Gkoufa [1], Stylianos Tsagarakis [1], Dimitra-Argyro Vassiliadi [1]

[1] *Department of Endocrinology Diabetes and Metabolism, National Expertise Center for Rare Endocrine Diseases,* Evangelismos Hospital, Athens, Greece
[2] *Department of Endocrinology, Metabolism & Diabetes Mellitus,* General Hospital of Nikaia-Piraeus "Agios Panteleimon", Piraeus, Greece

Background: The optimal treatment of patients with mild autonomous cortisol secretion (MACS) and bilateral adrenal adenomas/hyperplasia (BAA/BAH) remains uncertain. While there is evidence that surgery may be more beneficial than conservative management for unilateral adenomas, management of BAA/BAH and MACS poses challenges. Bilateral adrenalectomy is not recommended but evidence suggests that unilateral adrenalectomy, with removal of the largest adenoma and/or guidance by AVS or scintigraphy, may offer advantages over observation. Objective: To assess the existing literature on the effect of unilateral adrenalectomy on the hormonal, cardiometabolic and bone health, in these patients. Methods: computerized literature search in MEDLINE, Scopus, and Web of Science covering the period until December 2022. Studies were included if they presented data on the outcome of cortisol-related comorbidities and/or postoperative adrenal insufficiency. Results: From a total set of 2686 records, 133 studies were initially identified, and 10 met the inclusion criteria, with a total number of 164 patients. Transient adrenal insufficiency was described in 5 studies at a rate of 25-100%. Postoperative remission of hypercortisolemia was documented in 7 studies at a rate of 92-100%. Improvement of arterial hypertension was reported in 60-95%, of glucose metabolism in 45-100% and of osteoporosis in 60 - 90%. In those few patients who were not operated (total 27 patients from 2 case-control studies) there was no improvement. Conclusion: Unilateral adrenalectomy for patients with BAA/BAH and MACS may improve comorbidities such as arterial hypertension, glucose metabolism and osteoporosis. However, current clinical studies on this subject are limited and highly heterogeneous.

PP51: RESULTS OF FOLLOW-UP IN A CASE SERIES OF BILATERAL MACRONODULAR ADRENAL DISEASE (BMAD)

Andreea-Gabriela Nuta [1], Ionela Baciu [1, 2], Cristina Capatana [1, 2], Simona Galoiu [1, 2], Iulia Burcea [1, 2], Ramona Dobre [1, 2], Dan Niculescu [1, 2], Larisa Buduluca [5], Anda Dumitrascu [5], Nicoleta Baculescu [1, 2], Raluca Trifanescu [1, 2], Roxana Dusceac [2], Radu Iorgulescu [4], Mircea Diculescu [3], Serban Radian [1, 2], Catalina Poiana [1, 2]

[1] *University of Medicine,* Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
[2] *Pituitary and Neuroendocrine Dpt,* Parhon National Institute of Endocrinology, , Bucharest, Romania
[3] *Gastroenterology Dpt,* National Clinical Institute Fundeni, Bucharest, Romania
[4] *General Surgery,* Sf Ioan Clinical Emergency Hospital , Bucharest, Romania
[5] *Imaging and Radiology,* Parhon National Institute of Endocrinology, , Bucharest, Romania

Introduction: BMAD is defined as the presence of bilateral adrenocortical benign macronodules larger than 1 cm. Patients and methods: Prospective study - clinical, hormonal data, screening for aberrantly expressed G-protein coupled receptors. Results: We identified 20 patients (16F/4M), with onset age 64(50-63) years, median(25th-75th percentile): 14 were incidentally discovered with bilateral macronodular nodules, one was screened for endocrine causes of high blood pressure and 5 presented with clinical signs of cortisol excess. Eleven patients presented obesity, 8 of them were diagnosed with diabetes mellitus and 16 presented high blood pressure. Four patients had osteoporosis. 15 patients presented with variable degrees of Cushing's syndrome (CS), 2 of which associated aldosterone co-secretion and 5 patients had nonfunctional macronodular adrenal disease. In patients with CS the cortisol in 1 mg overnight dexamethasone suppression test was 3.37 mcg/dL(2.34-5.96). Cortisol in low-dose-dexamethasone suppression test was 2.4 mcg/dL(1.84-7.32). Only 3 patients had a high level of urinary free cortisol(UFC). Ten patients were screened for the presence of aberrantly expressed G-protein coupled receptors using a modified version of Lacroix protocol: 3 patients presented a positive response after a mixed meal and 6 of them after stimulation with Diphereline. Enhanced CT showed bilateral macronodules with a diameter of 2.5 cm(2.09-4.65). 5 patients were submitted to adrenal surgery, 2 needed bilateral adrenalectomy. One patient with bilateral adrenalectomy for severe CS died after the second adrenalectomy due to pulmonary embolism. Two other patients died during follow-up due to myocardial infarction and gastrointestinal causes. Conclusions: Clinical presentation in BMAD is variable, from asymptomatic incidentalomas to severe CS.

UFC was within reference range in subclinical/mild CS, while dexamethasone suppression testing and ACTH were diagnostic. Biochemical dynamic testing for aberrant adrenal receptors has therapeutic implications and should be performed. Management should be individualized, with targeted medical therapy where appropriate and/or with steroidogenesis inhibitors.

PP52: METYRAPONE TREATMENT IN MILD HYPERCORTISOLISM

Carmen Aresta [1], Vittoria Favero [2], Sebiana Tuccitto [2], Alberta Dall'Antonia [2], Elisa Delle Donne [2], Chiara Parazzoli [2], Iacopo Chiodini [2, 3], Valentina Morelli [1]

[1] *Department of Endocrine and Metabolic Diseases,* Istituto Auxologico Italiano IRCCS, Milan, Italy
[2] *Department of Medical Biotechnology and Translational Medicine,* University of Milan, Milan, Italy
[3] *Endocrinology Department,* ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy

A medical therapy could be a therapeutic option for patients with mild hypercortisolism (mHC) not candidate for surgery, particularly in the presence of cortisol-related comorbidities. We enrolled 6 mHC patients (3F, aged 70.3±8.6, 1mgDST 5.4±6 mcg/dl) treated with metyrapone (mean dose 500 mg). Before and 6 months after starting the treatment, a clinical assessment was performed with a 24-hour ambulatory blood pressure monitoring, fasting plasma glucose (FPG) and HbA1c levels and oral glucose tolerance test (OGTT). At baseline all patients were affected with arterial hypertension (AH), 3 with type2 diabetes (T2D) and 2 patients with impaired fasting glucose (IFG). After six months of therapy, we observed: The remission of diabetes in one patient and an amelioration in T2D control in 2 patients (HbA1c from 80 to 61 mmol/mol and from 55 to 50 mmol/mol, respectively) without intensifying anti-diabetic treatment. One IFG patient showed normalization of FPG with persistence of a normal OGTT response. The other 2 patients (1 with IFG and 1 with normal glucose metabolism) remained stable in their respective categories. AH improved in all patients: 3 patients showed an improvement in mean blood pressure (BP) passing from a high-normal to an optimal BP, from a high-normal to a normal BP, from a grade 1 AH to a normal BP, respectively, without anti-hypertensive therapy changes; 3 patients maintained adequate BP control despite reducing the number of medications. Both non-dipper patients showed a restoration of nocturnal dipping. In all patients metyrapone was well tolerated without signs or symptoms of hypocortisolism.

PP53: ADRENAL INCIDENTALOMA CASE-CONTROL STUDY: HIGHLIGHT ON A DIFFERENT APPROACH TO IDENTIFY CONTROLS

Miriam Giordano Imbroll [1, 2], Sarah Craus [1, 2], Mark Gruppetta [1, 2]

[1] *Medicine,* Mater Dei Hospital, Msida, Malta
[2] *Medicine,* University of Malta, Msida, Malta

Introduction: Most studies that attempted to compare adrenal incidentaloma with a control group identified controls through databases selecting individuals who did not have reported adrenal disease. We attempted another method for identifying controls for adrenal incidentaloma patients. Methods: 252 patients with an incidentally discovered adrenal adenoma were identified. For each patient, a sex and aged-matched control (+/- 5 years), who underwent a CT scan for a similar indication and on the same day as the cases was recruited. The actual CT scan of the control group was specifically and retrospectively reviewed to exclude the presence of an adrenal incidentaloma. Mortality and various biochemical and haematological parameters were compared between the two groups. Results: From the total cohort, 44.2% were males. The mean age at diagnosis was 67.2 years. 212 patients (84.1%) had an overnight dexamethasone suppression test (ODST) performed, out of whom 138 (65.1%) had an ODST<50nmol/L. When comparing cases with controls, lymphocytes were significantly higher in cases than controls (p=0.002). NLR)/monocyte ratio was higher in controls (p=0.006), as was NLR/monocyte/platelet ratio (p=0.001) and total cholesterol (p=0.036). In our cohort, mortality was highest among the controls (12.7% controls vs 6.3% cases (p=0.015). Conclusion: We have attempted to study adrenal incidentaloma sequelae by using a different method to identify controls. Our cohort of adrenal adenomas did not exhibit a higher mortality rate compared to controls. Some of the haematological parameters linked with increased mortality were also more favourable among the adenoma cohort.

PP54: A SYSTEMATIC AND META-ANALYSIS REVIEW REGARDING PREVALENCE OF BILATERAL ADRENOCORTICAL INCIDENTALOMAS

Diana Sousa [1], Sofia S. Pereira [2, 3], Bernardo Sousa Pinto [4, 5], Duarte Pignatelli [1, 6, 7]

[1] *Cancer Signalling and Metabolism,* i3S, Porto, Portugal
[2] *Unit for Multidisciplinary Research in Biomedicine,* UMIB, Porto, Portugal
[3] *Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto,* ICBAS, Porto, Portugal
[4] *Centro de Investigação em Tecnologias e Serviços de Saúde,* CINTESIS, Porto, Portugal
[5] *Departamento Medicina da Comunidade, Informação e Decisão em Saúde,* MEDCIDS, Porto, Portugal
[6] *Departamento de Endocrinologia,* Centro Hospitalar Universitário de São João, Porto, Portugal
[7] *Faculdade de Medicina da Universidade do Porto,* FMUP, Porto, Portugal

The aim of the present systematic review followed by meta-analysis was to provide an accurate overview of the prevalence of bilateral adrenocortical incidentalomas (AI). A systematic review using PubMed, Scopus and Web of Science databases was performed. Studies with patients aged ≥18 years who were discovered to have an AI and in which prevalence of bilateral adrenal lesions could be extracted or calculated were included. Afterwards, a random-effects meta-analysis of log-transformed proportions was performed. Heterogeneity was assessed by means of the I2 statistic and of the Cochran Q test. Of the initial 4216 records, 26 studies were included in the analysis. The meta-analysis of the included studies resulted in an overall prevalence of bilateral AI of 16.7% (95%CI=14.4-19.4%, I2=86.1). Using univariable meta-regression models, 3 parameters-"year of publication", "year of end of data collection" and "number of years of data collection"- were found to significantly influence the bilaterally prevalence. Moreover, the sub-group analysis revealed that (i) country of origin from Europe, (ii) NMR or ultrasound as imaging method and (iii) prospective nature of study, independently increased the prevalence of bilateral AI. Importantly, in the most recent period evaluated (2011-2020), the prevalence of bilateral AI increased to 21.5%. In conclusion, we found that the overall prevalence of bilateral AI is 16.7%, the percentage being higher in the last decade. This evidence should be considered in future studies describing AI prevalence.

PP55: RADIOLOGICAL AND CLINICAL INTERFACES OF ADRENAL TUMORS

Inesa Galinaityte [1], Urte Lukoseviciute [1], Kristina Zviniene [2], Rytis Stakaitis [3], Valentinas Matulevicius [4]

[1] *Faculty of Medicine,* Lithuanian University of Health Sciences, Kaunas, Lithuania
[2] *Department of Radiology,* Lithuanian University of Health Sciences, Kaunas, Lithuania
[3] *Neuroscience Institute,* Lithuanian University of Health Sciences, Kaunas, Lithuania
[4] *Department of Endocrinology,* Lithuanian University of Health Sciences, Kaunas, Lithuania

Aim: To evaluate the clinical, laboratory and radiological data of patients diagnosed with adrenal tumors. Methods: Analysis of medical histories of patients diagnosed with adrenal tumor in 2017-2018. 40 males and females with a mean age of 59.48 ± 12.45 and 63.83 ± 14.92, respectively. Results: 35% of the patients were overweight, and 41.3% - obese. Arterial blood pressure was elevated in 66 (82%) patients. The average heart rate in female patients was 77.48 ± 11.43 bpm, while in male – 78.53 ± 13 bpm. The most common: 1) complaint was high blood pressure (63,8%); 2) comorbidity - arterial hypertension (42,5%); 3) adrenal tumor - adenoma (42,5%); 4) tumor size - 3 cm (25%); and the least common adrenal tumor was aldosteronoma (3,8%). 37 tumors were enhanced according to the CT with a contrast enhancement. Males (30%) were more often diagnosed with bilateral tumors than females (5%). Finally, percental increase of metanephrine and noretanephrine was significantly higher in pheochromocytoma than in other tumors. Conclusions: 1) Differences between gender in BMI, blood pressure and heart rate were not significant. No significant differences were found between male and female complaint analysis. 2) The most common adrenal tumors were adenomas, and the changes in the hormone concentration of adenomas were not statistically significant. Pheochromocytomas were significantly larger than adenomas. Pheochromocytomas enhance statistically significantly more compared to other adrenal tumors. 3) In case of pheochromocytoma, the percentage of increase of metanephrine and normetanephrine is significantly higher than in other tumors.

PP56: PLASMA ACTH LEVELS AS A CONFOUNDING PARAMETER IN DIAGNOSING ADRENAL CUSHING SYNDROME, DUE TO ASSAY-SPECIFIC ISSUES: THE PARADIGM OF A CLINICAL CASE

Nikolaos Katechakis [1], Olga Papalou [1], Chrysoula Koukoula [1], Dimitra Vassiliadi [1], Stylianos Tsagarakis [1]

[1] *Endocrinology,* General Hospital Evangelismos, Athens, Greece

Background: In patients with Cushing syndrome (CS), ACTH measurement differentiates adrenal from pituitary/ectopic CS. However, there are reports of erroneous results of the two-site "sandwich" immunometric assays for ACTH due to interference with heterophile antibodies and/or proopiomelanocortin (POMC) and ACTH fragments. Objective: This is a clinical case of a patient with CS, highlighting the need for cautiously assessing plasma ACTH levels to avoid misdiagnosis. Case presentation: A 45-year-old woman presented with a 2-year history of obesity, hypertension, dyslipidemia, diabetes mellitus and menstrual irregularities. A previous abdominal CT scan had incidentally identified a 2,5 cm left adrenal nodule of <10HU. Cortisol post-1-mg dexamethasone was 17,1 μg/dL, with a baseline ACTH of 30,8 pg/ml, consistent with ACTH-dependent CS. DHEA-S levels were low (32,6 μg/dl). Pituitary MRI showed an equivocal 2,3 mm cystic lesion. Further testing included desmopressin (DDAVP) test, in which ACTH increased by 103% but there was no increase in cortisol levels. Due to these disparate results and before proceeding to more invasive procedures such as IPSS, we remeasured ACTH levels with another assay, revealing suppressed plasma ACTH levels (ACTH<1pg/ml). Consequently, the patient underwent laparoscopic adrenalectomy, pathology was consistent with adrenal cortical adenoma and postoperative serum cortisol was undetectable. At the last follow-up, she was in remission and hydrocortisone replacement. Conclusion: Assay-specific falsely elevated plasma ACTH measurement can lead to misdiagnosis and improper therapeutic management of patients with CS. When ACTH results are ambiguous, measurement with an alternate assay method may save our patients from unnecessary diagnostic and therapeutic interventions.

PP57: BILATERAL ADRENAL HEMORRHAGE: LEARNING NOTES FROM CLINICAL PRACTICE AND LITERATURE REVIEW

Maria Elena Aloini [1], Roberta Maggio [1], Irene Biondo [1], Guido Roberto [1], Francesca Ricci [1], Pina Lardo [1], Antonio Stigliano [1]

[1] *Clinical and Molecular Medicine,* Endocrinology, Rome, Italy

Adrenal hemorrhage is rare but very important to be recognized, especially when it involves both the adrenal glands. Bilateral adrenal hemorrhage in fact can lead to adrenal insufficiency, whose consequences can be dramatic if it is not promptly recognized and treated. It is usually caused by systemic conditions leading to the vasoconstriction and thrombosis of the adrenal vein. More often, clinical diagnosis of this condition can be very challenging, because its signs and symptoms are unusual and non-specific (abdominal pain, nausea, fatigue). Here we are presenting the cases of two patients who were admitted to the Emergency Department in 2016 and 2022 respectively, because of acute abdominal pain; they both had recently undergone surgery and had been prescribed with Low Molecular Weight Heparin. In both cases laboratory results revealed neutrophilic leukocytosis and unexplained anemia. Because of the persistence of abdominal pain despite medications, a CT scan was performed, showing an enlargement of both adrenal glands, suggestive for bilateral adrenal hemorrhage. Adrenal function was tested, showing adrenal insufficiency, and both patients were immediately administered parenteral hydrocortisone. After 5 years from the acute event, the first patient has normal adrenal function and does not need adrenal replacement therapy; patient 2 only demonstrated the persistence of adrenal failure, requiring replacement therapy. In this paper, through our experience and an analysis of literature, we will try to underline some clues to identify patients who are potentially at risk for bilateral adrenal hemorrhage.

PP58: NIVOLUMAB-INDUCED PRIMARY ADRENAL INSUFFICIENCY: A CASE REPORT AND A REVIEW OF THE LITERATURE

Anna Angelousi [1], Dimitrios Ziogas [2], Athina Karampela [1], Petros Papalexis [1], Vasiliki Siampanopoulou [1], Alexandros Lafioniatis [1], Chrysoula Mytareli [1], Helen Gogas [2]

[1] *Unit of Endocrinology, First Department of Internal Medicine,* National and Kapodistrian University of Athens , Athens, Greece
[2] *Unit of oncology, First Department of Internal Medicine,* National and Kapodistrian University of Athens , Athens, Greece

Background: Immune checkpoint inhibitors (ICIs) have recently become a cornerstone for the treatment of different advanced cancers. Endocrine-related adverse events (AEs) are among the most frequent AEs with an incidence ranging from 5-20%. Primary adrenal insufficiency (PAI) has been reported in few cases so far . Aim: We describe a case of a 71 years old female patient presenting with adrenal crisis after 2 cycles of nivolumab monotherapy for melanoma treatment. A review of the existing data in the literature was also performed. Result: Clinical and biochemical exam revealed a sudden onset of pigmentation of gingivitis as well as severe hyperkaliemia and hypotension. Adrenal crisis was suspected and confirmed by a low morning baseline cortisol level and a five-fold higher levels of adrenocorticotropic hormone (ACTH). Aldosterone levels were also decreased with abnormally high plasma renin activity levels. Adrenal cortex autoantibodies were found positive. Symptoms improved after hydrocortisone and fludrocortisone substitution. Imaging showed bilateral atrophy of the adrenals. Autoantibodies were found negative 6 months after the interruption of nivolumab treatment. Literature systematic review of the last 10 years found 27 studies describing 29 patients who experienced PAI following ICI therapy for various types of malignancy. In the vast majority the PAI was permanent. Conclusion: Endocrine-related AEs due to ICIs require a multidisciplinary approach in order to anticipate for the early detection and the prompt treatment. PAI is a rare but potentially life-threatening associated with substantial morbidity and mortality requiring hospitalisation and urgent management.

PP59: A CASE OF ELEVATED 17-HYDROXYPROGESTERONE AND MILD AUTONOMOUS CORTISOL SECRETION (MACS)

Vasiliki Siampanopoulou [1], Athina Karampela [1], Petros Papalexis [1], Alexandros-Aristeidis Lafioniatis [1], Chrysoula Mytareli [1], Foteini` Thanasoula [1], Anna Angelousi [1]

[1] *Unit of endocrinology, First Department of Internal Medicine,* Laiko General Hospital of Athens, National and Kapodistrian University of Athens, Athens, Greece

Introduction: The 17-hydroxy-progesterone is an established marker indicative of congenital adrenal hyperplasia (CAH). However, it may be misleading in cases of bilateral adrenal hyperplasia with mild autonomous cortisol hypersecretion (MACS). Objective: We present the case of a 68-year-old male patient diagnosed with multiple adrenal nodules in the left and a single in the right adrenal gland diagnosed since 2006. Results: Baseline hormonal work-up revealed an elevated 17-OH progesterone, a low dehydroepiandrosterone sulphate and Δ4-androstenedione and normal baseline adrenocorticotropic hormone (ACTH) and cortisol levels. Synacthen stimulation test revealed a 12 % increase of 17-OH-progesterone. However genetic analysis was negative for 21-hydroxylase deficiency. The 1 mg-overnight dexamethasone suppression test (ODST) and the low dexamethasone suppression test (LDDST) were suggestive of MACS (3,4 and 2,8 mcg/dl respectively). Urinary free cortisol and midnight salivary cortisol were within the normal range. The patient presented no signs or symptoms of Cushing's syndrome except for un uncontrolled diabetes mellitus. Imaging analysis demonstrated three benign adrenal nodules in the left (maximum diameter of 3,8 cm, 1,8 cm and 0,7 cm) and one in the right adrenal gland (1,6 cm), with low density (<10 UH) and presence of normal adrenal tissue between the adrenal nodules. Aberrant receptor analysis revealed a cortisol response of > 50% increase after posture test. Further genetic analysis (ARMC5) is pending. Conclusion: Elevated 17-OH-progesterone levels can indicate CAH, a state of potential cortisol deficiency. However, it may be misleading in cases of bilateral adenomas or MACS. In these cases, genetic counselling could be required.

PP60: ECTOPIC CUSHING'S SYNDROME IN THE PEDIATRIC AGE

Irene Biondo [1], Maria Elena Aloini [1], Guido Roberto [1], Francesca Ricci [1], Roberta Maggio [1], Pina Lardo [1], Antonio Stigliano [1]

[1] *Clinical and Molecular Medicine,* Endocrinology, Rome, Italy

Cushing's syndrome (CS) due to ectopic ACTH secretion (EAS) is a rare clinical condition resulting from a dysregulated ACTH secretion by neuroendocrine tumors which can have various localizations and different histological differentiations. The overall incidence of endogenous CS is 0.7–2.4 per million people per year. Only approximately 10% of the new cases each year occur in children. We present a 17 year-old patient affected by ectopic CS. At the onset patient showed psychotic crises, then she treated with oral antipsychotics. Clinical picture was also complicated by arterial hypertension and osteporosis with D9-D10 and L1-L5 vertebral collapses. After some months she was referred to the endocrinologist who requested blood tests. In particular her plasma ACTH was 305 pg/mL, and her serum cortisol was 1711 nmol/l. The 1 mg dexamethasone suppression test confirmed the presence of ACTH-dependent Cushing's syndrome. The total body CT scan showed left adrenal hyperplasia. 68Ga-PET-DOTA was negative. Thus, she started metyrapon 250 mg qid. After one year of therapy a new 68Ga-PET-DOTA showed a nodule in the thymic lodge. This finding was also confirmed by chest MRI. Despite the patient performed thymectomy the cortisol levels was elevated; so she continued Metyrapon. After three months from surgery a new 68Ga-PET scan showed disease recurrence in the thymic lodge which required another surgery, with definitively healing. This case underlines the difficult of the diagnosis of ectopic Cushing's syndrome diagnosis due to the heterogeneity of the presentation of this condition. It also shows the need for close biochemical and imaging follow-up for tumor's localization.

PP61: INTELLIGENT CHATBOTS FOR PROGNOSING ADRENAL TUMOUR DISEASE IN ENDOCTRINE PATIENTS WITH HEREDITARY CONDITIONS BASED ON CERTAIN PRECLINICAL SYMPTOMS

Angel Montes Olguín [1], *Jolanta Mizera-Pietraszko* [2]

[1] *Computer Science,* Tecnológico Nacional de México Campus Zacatecas Norte, Río Grande, Mexico
[2] *Institute of Management,* Military University of Land Forces, Wrocław, Poland

Domain-oriented medical chatbots are artificial intelligence (AI) programs designed to offer medical assistance and advice to patients. Typically, they use natural language processing (NLP) and machine learning (ML) algorithms to understand the user's symptoms, analyze medical history, provide relevant information, and suggest treatments or basically recommend a doctor's appointment if necessary. This technology can assist endocrine specialists by quickly screening patients and providing data-driven recommendations. As a result, healthcare professionals can provide more efficient and precise treatments for patients with adrenal tumours. Furthermore, chatbots can aid in educating patients about hereditary conditions and their impact on their overall health. One of the greatest advantages of these chatbots is that they are available 24/7, accessible from anywhere, and can handle multiple user requests simultaneously. This way it can substantially reduce healthcare wait times, minimize the workload of healthcare providers, improve the patient experience, and save costs for both patients and healthcare systems. Using a database that provides evidence-based recommendations for the diagnosis, treatment, and management of patients with neuroendocrine tumours (NETs), adrenal tumors, pheochromocytomas, paragangliomas, and multiple endocrine neoplasia, we propose an approach to personalized testing and evaluation of some medical chatbots with the aim at the functionalities oriented towards specific endoctrine patients' needs. Our research objective is to defuse most of the controversy and fears surrounding the chatbots causing revolutionary changes in medicine and many other branches including the course of litigation.

PP62: HUMAN SECURITY APPROACH IN ETHICAL AND LEGAL ASSESSMENT OF HUMAN SUBJECTS RESEARCH

Serghei Sprincean [1]

[1] *Center for Strategic Studies,* Institute of Legal, Political and Sociological Research, Chisinau, Moldova

The correlation between the field of ethics and moral norms with the sphere of security, political and social action with the aim of ensuring personal and social security ans safety is transposed, in a natural way, in the legal-normative level, thus manifesting a phenomenon much discussed by jurists: the emergence of norms of law with legal power of recommendation (soft law) and imperative and mandatory legal norms (hard law). In particular, this phenomenon manifests itself in international law, where the recommended legal norms include various conventions, multilateral treaties, guidelines, directives, etc., and the mandatory legal norms are those that provide for direct sanctioning mechanisms in case of deviation or non-compliance with person or state level as legal subjects. In this practical aspect, bioethics has advanced substantially, adopting in the last three decades an imposing base under the auspices of numerous specialized international bodies, both regional and global, including a series of very vast and generalizing conventions and international legal acts, regarding various human security and bioethical aspects, such as the field of ethical expertise of biomedical research with human or animal subjects.

PP63: DEEP LEARNING APPROACHES APPLIED TO IMAGE CLASSIFICATION OF KIDNEY TUMOURS

Sandra Amador [1], José Perona [1], Jorge Azorin [1], David Gil [1]

[1]*Department of Computer Science Technology and Computation,* University of Alicante, Alicante, Spain

Kidney cancer, also known as renal cancer, is one of the ten most common cancers in adults. They are a major and growing health problem worldwide, with increasing incidence and mortality rates in recent years. The use of artificial intelligence techniques may represent a new advance in the accurate detection of renal tumours. Artificial intelligence, specifically computer vision, enables the recognition of complex patterns in images. Currently, computer vision solutions are tied to deep learning because they have proven their superiority among different approaches. Deep learning uses deep artificial neural networks to learn, classify, or backtrack from complex data that has several advantages over traditional machine learning methods. This set of techniques applied to kidney cancer is not widespread. Currently, only 8 papers have been found in the last 5 years that meet these requirements. In this work, a deep learning method has been carried out, specifically convolutional neural networks, to predict the pathology or classify the renal tumour. Data augmentation has been applied to increase the amount of data, since it is difficult to find a large number of quality medical images. We used transfer learning to take advantage of pre-trained models, particularly important in computer vision applications. Furthermore, we develop with Keras environment that provides convenient access to several high-performing models on the image recognition tasks such as VGG, Inception, and ResNet. Promising results were obtained for the improvement of patient care, being able to predict the diagnosis of patients with renal tumours.

PP64: CREATING THE BEST DECISION TREE FOR ASSISTING PRACTITIONERS TO DIAGNOSE CANCER

Avi Rosenfeld [1]

[1] *Computer Science,* Jerusalem College of Technology, Jerusalem, Israel

The goal of our research is to develop algorithms to determine the best decision tree to use in order to teach a medical practitioner to perform a classification task. Specifically, we taught people how to diagnose suspected cancer using a decision tree. After the learning process, we tested the diagnostic ability of the people via new cases presented to them. Based on the results of the experiment, we built an agent that used Machine Learning to determine which decision tree was best to present so that the people's cancer understanding was maximized as quantified by their predictive accuracy. We found that too complicated tree was hard to understand, thus impairing the ability to correctly diagnose cancer. Conversely, too simple tree was easy to understand, but also impaired performance as it oversimplified the medical information. Thus, the agent needed to find the best balance between these extremes so it could maximize his performance while not overloading practitioners with too much information. We present initial results of this agent's success in helping people predict esophageal cancer.