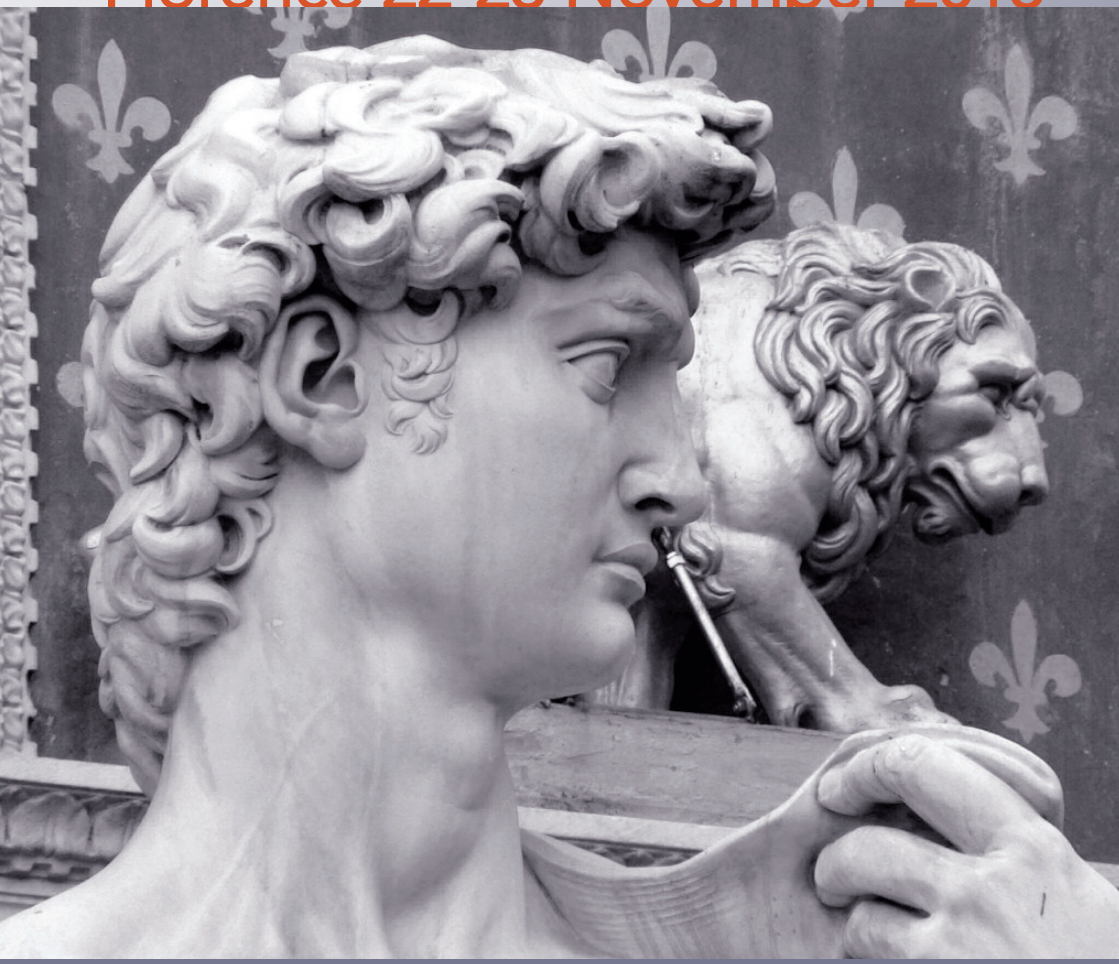
17th ENS@T Scientific Meeting

Florence 22-23 November 2018



PROGRAM & ABSTRACT BOOK

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UNIVERSITÀ DEGLI STUDI FIRENZE

Azienda Ospedaliero Universitaria Careggi

FONDAZIONE CR FIRENZE

sie Società Italiana Endocrinologia

AME

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General Information

Venue
Azienda Ospedaliero-Universitaria Careggi
Auditorium Centro Traumatologico Ortopedico
Largo Palagi 1, 50139 Firenze

Country Dialing Code
+39

Electrical Voltage
The voltage in Italy is 220 volt.

Liability and Insurance
The organizers have no responsibility whatsoever for injury or damage involving persons and property during the Conference. Participants are advised to carry their own personal insurance during their stay in Italy.

Name Badges
All participants and accompanying persons must wear the Conference identification badge in a visible place. Entrance to meeting hall, poster and exhibition areas will not be permitted to any person without badge.

Official Language
The official Language of the Conference will be English. Simultaneous translation will not be provided.

Travel Information

To attend the whole meeting, we suggest to arrive in the evening of Wednesday November 21st.
The closure of the meeting is planned early in the afternoon of Friday 23rd, to allow most of the delegates to fly back home on the same day. Nonetheless, we invite you to take the chance to spend the week-end visiting our beautiful historical town.

How to reach the conference venue:

Urban bus/tram
The Careggi hospital is served by the following new tramline **T1** connecting the congress venue to the main central railway station (Santa Maria Novella).

Suburban bus
The Careggi hospital is served by several suburban lines that connect it with the neighboring municipalities.

Taxi
Telephone: 055 4390 - 055 4242 - 055 4798
For text messages to 334 66 22 550: write in the message the street and the street number where you are (or a known reference); you will receive a confirmation text message.

Train
From the train station of Florence S.M.N. (Santa Maria Novella): take the Tramvia T1.
From the train station Florence Rifredi: take the ATAF shuttle bus 33.

Airplane
From Florence Peretola Airport (Amerigo Vespucci):
The city's airport, which is located approximately 6 km from the hospital, is served by taxi, bus and car rentals.

From Pisa Airport (Galileo Galilei):
The airport of Pisa, which is located approximately 90 Km from Careggi hospital, is connected to Florence by train and bus; car rental is also possible and in this case it is recommended the route via SGC FI-PI-LI.

Secretariat information

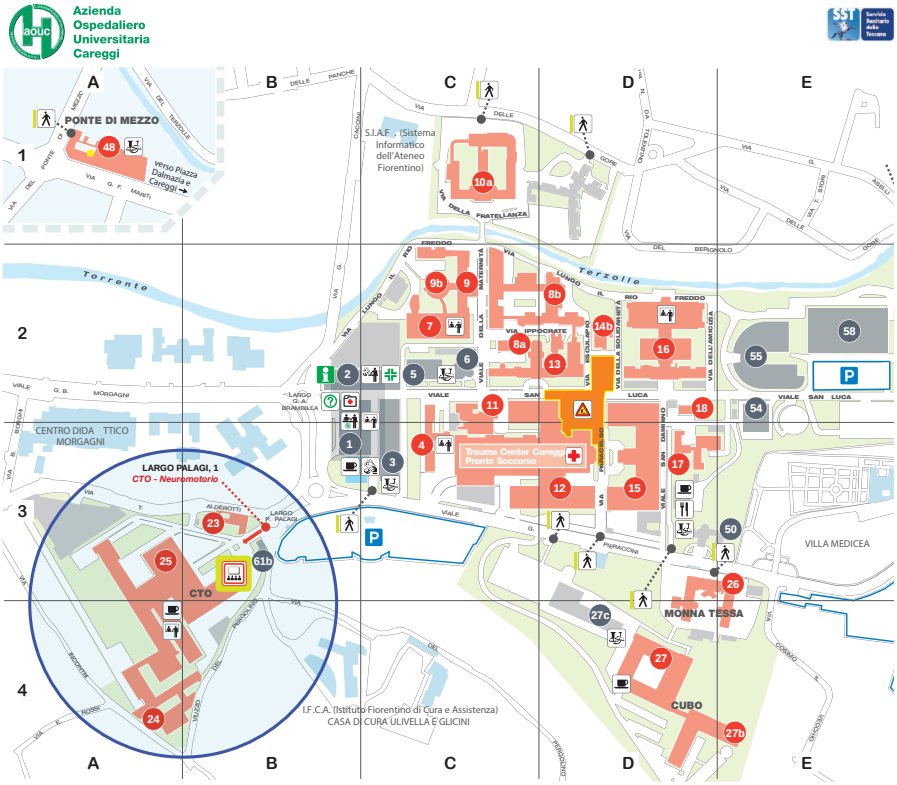
Registration Desk and Opening Hours
Thursday 22 November: 08.00-19.00
Friday 23 November: 08.00-16.30

Certificate of Attendance
Certificates of attendance will be available at the registration desk for all participants.

Social program

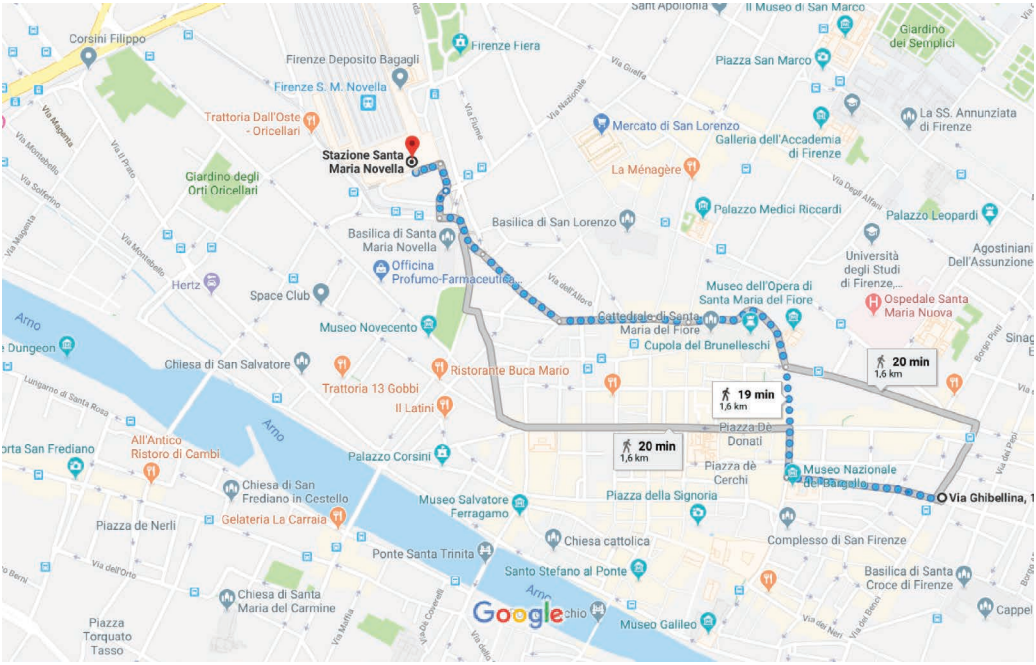
Festive Award Dinner
Palazzo Borghese, Via Ghibellina 110, 50122 Firenze

Maps



Conference venue

Azienda Ospedaliero Universitaria Careggi,
Auditorium Centro Traumatologico Ortopedico,
Largo Palagi 1, 50139, Firenze



Festive Award Dinner

Palazzo Borghese, Via Ghibellina 110, 50122 Firenze

17th ENS@T SCIENTIFIC MEETING

PROGRAM

Thursday 22 November 2018

08.00-08.30	Registration
08.30-09.00	Welcome: *Authorities, Massimo Mannelli, Florence, Italy*
09.00-10.45	**ACC Oral communications OC1-8** Chairs: *Michaela Luconi (Italy), Peter Igaz (Hungary)*

OC1 - NCI-H295R cell xenografts in zebrafish embryo as an experimental model to evaluate the in vivo cytotoxicity of abiraterone
A. Gianoncelli, M. Guarienti, M. Fragni, M. Bertuzzi, E. Rossini, A. Abate, M. Memo, A. Berruti, S. Sigala

OC2 - Urine steroid metabolomics for the detection of malignancy in adrenal incidentaloma - Results of the ENSAT EURINE-ACT test validation study
I. Bancos, A. Taylor, V. Chortis, A. Sitch, K. Lang, A. Prete, M. Terzolo, M. Fassnacht, M. Quinckler, D. Kastelan, D. Vassiliadi, F. Beuschlein, U. Ambroziak, M. Biehl, J. Deeks, W. Arlt

OC3 - Prognostic role of modified GRAS score in adrenocortical carcinoma: validation in a large cohort of patients
C. L. Ronchi, B. Altieri, Y. S. Elhassan, J. Lippert, V. Chortis, S. Hahner, M. O'Reilly, M. Kroiss, M. Fassnacht

OC4 - Comparative Drug Screening of Classical Chemotherapeutic and Targeted Therapies in Two Preclinical Models for ACC
A. Sharma, I. Shapiro, P. Perge, A. Decmann, P. Igaz, C. Hantel

OC5 - The igf2 methylation score as an objective marker for adrenocortical carcinoma: a european network for the study of adrenal tumors (ensat) validation study
S. Creemers, R. Feelders, N. Valdes, C. Ronchi, M. Volante, B. van Hemel, M. Luconi, M. Ettaieb, M. Mannelli, M. Chiara, M. Fassnacht, M. Papotti, M. Kerstens, G. Nesi, H. Haak, F. van Kemenade, L. Hofland

OC6 - Cabozantinib treatment of advanced adrenocortical carcinoma: results from an international case series and preview of two phase II trial protocols
M. Kroiß, F. Megerle, J. Wendler, J. Schreiner, M. A. Habra, M. Fassnacht

OC7 - Intra-tumor heterogeneity of molecular alterations in adrenocortical carcinoma
A. Jouinot, B. De La Villeon, M. Neou, S. Garinet, L. Groussin, M. Sibony, S. Gaujoux, B. Dousset, K. Perlemoine, R. Libé, G. Assié, J. Bertherat

OC8 - The role of filamin A (FLNA) in the regulation of IGF2/IGF1R pathway in adrenocortical carcinomas
R. Catalano, E. Giardino, F. Mangili, D. Treppiedi, P. Vercesi, V. Morelli, M. Mannelli, A. Spada, M. Arosio, G. Mantovani, E. Peverelli

10.45-11.15	*Coffee Break*
11.15-12.45	**Poster Guided Session/Networking** (Delegates, Patients Associations)
PO01-32	ACC Chairs: *Jerome Bertherat (France), Enzo Lalli (France)*
PO33-51	PPGL Chairs: *Graeme Eisenhofer (Germany), Rodrigo Toledo (Spain)*
PO52-56	APA Chairs: *Tracy Ann Williams (Italy), Mitsuhide Naruse (Japan)*
PO57-65	NAPACA: *Guillaume Assie (France), Iacopo Chiodini (Italy)*
	Tutorial ENS@T Registry: *Anthony Stell (Australia)*
12.45-13.30	*Lunch/Networking*
13.30-15.15	**PPGL Oral Communications OC9-16**
	Chairs: *Judith Favier (France), Henry Timmers (Netherlands)*

OC9 - Identification of a long non-coding RNA signature predictive of metastasis in SDH-mutated paraganglioma
L. J. Castro-Vega, S. Job, A. Georges, N. Burnichon, A. Buffet, L. Amar, J. Bertherat, A. de Reyniès, J. Favier, A. Gimenez-Roqueplo

OC10 - Genotype-phenotype correlations in the SDH genes: elevated phaeochromocytoma-paraganglioma risk, multifocal disease and a new intermediate phenotype
J.P. Bayley, B. Bausch, D.B. Ascher, D.E. Pires, J.A. Rijken,

L. T. Hulsteijn, J. C. Jansen, F. J. Hes, E. F. Hensen, E. P. Corssmit, P. Devilee, H. P. Neumann

OC11 - Tumor microenvironment stroma cells increase aggressiveness of pheochromocytoma spheroids
S. Martinelli, M. Riverso, T. Mello, L. Canu, G. De Filpo, S. Richter, A. S. Tischler, K. Pacak, M. Mannelli, E. Rapizzi

OC12 - Possible role of a CMV-like virus in paragangliomas
M. R. Pantalone , F. Verginelli, S. Perconti, M. K. Arzenani, D. L. Esposito, S. Vespa, F. Schiavi, A. Sheu, S. Valentinuzzi, S. De Fabritiis, E. Taschin, V. Tarantini, S. Soliman, A. Ramassone, M. Di Marco, M. Serluca, D. Vlad, A. Veronese, R. Visone, A. Zangrandi, G. Opocher, C. T. Paties, A. Rahbar, M. Sanna , C. Söderberg Nauclér, L. V. Lotti, R. Mariani-Costantini

OC13 - Generation and functional characterization of a zebrafish sdhb knock-out model
M. Dona, S. Waaijers, T. van Herwaarden, S. Richter, G. Eisenhofer, R. Giles, R. Rodenburg, H. Timmers, P. Deen

OC14 - Evaluation of circulating tumor cells and circulating tumor dna as liquid biopsy in pheochromocytoma/paraganglioma
F. Schiavi, C. Michele, F. Antonella, R. Vidotto, C. Poggiana, E. Taschin, G. Barbon, M. Ferrara, F. Ceccato, S. Zovato, G. Opocher, R. Zamarchi

OC15 - Clinical and phenotypic features of pheochromocytomas and paragangliomas with somatic mutations in HRAS
N. Bechmann, B. Calsina, E. Rapizzi, S. Richter, M. Peitzsch1, K. Langton, S. Fliedner, K. Pacak, M. Kroiß, M. Fassnacht, H. J. Timmers, F. Beuschlein, A. Prejbisz, J. W. Lenders, M. Mannelli, M. Robledo, G. Eisenhofer

OC16 - Preliminary secondary mutational events analysis in metastatic PPGLs
B. Calsina, E. Piñeiro-Yañez, C. Fustero-Torre, Á. M. Martínez-Montes, N. Bechmann, M. Fassnacht, F. Beuschlei, H. Timmers, S. Fliedner, G. Eisenhofer, K. Pacak, F. Al-Shahrour, M. Robledo

15.15-16.30 **ACC-WG**

ACC WG Committee Members:
Felix Beuschlein (Switzerland) (Chair),
Martin Fassnacht (Germany),
Guillaume Assie (France),
Alfredo Berruti (Italy),
Peter Igaz (Hungary)

16.30-16.50 *Coffee Break*

16.50-18.10 **PPGL-WG**

PPGL-WG Committee Members:
Mercedes Robledo (Spain) (Chair),
Henry Timmers (Netherlands),
Judit Favier (France),
Anne-Paule Gimenez-Roqueplo (France),
Elena Rapizzi (Italy),
Rodrigo Toledo (Spain)

19.30-22.30 Conference Dinner

Friday 23 November 2018

08.00-08.45 **ENS@T General Assembly**

ENS@T Executive Committee Members:
Martin Fassnacht (Chair),
Anne-Paule Gimenez-Roqueplo,
Giuseppe Opocher, Mercedes Robledo,
Tracy Ann Williams, Martin Reincke,
Felix Beuschlein, Wiebke Arlt,
Peyman Bjorklund, Massimo Mannelli

08.45-10.05 **APA Oral Communications OC17-22**

Chairs: *Laurence Amar (France),*
Marcus Quinkler (Germany)

OC17 - Untargeted Metabolomics of Adrenal Hypertension using plasma NMR
N. Bliziotis

OC18 - Contribution of the retinoic acid receptor signalling to adrenal cortex morphology and functional zonation
R. El Zein, A. Rickard, F. L. Fernandes-Rosa, J. F. Golib-Dzib, B. Samson-Couterie, I. Giscos-Douriez, A. Rocha, M. Poglitsch, H. LeFebvre, C. E. Gomez-Sanchez, L. Amar, N. B. Ghyselinck, A. Benecke, E. Lalli, M. Zennaro, S. Boulkroun

OC19 - Accumulated Evidence for Clinical Practice of Primary Aldosteronism By the National PA Registry as a Sustainable Platform in Japan
M. Naruse, Y. Takeda, I. Kurihara, T. Katabami, T. Ichijo, N. Wada, Y. Ogawa, M. Sone, M. Tsuiki, A. Tanabe, A. JPAS/JRAS Investigators

OC20 - Observational study: adrenalectomy but not mineralocorticoid receptor antagonist treatment is associated with reduced salt intake in primary aldosteronism

C. Adolf, D.A. Heinrich, F. Holler, N. Nirschl, L. Sturm, F. Beuschlein, M. Reincke

OC21 - Safety of medical adjustment and confirmatory testing in the diagnostic workup of primary aldosteronism
D. Heinrich , C. Adolf, M. Quinkler, F. Holler, B. Lechner, N. Nirschl, L. Sturm, V. Görge, F. Beuschlein, M. Reincke

OC22 - Expression of clock-related genes in benign and malignant adrenal lesions
A. Angelousi, N. Nasiri-Ansari, A. Karapanagioti, C. Parianos, C. Aggeli, G. Zografos, G. Kyriakopoulos, T. Choreftaki, T. Kounadi, H. S. Randeva, G. Kaltsas, A. G. Papavassiliou , E. Kassi

10.05-11.20 **APA-WG**

APA-WG Committee Members:
Martin Reincke (Germany)(Chair),
Marcus Quinkler (Germany),
Tracy Ann Williams (Italy),
Jacques Lenders (Netherlands),
Mitsuhide Naruse (Japan),
Aleksander Prejbisz (Poland)

11.20-11.40 *Coffee Break*

11.40-13.00 **NAPACA Oral Communications OC23-28**

Chairs: *Cristina Ronchi (Germany),*
Antoine Tabarin (France)

OC23 - Untargeted Metabolomics of Adrenal Hypertension using plasma NMR
N. G. Bliziotis, J. Deinum, G. Eisenhofer, M. Zennaro, P. Mulatero, A. Prejbisz, F. Beuschlein, M. Fassnacht, L. Lenzini, M.C. Dennedy, M. Boscaro, F. Ceccato, G.P. Rossi, J. M. Connell, T. Williams, M. Azizi, L. Amar, J. Bertherat1, A.G. Roqueplo, C.K. Larsen, E. Davies, U. Engelke, R. A. Wevers, L.J. Kluijtmans, H. Timmers

OC24 - Levoketoconazole, the Single 2S,4R Enantiomer of Ketoconazole, is a Potential Novel Steroid Synthesis Inhibitor for Medical Treatment of Cushing's Syndrome
S. G. Creemers, R. A. Feelders, F. H. de Jong, G. J. Franssen, Y. B. de Rijke, P. M. van Koetsveld, L. J. Hofland

OC25 - Measurement of dexamethasone levels with LC-MS/MS during 1-mg dexamethasone suppression test in routine clinical practice
F. Ceccato, S. Niero, M. Barbot, G. Antonelli, C. Artusi, M. Plebani, M. Boscaro, C. Scaroni

OC26 - The Impact of Autonomous Cortisol Secretion on body composition
D. Delivanis, M. Hurtado, T. Cortes, K. Aakanksha, E. Atkinson, N. Takahashi, M. Moynagh, I. Bancos

OC27 - Could ARMC5 status be predicted from the clinical, hormonal and radiological investigations in primary bilateral macronodular adrenal hyperplasia (PBMAH) ? Analysis of a large European series of patients
L. Bouys, A. Vaczlavik, S. Espiard, M. North, A. Tabarin, G. Assié, L. Guignat, T. Brue, P. Touraine, P. Chanson, O. Chabre, H. Lefebvre, J. Sadoul, F. Borson-Chazot, S. Christin-Maitre, M. Vantyghem, M. Terzolo, W. Arlt, M. Fassnacht, M. Reincke, F. Beuschlein, J. Bertherat

OC28 - Differentiation of adrenal myelolipoma from adrenocortical adenoma and cancer by microRNA expression profiling
A. Decmann, P. Perge, G. Nyírő, O. Darvasi, I. Likó, K. Borka, T. Micsik, Z. Tóth, I. Bancos, R. Pezzani, M. Iacobone, P. Turai, A. Patócs, P. Igaz

13.00-13.45	*Lunch/Networking*
13.45-15.00	**NAPACA-WG** NAPAPA-WG Committee Members: *Wiebke Arlt (United Kingdom) (Chair),* *Felix Beuschlein (Switzerland),* *Iacopo Chiodini (Italy),* *Cristina Ronchi (Germany),* *Antoine Tabarin (France)*
15.00-15.50	*Martin Fassnacht (Germany),* *Massimo Terzolo (Italy)* **Highlights on ENS@T ACC guidelines** All Participants **Question & Answers on ACC guidelines**
15.50-16.20	**Poster Awards and Closing**

ABSTRACT BOOK

OC1 - NCI-H295R CELL XENOGRAFTS IN ZEBRAFISH EMBRYO AS AN EXPERIMENTAL MODEL TO EVALUATE THE IN VIVO CYTOTOXICITY OF ABIRATERONE

A. Gianoncelli[1], M. Guarienti[1], M. Fragni[1], M. Bertuzzi[1], E. Rossini[1], A. Abate[1], M. Memo[1], A. Berruti[2], S. Sigala[1]

[1]Department of Molecular and Translational Medicine Brescia, [2]Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health Brescia

Abiraterone acetate (AbiAc) is able to inhibit tumor growth when administered to immunodeficient mice engrafted with NCI-H295R cells, the widely used experimental cell model of human adrenocortical carcinoma (Fiorentini C et al JCEM 2016).

Several zebrafish xenograft models are currently used for pre-clinical anti-cancer drug screening. Here, we developed and validated a zebrafish embryo model engrafted with NCI-H295R cells to study the effects of AbiAc on tumor growth.

Zebrafish embryos at 48 h post fertilization (hpf) were anesthetized and microinjected with NCI-H295R cells into the subperidermal space of the yolk sac. Cells were previously treated with the vital fluorescent dye CellTrackerTM CM-DiI (Thermo Fisher). A picture of each embryo was acquired under a Leica MZ16F fluorescence stereomicroscope 2 hours post treatment (T0); then embryos (N=80) were divided into two groups: one treated (N=40) with 1μM AbiAc and one (N=40) left untreated. AbiAc was added directly to the embryo medium. After 3 days (T3) pictures were taken as above described. The tumor area was then measured with Noldus DanioScope software and analyzed by GraphPad Prism 6. Results demonstrated that in untreated embryos the tumor area was significantly increased at T3 compared to T0, indicating that injected NCI-H295R were viable and proliferating, while in AbiAc-treated embryos, the tumor area was superimposable to that measured in T0 embryos.

AbiAc absorption from embryos was evaluated by quantifying the concentration of AbiAc and its metabolites by high-performance liquid chromatography–tandem mass spectrometry (LC-MS/MS). Analytes were extracted from batch of 25 embryos, each treated with 1μM AbiAc at different times (24, 48 or 120 hpf) and collected after 1,5 h (T1), 3 h (T2), 6 h (T3) and 24 h (T4) of treatment. Results shown that AbiAc absorption was embryos stage-dependent (24-48 and 120 hpf) and that there was an initial increase of abiraterone concentration (from T0 to T4), followed by its decrease in favor of the increase of other metabolites, in particular Δ4A. Accordingly, by q-RT-PCR we demonstrated that zebrafish embryos expressed the enzyme 3β-HSD mRNA, that converts abiraterone in Δ4A.

Identification of other metabolites is still ongoing.
These results indicated that zebrafish embryo xenografted with ACC tumor cells could be a useful, fast and reproducible experimental model to test preclinically the activity of new drugs in ACC in vivo.

OC2 - URINE STEROID METABOLOMICS FOR THE DETECTION OF MALIGNANCY IN ADRENAL INCIDENTALOMA – RESULTS OF THE ENSAT EURINE-ACT TEST VALIDATION STUDY

I. Bancos[1], A. Taylor[2], V. Chortis[2], A. Sitch[2], K. Lang[3], A. Prete[2], M. Terzolo[4], M. Fassnacht5, M. Quinckler6, D. Kastelan[7], D. Vassiliadi[8], F. Beuschlein[9], U. Ambroziak[10], M. Biehl[11], J. Deeks[2], W. Arlt[2]
[1]Mayo Clinic Rochester, [2]University of Birmingham Birmingham, [3]University of Birmingham Bi, [4]University of Turin Turin, [5]University hospital Wurzburg Wurzburg, [6]Charite University Hospital Berlin, 7University of Zagreb Zagreb, [8]Evangelismos Hospital Athens, [9]Zurich University Hospital Zurich, [10]University of Warsaw Warsaw, [11]University of Groningen Groningen

Background: Adrenal masses are discovered in 5% of abdominal imaging scans. Accuracy of currently available imaging tests to diagnose malignancy is poor. In a proof-of-concept study (JCE&M 2011;96(12):3775-84), we had demonstrated 90% sensitivity and specificity in detecting adrenocortical carcinoma (ACC) for urine steroid metabolomics, the combination of steroid profiling and machine learning-based analysis. Implementation of our novel test in routine practice requires prospective validation.
Methods: We undertook a prospective multi-center test validation study of patients with newly diagnosed adrenal mass, biochemical exclusion of pheochromocytoma and 24-h urine collection. Recruitment was carried out in 14 centers (11 countries) of the European Network for the Study of Adrenal Tumors (ENSAT). Urinary steroid excretion was quantified by high-throughput liquid chromatography-tandem mass spectrometry and results processed by an algorithm based on generalized matrix relevance learning vector quantization. Reference standard was based on histology and imaging follow up.
Results: We enrolled 2017 patients, 1767 (87.6%) with a benign adrenocortical adenoma (ACA), 98 (4.9%) with ACC, and 87 (4.3%) and 65 (3.2%) with other benign and malignant adrenal masses, respectively. Unenhanced CT imaging of the adrenal mass was available for 1328/1767 patients with ACA; 68% of masses had a radiodensity <10HU indicative of a benign lesion; 17% had borderline results (10-20HU) and 15% were suspicious of ACC (>20HU). MRI with chemical shift indicated suspicion of ACC in 22% of 273 benign ACA. Adrenalectomy was performed in 21%

(370/1767) of ACA patients. Urine steroid metabolomics demonstrated an excellent diagnostic performance with AUROC of 94.56% for 15 steroids (Sens=Spec 87.1%). A diagnostic strategy combining size, urine steroid metabolomics and imaging had a post-test ACC probability of 76.4% (95% CI 67.2-84.1%) for those with positive result and 0.3% (95% CI 0 to 0.6%) for those with negative result. This strategy identified 93 of 98 adrenocortical carcinomas and only required urine steroid metabolomics in 488 (24.2%) and non-contrast CT imaging in 270 (13.4%) of 2017 patients.
Conclusions: Overall risk of ACC in adrenal tumors is 4.9% and almost exclusively relates to adrenal masses >4 cm. Urine steroid metabolomics demonstrates high accuracy for detection of ACC and should become standard-of-care in patients with indeterminate adrenal tumors.

OC3 - PROGNOSTIC ROLE OF MODIFIED GRAS SCORE IN ADRENOCORTICAL CARCINOMA: VALIDATION IN A LARGE COHORT OF PATIENTS

C. L. Ronchi[1], B. Altieri[2], Y. S. Elhassan[1], J. Lippert[3], V. Chortis[1], S. Hahner[2], M. O'Reilly[1], M. Kroiss[2], M. Fassnacht[2]
[1]Institute of Metabolism and System Research Birmingham, [2]Division of Endocrinology and Diabetes Wuerzburg, [3]Institute of Human Genetics Wuerzburg

Adrenocortical carcinoma (ACC) is a rare cancer with a generally poor, but heterogeneous prognosis. Some molecular patterns may be observed in tumours associated with worst clinical outcomes, but they are not currently used in the clinical setting. A previous study on patients with advanced ACC showed that a combination of clinical and histopathological parameters (GRAS score) may stratify patients according to prognosis (Libe et al 2015). Recently, we demonstrated that a modified combination (mGRAS) may play a prognostic role for all patients with ACC.
In this study, we evaluated the impact of mGRAS in predicting clinical outcomes in a large cohort of 457 ACC patients followed in two referral centres since 2000, and with available clinical and histopathological data as well as sufficient follow-up. Age at diagnosis, symptoms at presentation, ENSAT tumour stage, resection status, and ki67 proliferation index were collected from clinical records in order to calculate the mGRAS score (range: 0-9, as previously reported, Lippert et al 2018). Major endpoints were progression-free survival (PFS) and disease-specific survival (DSS). All the single parameters showed a significant association with both PFS and DSS (P value from <0.05 to <0.0001, chi-square value from 5.5 to 177). However, the combination of all paramenters compriosed in the mGRAS score gave the best risk stratification in terms of either

PFS (median 106 vs 33 vs 7 vs 3.5 months for scores 0-1 vs 2-3 vs 4-5 vs 6-9, respectively, P<0.0001, chi-square value 235) or DSS (median undefined vs 116 vs 40 vs 16 months for score 0-1 vs 2-3 vs 4-5 vs 6-9, respectively, P<0.0001, chi-square value 247). In particular, the mGRAS score better predicted occurrence of both early recurrences/progress and disease-specific death than ki67 index alone (area under the ROC curve 0.798±0.021 vs 0.733±0.025 and 0.892±0.020 vs 0.781±0.029).
To conclude, this study demonstrates that, when the tumor material is not available for molecular investigations, the combination of parameters included in the mGRAS score may allow the clinicians to identify subgroups of ACC patients with different prognosis. Thus, this can be used in the clinical practice for guiding follow-up planning and therapeutic decisions.

OC4 - COMPARATIVE DRUG SCREENING OF CLASSICAL CHEMOTHERAPEUTIC AND TARGETED THERAPIES IN TWO PRECLINICAL MODELS FOR ACC

A. Sharma[1], I. Shapiro[1], P. Perge[2], A. Decmann[2], P. Igaz[2], C. Hantel[3]
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Current clinical gold standards are not satisfying for the treatment of adrenocortical carcinoma (ACC). However, translation of preclinically promising approaches were unfortunately disappointing in recent years indicating that existing tumor models also might have inadequately predicted clinical applicability. Thus, our workgroup initiated a comparative drug screen of relevant chemotherapeutic agents and therapies targeting IGFR, EGFR, VEGFR/PDGFR and Wnt signalling pathway in both, the classical NCI-H295R and the recently developed MUC-1 tumor model. While e.g. Etoposide (E) and Cisplatin (P) inhibited cell viability of NCI-H295R cells in a highly significant (E 180uM: 2.5%; P 160uM: 17%) and dose-dependent manner, even at extraordinary high drug concentrations cell viability remained high for MUC-1 (E 180uM: 67%; P 160uM: 70%, both p<0.001 vs NCI-H295R). Moreover, for single treatments with Doxorubicin (D), 9-cis-Retinoic acid (RA), Gemcitabine (G), Erlotinib (E), XAV-939 (X) and Isoquercitrin (I) we detected comparably low or even a complete lack of toxicity in either MUC-1 alone (for D and E) or both tumor models (for I). Of note, Mitotane (M), Paclitaxel (PTX), Linsitinib (L) and Sunitinib (S) displayed overall improved toxicities. However, as

single drugs, at clinically relevant dosages, they were still not potent enough to result in complete tumor cell cytotoxicity responses for both tumor models. Interestingly, among combinatory approaches tested so far (such as EDP-M, P + RA, PTX + G, P + G and P + PTX) the latter e.g. P + PTX led to slightly improved combinatory effects at higher drug dosages and P + G to additive reduction of cellular viability (NCI-H295R: G: 89%; P: 27%; G + P: 18% and MUC-1: G: 91%; P: 88%; G + P: 58%; both $p<0.001$ vs 100% controls) as well as to reduced clonogenicity in both tumor models. Mechanistically, G alone induced, as expected, a dose-dependent increase in expression of, both RRM1 and RRM2, genes that are known to be involved in the development of clinically relevant G resistance. However, in combination with P these effects were significantly reversed down to basal levels or even below (NCI-H295R RRM1, G: 791% $p<0.001$; G + P: 188%; RRM2, G: 336% $p<0.001$; G + P: 60% and in MUC-1 RRM1, G: 275% $p<0.01$; G + P: 97%; RRM2, G: 474% $p<0.01$; G + P: 175%; vs. 100% controls). In summary therefore, a combination of both models might help to identify potentially new drug combinations for the treatment of ACC in the future.

OC5 - THE IGF2 METHYLATION SCORE AS AN OBJECTIVE MARKER FOR ADRENOCORTICAL CARCINOMA: A EUROPEAN NETWORK FOR THE STUDY OF ADRENAL TUMORS (ENSAT) VALIDATION STUDY

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Introduction: Diagnosis of adrenocortical carcinoma (ACC) is based on the histopathological Weiss Score (WS), but remains clinically elusive unless it has metastasized. Previously, we proposed the use of a methylation score of IGF2 regulatory regions as an objective diagnostic tool for malignancy of adrenocortical tumors. The aim of this study was to validate this assay in a multicenter European cohort study.

Patients and methods: Patient and tumor characteristics were obtained from all patients. DNA was isolated from frozen specimens. After pyrosequencing of DMR2, CTCF3, and H19, a standard deviation score was calculated compared to normal adrenals. Uni- and multivariate logistic and cox regression analysis were used to identify predictors for malignancy, defined either by the WS or the development of metastases. The discriminative value was assessed using Receiver Operating Characteristic curves. Patients with ENSAT tumor stage IV ACC at diagnosis were excluded from these analyses.

Results: Seventy-eight patients with ACC and 118 patients with adrenocortical adenoma were included from seven participating centers. The methylation score and the tumor size were independently associated with the pathological diagnosis of ACC (OR 3.745 95% CI 2.247 – 6.240; OR 1.498 95% CI 1.220 – 1.839, respectively; Hosmer-Lemeshow test P= 0.943). The discriminative value of this model resulted in an area under the curve (AUC) of 0.950 (95% CI 0.919 – 0.980). The methylation score alone resulted in an AUC of 0.908 (95% CI 0.864 – 0.951). Cox regression analysis revealed that the methylation score, WS and tumor size predicted development of metastases in univariate analysis, whereas in multivariate analysis only the WS was predictive for development of metastasis during follow-up (OR 1.682 95% CI 1.285 – 2.202; $P < 0.001$).

Conclusion: We validated the high diagnostic accuracy of the previously proposed IGF2 methylation score for confirming the pathological diagnosis of ACC in a multicenter European cohort study. Considering the known limitations of the WS, the objective IGF2 methylation score could potentially provide extra guidance on decisions on postoperative strategies in patients with adrenocortical tumors.

OC6 - CABOZANTINIB TREATMENT OF ADVANCED ADRENOCORTICAL CARCINOMA: RESULTS FROM AN INTERNATIONAL CASE SERIES AND PREVIEW OF TWO PHASE II TRIAL PROTOCOLS

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Background: Objective response of advanced adrenocortical carcinoma to mitotane and cytotoxic chemotherapy regimens is only ~20% and early tumor progression is frequent.

Objective: To investigate the potential of cabozantinib monotherapy in ACC patients with progressive disease after mitotane and chemotherapy.

Design: Retrospective cohort study in two tertiary care centers. Presentation of protocols for phase II trial.

Results: Twelve patients (10 female) with progressive disease after mitotane (11/12 patients) and three (median; range 1-8) other systemic therapies were treated with a starting dose of 60 mg (60-140) cabozantinib. Mitotane had been stopped in all patients. Plasma concentration was documented to be <2 mg/L in 5/12 patients, mitotane stop was >270 days in four additional patients and one patient never received mitotane. Grade 1/2 adverse events (AE) were observed in 9/12 patients and consistent with the known safety profile of cabozantinib. There were no grade 3/4 AE. At the time of analysis, 2 patients were still treated with cabozantinib. Best response was partial response in two, stable disease in 4 and progressive disease in 6 patients. A progression-free survival of >16 weeks was observed in 4/10 patients evaluable for this end point. Median PFS and OS was 13 and 49 weeks, respectively.

Conclusion: Cabozantinib monotherapy appears to be active in monotherapy for the treatment of adrenocortical carcinoma. Patient recruitment in two parallel phase II trials is ongoing (NCT03370718, MD Anderson Cancer Center) and expected in Q1/2019 (NCT03612232, University Hospital Würzburg), respectively.

OC7 - INTRA-TUMOR HETEROGENEITY OF MOLECULAR ALTERATIONS IN ADRENOCORTICAL CARCINOMA

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Background:

The prognosis of adrenocortical carcinoma (ACC) is heterogeneous. Recently genomic studies have identified 3 subgroups of ACC characterized by specific molecular alterations and associated with very different prognosis. Molecular classification can be routinely determined by targeted markers and improves prognostic assessment. The aim of this study was to evaluate the intra-tumor heterogeneity of molecular alterations of ACC.

Methods:

Two different tissue samples were analyzed for 14 patients that underwent surgery for primary or recurrent ACC, either from primary tumor and metastasis (N=9, including 5 metachronous metastases), from two regions of bulky primary tumor (N=2), or from two metastases (N=3). Somatic mutations of 20 driver genes were assessed using targeted next generation sequencing (NGS, AmpliSeq) and chromosome alteration profiles were determined using SNP arrays (Illumina).

Results:

The most frequently altered genes were ZNRF3 (25%), TP53 (21%), CTNNB1 (18%), CDKN2A (21%) and TERT (21%). Intra-tumor heterogeneity was observed in 4 patients: alterations of CDKN2A (N=2), CTNNB1 (N=1) and ZNRF3 (N=1) were present either in metastasis but not in primary tumor samples (N=3), or in one but not the other of the two samples from bulky primary tumor (N=1). Intra-tumor heterogeneity was not associated with stage at diagnosis or tumor grade.

Chromosome alteration profiles were “Noisy” (numerous and anarchic alterations) in 67% and “Chromosomal” (extended patterns of loss of heterozygosity) in 33% of the study samples, and were similar for the two samples of each patient.

Conclusion:

Somatic mutations analysis reveals intra-tumor heterogeneity in 4/14 patients (28%), evoking the presence of tumor sub-clones with different aggressiveness profiles. However, the chromosome alteration profiles remain similar and suggest a global genomic stability in tumor progression and metastatic spread compared to other tumor types. Targeted molecular

markers reflecting the pan-genomic profiles should be preferred to somatic mutation analysis alone for prognosis assessment of ACC. Intra-tumor heterogeneity may also represent a limitation for personalized medicine based on mutational status.
All authors declare that they have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

OC8 - THE ROLE OF FILAMIN A (FLNA) IN THE REGULATION OF IGF2/IGF1R PATHWAY IN ADRENOCORTICAL CARCINOMAS

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Adrenocortical carcinomas (ACCs) are rare endocrine tumors with poor prognosis. The insulin-like growth factor 2 (IGF2) is overexpressed in the great majority of ACC, and IGF2/IGF1R pathway acts as a proliferative autocrine loop, but to date IGF1R-targeted therapies have demonstrated a limited efficacy and the molecular mechanisms regulating this pathway are still unknown.
The cytoskeleton acting-binding protein filamin A (FLNA), determinant in cancer progression and metastasis in different tumors, affects the intracellular trafficking and signalling of many receptors, including growth factor receptors such as EGFR, but a possible role of FLNA in regulating IGF1R has never been investigated.
The aims of this study are: 1) to test FLNA involvement in modulating IGF1R signaling in human ACC cell lines H295R and SW13; 2) to evaluate the expression of FLNA in ACCs and a possible correlation with IGF1R pathway activation.
By immunoprecipitation we found that IGF1R interacted with FLNA in basal condition, with an increased recruitment of FLNA after IGF2 stimulation, in both cell lines.
As expected, IGF2 promoted H295R and SW13 cell proliferation and migration and accordingly increased ERK and cofilin activation. Interestingly, all these tumorigenic actions of IGF2 were potentiated in the absence of FLNA. Indeed, in cell silenced for FLNA, IGF2 induced a further increase of proliferation (+69±40% in SW13 and +11±1.3% in

H295R, p<0.05), migration (+10±7% in SW13 and +17±8% in H295R, p<0.05), p-ERK/tot-ERK ratio (+1.14±0.2 fold in SW13, p<0.05) and a decrease of p-cofilin/tot-cofilin ratio (-0.20±0.02 fold in SW13, p<0.05) vs IGF2-stimulated control cells.
Furthermore, FLNA silencing in SW13 cells was associated to an increase of IGF1R expression after IGF2 stimulation (+1.60±0.7 fold vs IGF2-stimulated control cells, p<0.05), suggesting that FLNA is involved in receptor downregulation.
Finally, western blot analysis showed significantly lower FLNA expression in ACCs (n=5) than in adrenocortical adenomas (n=19) (FLNA/GAPDH ratio 0.98±1.4 and 4.37±2.5, respectively, p<0.01). Moreover, FLNA expression levels in ACC samples were negatively correlated with ERK phosphorylation status.
In conclusion, we demonstrated that low levels of FLNA enhance IGF2/IGF1R pathway activation in adrenocortical tumor cells, suggesting FLNA as a new factor possibly influencing the response to the therapy with IGF1R-target drugs.

OC9-16 PPGL

OC9 - IDENTIFICATION OF A LONG NON-CODING RNA SIGNATURE PREDICTIVE OF METASTASIS IN SDH-MUTATED PARAGANGLIOMA

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Background: Pheochromocytomas and paragangliomas (PPGL) are neuroendocrine tumors caused by germline or somatic mutations in about 70% of cases. Patients with SDHB mutations are at high-risk of developing a metastatic disease, yet no biomarkers are available to predict tumor aggressiveness. Here, we investigated whether long non-coding RNAs (lncRNAs) could be markers of poor prognosis.
Methods: lncRNA expression was analyzed in a well-characterized series of 187 PPGL using a mining approach of transcriptome data (ArrayExpress, E-MTAB-733). Unsupervised and cluster consensus analyses were carried out to identify lncRNAs specific for molecular groups. Receivers operating characteristic curve analysis was applied to identify the best discriminators of metastasis in SDHB-mutated tumors. Univariate/multivariate cox regression models and metastasis-free survival (MFS) analysis were performed for assessment of clinical outcome.

Results: lncRNA-based subtypes strongly correlated with mRNA expression clusters (chi² p-values from 1.38e-32 to 1.07e-67). This classification was validated in an independent series of 51 PPGL (GEO, GSE67066). Co-expression analyses suggest that differentially expressed lncRNAs participate in regulating signaling pathways that characterize PPGL subtypes. Remarkably, we identified three putative lncRNAs (GenBank: BC063866, BC047922 and AL833059) that accurately discriminates metastatic from benign tumors in patients at high-risk of progression (AUC 0.95, 0.91 and 0.89; P = 4.59E-05, 3.73E-04 and 6.70E-04, respectively). These lncRNAs were significantly associated with poor prognosis (hazard ratio, 5.8, 2.6 and 2.4; P = 3.5E-03, 2.8E-03 and 3.2E-03, respectively) and short MFS (logrank tests p-values <0.001). Of these, BC063866 further appeared as independent risk factor (hazard ratio, 9.4; P = 0.01). Notably these lncRNAs have been also reported in aggressive neuroblastoma, a tumor related in origin to PPGL. Finally, we found that BC063866 might interact with genes involved in neural crest and peripheral nervous system development, as suggested by its nuclear localization, co-expression analyses and in silico lncRNA/DNA binding predictions.
Conclusion: In summary, our findings extend the spectrum of transcriptional dysregulations in PPGL to lncRNAs and provide insights into the roles of these transcripts in tumorigenesis and metastasis. Remarkably, we identified a novel signature of three-lncRNAs that could be useful to identify potentially metastatic PPGL in patients with SDHB mutations, thus providing a clinical value to better stratify this group of patients.

OC10 - GENOTYPE-PHENOTYPE CORRELATIONS IN THE SDH GENES: ELEVATED PHAEOCHROMOCYTOMA-PARAGANGLIOMA RISK, MULTIFOCAL DISEASE AND A NEW INTERMEDIATE PHENOTYPE

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Background

Previously established genotype-phenotype correlations for the SDH genes include the association of PPGL with SDHB and HNPGL with SDHD, but variant class (i.e. missense/nonsense) has not previously been investigated.

Methods

Three independent datasets (DE, n=383; GB, n=366; NL, n=280) including 1029 affected cases were combined. All PPGL and/or HNPGL patients were carriers of germline variants in the SDHB, SDHC or SDHD genes. Association of variant class with disease incidence and age-related tumour risks was analysed.

Results

All three SDH genes displayed a trend towards greater clinical detection of nonsense compared to missense variants. Carriers of SDHD nonsense variants showed a significantly higher risk for PPGL, an earlier age of diagnosis and a greater risk for multifocal disease compared to carriers of missense variants. Specific SDHD missense variants were associated with a distinct phenotype. Carriers of SDHB nonsense variants showed a smaller but still significantly increased PPGL incidence and earlier age of tumour diagnosis compared to carriers of SDHB missense variants. All three SDH genes showed a trend towards overrepresentation of missense variants in HNPGL cases. In addition, we show that SDHD nonsense variants represent an intermediate class, clinically more closely associated with SDHB than the traditional SDHD phenotype.

Conclusions

Nonsense SDHx variants are clinically overrepresented and confer an elevated risk for PPGL, and in the case of SDHD, earlier age of diagnosis and greater multifocality of disease. SDHD nonsense variants result in a phenotype intermediate between SDHB and SDHD missense variants. These findings suggest that the clinical screening and counselling of carriers of SDHC and SDHD may need to be tailored according to their variant class.

OC11 - TUMOR MICROENVIRONMENT STROMA CELLS INCREASE AGGRESSIVENESS OF PHEOCHROMOCYTOMA SPHEROIDS

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Pheos/PGLs are generally benign tumors and surgery is the current therapy but SDHB mutated Pheos/PGLs are an exception, since up to 80% of these tumors are metastatic and to date there is no effective treatment.

In this study, we evaluated the different effect of tumor microenvironment on tumor cell migration/invasion by co-culturing tumor cell spheroids with primary cancer-activated fibroblasts (CAFs). To this end, we used three dimension (3D) cultures of a mouse pheochromocytoma cell line (MTT) silenced or not (wild type = Wt) for the catalytic SDHB or the anchoring SDHD subunit.

We measured matrigel invasion of spheroid cells by the computation of the migratory areas. Intriguingly, we observed that SDHD silenced spheroids had an intermediate migration pattern compared to the highest migration capability of SDHB and the lowest one of the Wt spheroids.

Interestingly, by confocal microscopy, we found that all the conditioned spheroids (Wt, SDHB and SDHD silenced ones) developed long filamentous formations, but only SDHB silenced cells invaded the surrounding space moving collectively probably using those filamentous as binaries. Wt and SDHD silenced spheroids tended to move individually. Ongoing experiments are aimed to understand if these long outgrowths are neuronal structures (dendrites or axons) or not.

To better characterize the molecules involved in promoting tumor cell migration, conditioned medium of CAFs was divided into two fractions depending on molecular weights of its components by using cut-off filters. The upper fraction contains molecules with a molecular weight higher than 3000 Da, while the lower fraction is constituted by small molecules (MW < 3000 Da). Surprisingly, when SDHB silenced spheroids were conditioned with the upper or the lower fraction of conditioned medium we did not observe an increase in migration areas. Areas were similar to those of unconditioned spheroids, and we were able to detect a significant migration only when the whole, undivided medium was used. On the contrary, we found that conditioning Wt and SDHD silenced spheroids by the upper fraction was sufficient to induce migration, migration areas were similar to those observed when the whole medium was used.

These results suggest that the interplay between tumor microenvironment and SDHB silenced tumor cells is peculiar and that specific factors are responsible for the higher aggressiveness of SDHB mutated Pheos/PGLs. The characterizations of these factors may open new approaches for medical therapy.

OC12 - POSSIBLE ROLE OF A CMV-LIKE VIRUS IN PARAGANGLIOMAS

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The etiology of paragangliomas (PGLs) is unclear, as recent data suggest low penetrance of SDHxmutations (1), and mice mutated in Sdhb/d are vulnerable to aberrant differentiation but do not develop PGLs (2-3). Studying prospectively-collected head and neck PGLs by electron microscopy (EM), we found cytomegalovirus-like particles in all tumors. To confirm CMV infection we analysed a series of over 140 PGL cases for SDHxmutational status and CMV infection. Germline point mutations and large deletion/rearrangements of the SDHxgenes were analysed by PCR, sequencing and MLPA. Blood and tumor DNAs were analysed for CMV sequences using PCR. Tumor tissues were analysed by rapid in situ hybridization (RISH) with a large CMV probe, EM, cryo-immuno EM, IF, IHC and WB for several CMV proteins. Viral transmissibility to HELF, MRC5, and HEK293T cells from PGL cells or PGL tissue extracts was tested with protocols modified from Rous (4) and Heine et al. (5). PGL-derived cells were treated with ganciclovir/valganciclovir and imatinib in vitroand in vivo. SDHxmutational analysis showed a mutation frequency of 34.3%. CMV-like particles were observed in all the 51 cases analysed by EM. The CMV reactive proteins IE, gB, pp65, vMIA and their partner cellular proteins (ZEB1, PDGFRA, viperin) were detected by IF, IHC, immuno-EM, and WB in all cases. In our attempts to amplify the viral DNA using CMV-specific primers, we detected a viral glycoprotein B sequence stretch in 68.2% and 82.3% of the tested PGL and matched blood samples respectively. Overall, 36/40 PGLs examined by RISH resulted positive. Exposure of HELF, HEK293T, and MRC5 cell lines to PGL cells or tissue extracts resulted in cytopathic effects and PCR positivity for CMV sequences. PGL-derived xenografts presented the same viral particles and proteins of the original tumors, while CMV sequences

were retrievable from the blood of the xenografted mice. Ganciclovir and imatinib inhibited paraganglioma cell growth, downregulated the expression of CMV proteins and their host partners, and significantly prevented engraftment of paraganglioma cells. Overall, this suggests that infection with a CMV-like virus is common in PGLs.

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OC13 - GENERATION AND FUNCTIONAL CHARACTERIZATION OF A ZEBRAFISH SDHB KNOCK-OUT MODEL

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Background: Pheochromocytomas or paragangliomas (PPGLs) caused by mutations in the β-subunit of the succinate dehydrogenase (SDHB) have the highest metastatic rate, for which effective systemic therapy is lacking. To unravel the underlying pathogenic mechanisms and to evaluate therapeutic strategies, suitable in vivo models are needed. The available Sdhb knock-out mice models do not mimic the human PPGL phenotype, as also seen for the Von Hippel Lindau gene. In contrast to mice, zebrafish vhl knockouts showed several features of VHL in humans1. Despite absence of obvious PPGL-like tumours in vhl mutants, we detected increased metanephrine levels in homogenates of vhl knock-out larvae and adult fish, as well as their swimming water. Similar to VHL, we hypothesized that, possibly, features of SDHB-related diseases in humans could also be replicated in a zebrafish model.

Aim: To generate and characterize a zebrafish sdhb mutant as a potential model for SDHB-related disease in humans.

Results: Using CRISPR-cas9 technology, we successfully introduced a germline lesion in the zebrafish sdhb gene resulting in a frameshift and early stop codon. Homozygous sdhb-/- larvae are viable, but exhibit a shorter lifespan. Biochemical analysis revealed decreased mitochondrial complex II activity and significant succinate accumulation in sdhb-/- larvae as compared to their heterozygous sdhb+/- and wild-type siblings. In line with the anticipated inability of our fish to produce sufficient energy, behavioral analysis showed a lower basic activity as well as a faster decrease in activity after labor in our sdhb-/- fish. These features are similar to those found in patients born with inactive SDHB due to bi-allelic SDHB mutations and as found in SDHB-associated PPGL.
Conclusion: we have created a first vertebrate animal model that mimics the metabolic effects of SDHB-associated PPLGs and allows us for the first time to study the metabolic effects of the lack of sdhb in vivo.

OC14 - EVALUATION OF CIRCULATING TUMOR CELLS AND CIRCULATING TUMOR DNA AS LIQUID BIOPSY IN PHEOCHROMOCYTOMA/PARAGANGLIOMA

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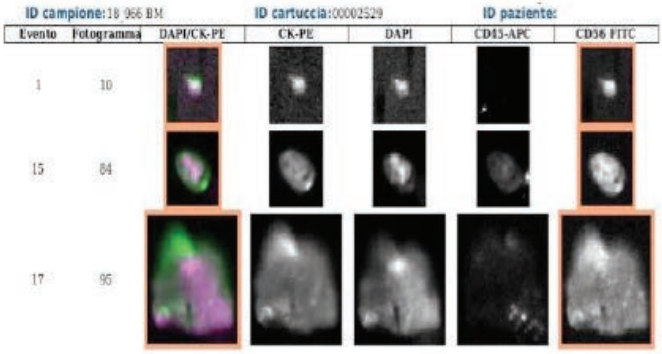
Background – According to an evolving concept of liquid biopsy, Circulating Tumor Cells (CTCs) and circulating-free tumor DNA (ctDNA) constitute novel biomarker classes to monitor disease and treatment efficacy over time, even when tumor or metastases material is not available (e.g. in early or minimal residual disease) or taking a biopsy is associated with significant risks to the patient.
The presence of CTCs in neuroendocrine tumors (NETs) has been provided demonstrating the feasibility of CellSearch assay, because of EpCAM expression by NETs [1,2]. Data were then confirmed and extended by M. Schott and coll., which reported the presence of EpCAM-positive, CD45-negative CTCs in Pheochromocytoma [3].
Methods – Eight patients with metastatic pheochromocytoma or paraganglioma has been recruited in 2018 and monitored by enumeration of CTCs and ctDNA analysis.
We have enumerated CTCs in whole blood by the CellSearch CTC Kit. We have integrated the standard CTC assay with an anti-CD56 and anti-Synaptophysin mAb in order to disclose the neuroendocrine origin of

CTCs. We have also evaluated the neuroendocrine origin of EpCAM-negative fraction of CTCs by the ASCD device, an accessory of the CellSearch platform suited to collect and characterize EpCAM low/neg tumor cells.
Moreover, we have interrogated mutations or hotspots in 56 clinically-relevant oncology-related genes using the Accel-Amplicon 56G Oncology Panel v2.
Results – CTCs has been detected in 4 out of 8 patients. CTCs exhibit the phenotype EpCAM+, CK+, DAPI+, CD45- and CD56/Synaptophysin +. In 2 CTC-positive patients we have also detected in the ASCD device additional cells with the phenotype EpCAM-, CK+, DAPI+, CD45- and CD56/Synaptophysin +.
In 2 out of 7 patients analyzed, we identified mutations in PIK3CA, TP53, NRAS and SMAD4 genes in ctDNA.
Conclusions – ctDNA and CTCs detecting may serve as a promising liquid biopsy in pheochromocytoma/paraganglioma management with broad application including early diagnosis, disease progression prediction, and personalized treatment.



CD56/synaptophysin immunostaining of human CTCs from a PCC patient
The photo gallery shows analysis of 2 rare cells (events #: 1 and 15) and of 1 tumor micro-embolus (event #: 17) in a blood sample of a PPGL patient, using an Analyzer II device. Horizontally, the photos show the same cell, imaged separately, and stained for different fluorophores: [1] combination of DAPI (violet, nucleated cells) and CK-FLU (green, epithelial marker), [2] CK-FLU only; [3] DAPI only, [4] CD45-APC only (specificity control), [5] CD56-PE / synaptophysin only.
The orange squares indicate positively stained cells both for DAPI and CK (first column at links), and CD56/synaptophysin-positive cells (first column at right): both dim (# 15) and strong CK staining (#1) are presented.
In the tumor micro-embolus (event #: 17), we can recognize at least 4 nuclei of different size.
Based on CD56/synaptophysin staining profile (sufficient signal relative to background), all the events are classified as CD56/synaptophysin -positive CTCs.

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OC15 - CLINICAL AND PHENOTYPIC FEATURES OF PHEOCHROMOCYTOMAS AND PARAGANGLIOMAS WITH SOMATIC MUTATIONS IN HRAS

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An increased number of gene mutations associated with pheochromocytomas and paragangliomas (PPGLs) have been identified, and therefore it is important to define phenotypic features including biochemical and metabolomic phenotypes, tumor location, malignancy and clinical presentation associated with each PPGL-related gene for an early diagnosis and individualized treatment. Gene expression profiling has identified two main groups of PPGLs, with alterations to either pseudohypoxic (cluster 1) or kinase signaling pathways (cluster 2). Somatic mutations in Harvey rat sarcoma viral oncogene homolog

(HRAS) in sporadic PPGLs cluster together with NF1-and MEN2-mutated tumors associated with an activation of kinase signaling pathways.
In order to further establish biochemical and clinical features, a total of 226 PPGLs including 26 (11.5%, male:female 9:17) patients with confirmed somatic HRAS mutations were collected and tissue catecholamines, PNMT activity, and Krebs cycle metabolites were analyzed. The mean age at initial diagnosis was 51.7 years in patients with somatic HRAS mutation in our cohort (vs. cluster 1: 37.1 vs. cluster 2: 47.6). Interestingly, tumors bearing a HRAS mutation were smaller (mean 33.7 cm3) in comparison to other cluster 2 (72.6 cm3) and cluster 1 (50.5 cm3) tumors, and tended to a higher prevalence for metastatic disease (absence:present 23:3; 13%) in comparison to the other cluster 2 (53:0) tumors (chi square test, p=0.0117). Relatively unusual for cluster 2 tumors, three out of 26 HRAS cases developed in extra-adrenal tumors (11.5%). Like other patients with mutations in cluster 2 genes, HRAS patients showed an adrenergic phenotype that could be confirmed by measuring tissue catecholamines. HRAS tumors showed an elevated PNMT activity also in comparison to the other cluster 2 tumors. Microarray analysis gave the first hint to an impairment of the MYC/MAX interaction in HRAS tumors. MYC plays an important role in cancer cell metabolism. Analysis of Krebs cycle metabolites showed an accumulation of citrate, α-ketoglutarate, fumarate and malate in tissues with HRAS mutations compared to other cluster 2 and 1 tumors, whereas the pyruvate levels were similar.
In summary, PPGL patients with somatic HRAS mutation show distinct phenotypic features, including an adrenergic phenotype in line with other cluster 2 tumors. However, they appear to show specific characteristics compared to both cluster 1 and 2 tumors, including smaller tumor volume and differences in Krebs cycle metabolites.

OC16 - PRELIMINARY SECONDARY MUTATIONAL EVENTS ANALYSIS IN METASTATIC PPGLS

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Pheochromocytoma and paraganglioma (PPGL) is a heterogeneous genetic disease. Disease management continues to be complicated by the highly variable clinical behavior of these tumors. Approximately 15-20% of these tumors metastasize, usually to the lymph nodes (80%), bone (71%), liver (50%), and lungs (50%), leading to a decreased overall survival of only 60% at 5 years after the initial diagnosis of metastasis.

The aim of this work was to define secondary mutational events involved in pathogenesis and progression to metastatic lesions, with special attention to those found in SDHB-carriers, as 40% of SDHB patients develop metastatic disease.

To achieve this objective, we have gathered a series of 64 metastatic patients to perform Whole Exome Sequencing (WES). From these patients at least germline and primary tumor/metastasis DNA were available. Of note, germline, primary tumor and metastatic lesion tissues was available from nine of them, as well as multiple primary tumors for 10 additional ones. Exome variant calling were performed by RUbioSeq multiplatform pipeline and MuTect for somatic variation analysis. A customized automatic and manually curated analysis was applied to the mutations found for annotation, selection and prioritization for further study. Furthermore, transcriptional and methylation profiles are being used to assess the functional impact of the events found.

In this preliminary study, we present the results describing the most relevant somatic events appeared in tumors, as well as in metastatic tissues, in order to identify potential specific patient weaknesses and novel druggable targets.

OC17-22 APA

OC17 - UNTARGETED METABOLOMICS OF ADRENAL HYPERTENSION USING PLASMA NMR

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[1]*ENSAT-HT consortium ..*

Background: The ENSAT-HT project aims to establish a signature for different forms of adrenal hypertension based on a multi-omics approach, including untargeted metabobolomics of plasma. Profiling the metabolome using 1H-Nuclear Magnetic Resonance Spectroscopy (NMR) has led to the discovery of biomarkers for many diseases, such as inborn errors of metabolism. Our aim within the scope of ENSAT-HT is to define the metabolic signature of patients suffering from Primary Aldosteronism (PA), Cushing's Syndrome (CS) and Pheochromocytoma and Paraganglioma (PPGL), and compare it to primary hypertensives (PHT) and healthy volunteers (HV).

Methods: Heparin plasma samples were collected from patients and distributed to all centers collaborating on ENSAT-HT. These samples were analyzed, along with Quality Control samples (QC) using NMR spectroscopy. The resulting spectra from both sample types were processed using signal alignment and peak picking routines, so as to convert the spectra to a readable table by multivariate statistical analysis (MVA) tools. The resulting datasets were subsequently normalized, batch corrected and transformed. As a first step in MVA, Principal Component Analysis (PCA) was applied as an unsupervised method to check for outliers and trends within the datasets.

Results: Spectra collected were of high quality. Diagnostic plots of data processing gave away the robustness of the resulting peak data that were subsequently used for statistical analysis. Upon inspection of the PCA score plots of NMR plasma data, a tight clustering of the QC samples based on order of analysis was observed. This confounder was dealt with by using batch correction methods, leading to a significantly smaller amount of technical variation within the complete dataset. Finally, as shown in the 3D PCA score plot which describes 39% of the variation present in the dataset (Figure), by coloring samples based on their respective groups, three clusters of samples can be observed as PHTs (blue, n=106), HVs (green, n=132) and Adrenal Hypertensives (PA, PPGL, CS, all in red, n=230).

Conclusions: We have established a reliable method for untargeted metabolomics using plasma NMR. The initial analysis suggests that patient classification based on whether there is adrenal or primary hypertension appears to be feasible. However, removing other sources of variation, such as sample origin, age of sampling and patient gender is a necessary step before reaching any definitive conclusions concerning disease group differentiations. Future plans include, along with diminishing the effects of these confounders, the analysis of urine NMR and UHPLC-QTOF plasma data, and the application of supervised statistical methods to further understand the metabolism of patients with PA, PPGL and CS.

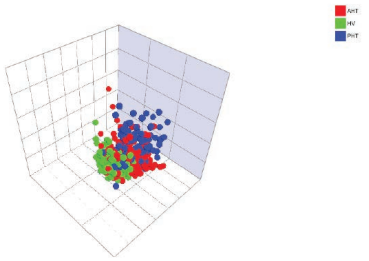

Figure: 3D PCA score plot comparing Adrenal Hypertensives (AHT) with Primary Hypertensives (PHT) and Healthy Volunteers (HV).

OC18 - CONTRIBUTION OF THE RETINOIC ACID RECEPTOR SIGNALLING TO ADRENAL CORTEX MORPHOLOGY AND FUNCTIONAL ZONATION

R. El Zein[1], A. Rickard[1], F. L. Fernandes-Rosa[1], J. F. Golib-Dzib[2], B. Samson-Couterie[1], I. Giscos-Douriez[1], A. Rocha[1], M. Poglitsch[3], H. LeFebvre[4], C. E. Gomez-Sanchez[5], L. Amar[6], N. B. Ghyselinck[7], A. Benecke[8], E. Lalli[9], M. Zennaro[10], S. Boulkroun[1]

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Primary aldosteronism is the major cause of secondary arterial hypertension. Recurrent somatic mutations in KCNJ5, CACNA1D, ATP1A1 and ATP2B3 have been identified in aldosterone producing

adenoma (APA). Although the role of these mutations in regulating aldosterone biosynthesis has been clearly established, the mechanisms involved in proliferation and APA formation still remain to be elucidated. The aim of our study was to identify pathways involved in adrenal cortex nodulation and APA formation.
Transcriptomic analysis from 48 APA and 11 control adrenal and correlations of gene expression with genetic, morphological and functional characteristics of the tumours allowed us to identify retinoic acid receptor (RAR) signalling as a central molecular network involved in APA formation independently of the mutation status.
Investigation of the adrenal phenotype of rarα-/- mice demonstrated that in young (12 weeks) and old (52 weeks) rarα-/- mice the characteristic cellular arrangement of the adrenal cortex was replaced by a reduction in the thickness of zona glomerulosa at 12 weeks and a hyperplasia of this zone at 52 weeks of age, and a disorganized zona fasciculata at 12 and 52 weeks of age. Furthermore, young rarα-/- mice displayed a decreased expression of steroidogenic enzymes. Interestingly, this was associated to a reduction in the expression of effectors and target genes of the Wnt signalling pathway. Transcriptomic analysis on adrenals from 12 and 52 weeks old rarα+/+ and rarα-/- male mice revealed a reduced expression of VegfA only in 12-week old rarα-/- mice. This was also associated to dilatation and disorganization of vessels' architecture, and a decreased expression of Laminin 1 b in the adrenals of 12 and 52 weeks old rarα-/- mice.
Our results suggest that retinoic acid receptor signalling contributes to the normal morphology and functional zonation of the adrenal cortex by modulating Wnt signalling as well as VegfA signalling and that any alterations in these pathways could lead to an abnormal cellular proliferation in the adrenal cortex, creating a propitious environment for the emergence of specific driver mutations in APA.

OC19 - ACCUMULATED EVIDENCE FOR CLINICAL PRACTICE OF PRIMARY ALDOSTERONISM BY THE NATIONAL PA REGISTRY AS A SUSTAINABLE PLATFORM IN JAPAN

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Background: Primary aldosteronism (PA) is a representative cause of endocrine hypertension characterized by an excess production of

aldosterone, hypertension, and cardiovascular complications. Although clinical practice guidelines have been published, diagnostic steps have not been standardized and heterogeneous clinical practice between centers remain to be the major issue of public health in Japan.

Aim: To produce diverse evidence for improvement of clinical practice of PA.

Methods: National PA registry was developed by Japan PA Study (JPAS) as one of the Incurable Disease Platform Project by the Japan Agency of Medical Research and Development (AMED) and the Ministry of Health, Labor and Welfare in Japan. 2850 PA patients who underwent AVS between 2006 and 2016 in 28 referral centers were registered. Major clinical questions were selected to be resolved.

Results: Diverse evidence produced was as follows. 1) Prevalence of cardiovascular diseases was higher in PA than essential hypertension. Hypokalemia, unilateral subtype and/or PAC > 12.5ng/dl were at greater risk of cardiovascular diseases. 2) Obesity-related factors contribute to the pathogenesis of IHA. 3) ACTH stimulation improved the success rate of AVS but did not contribute to better cllinical and biochemical outcomes of PA. 4) More than 90% of PA patients with normokalemia and bilateral disease on CT had bilateral subtype on AVS, needing AVS less weakly. 5) Those aged <35 yrs. with marked PA could be spared AVS. 6) While biochemical benefit after ADX was achieved solely with LI> 4 of AVS, clinical benefit was largely affected by clinical findings such as age, BMI, and blood pressure. 7) Since prevalence of cortisol co-secretion was high in PA with adrenal tumor > 2cm, DEX suppression test was mandatory. LI > 4 was applicable for PA subtype diagnosis even in patients with cortisol co-secretion, but not in those with overt Cushing syndrome. 8) ADX provided superior results in correcting hypertension and hypokalemia than medication in unilateral PA. 9) The treatment strategy should be determined considering the high postoperative incidence of persistent hypertension and hyperkalemia in elderly patients.

Conclusions: National PA registry was developed to create evidence for clinical practice of PA in Japan. Diverse evidence for elaboration and simplification of clinical practice guideline of PA was produced by excluding institutional bias. The PA registry has started to be operated as a part of more comprehensive measures by Japan Rare Adrenal Disease Study (JRAS) for a sustainable platform of future research and development. (Supported by AMED for the Practical Research Project for Rare/Intractable Disease under Grant Number JP17ek0109122; JP18ek0109352).

OC20 - OBSERVATIONAL STUDY: ADRENALECTOMY BUT NOT MINERALOCORTICOID RECEPTOR ANTAGONIST TREATMENT IS ASSOCIATED WITH REDUCED SALT INTAKE IN PRIMARY ALDOSTERONISM

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Context: High dietary salt intake is known to deteriorate blood pressure in hypertensive patients. In context with primary aldosteronism (PA), it leads to cardiovascular damage independent of blood pressure levels. The aim of the study was to objectify salt intake in PA patients before and one respectively three years after initiation of specific treatment for PA

Patients and Methods: Data of 149 consecutive PA patients, 63 patients with unilateral aldosterone-producing adenoma (APA) and 86 patients with bilateral adrenal hyperplasia (BAH) were extracted from the database of the German Conn's Registry. Salt intake was quantified by 24-hour urinary sodium excretion.

Study design: Observational longitudinal study.

Setting: Tertiary care hospital.

Results: At the time of diagnosis, patients had a median daily salt intake of 10.8 g. Higher salt intake correlated with an increased cardiovascular risk profile including impaired lipid and glucose metabolism and necessitated higher daily doses of antihypertensive drugs to achieve blood pressure control before and after treatment. One year after adrenalectomy daily salt intake dropped significantly in APA patients from 12.0 g pre-surgery to 9.5 g one year after surgery (p= 0.004) and remained stable at three years follow-up. In contrast, treatment with the mineralocorticoid receptor antagonist (MRA) spironolactone in BAH patients was not associated with a significant change in salt intake neither at one nor at three years follow-up.

Conclusion: Despite elevated cardiovascular risk, PA patients show high salt intake far beyond the threshold recommended by the WHO (<5 g/day). High salt diet correlates with parameters of increased cardiovascular risk. Treatment of PA by adrenalectomy, in contrast to MRA treatment, is associated with a significant reduction of salt intake and could be therefor favorable in reduction of cardiovascular risk.

OC21 - SAFETY OF MEDICAL ADJUSTMENT AND CONFIRMATORY TESTING IN THE DIAGNOSTIC WORKUP OF PRIMARY ALDOSTERONISM

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Context: Saline infusion (SIT) and captopril challenge tests (CCT) are standard confirmatory procedures in the diagnostic workup of primary aldosteronism (PA), for which patient safety has not yet been assessed.

Objective. We investigated the frequency and severity of side effects before, during and after SIT and CCT.

Design. Comparative, retrospective analysis of prospectively enrolled patients undergoing SIT and/ or CCT in two centers from 2016 until 2018.

Setting. 272 patients with suspected PA were enrolled at two outpatient centers in Germany (Berlin and Munich).

Main Outcome Measure(s). Frequency and severity of side effects associated with adjustment of medication before confirmatory testing and with test performance.

Results. For adjustment of medication prior confirmatory testing, side effects occurred in 18.4% of subjects. These were associated with higher defined daily doses (DDD) ($r= 0.25$, $p< 0.005$), number of antihypertensive drugs ($r=0.285$, $p< 0.005$) and higher blood pressure at SIT ($r= 0.145$ $p= 0.019$). During SIT, 17.5% of subjects had side effects, associated with higher blood pressure (systolic: $r=0.541$, $p< 0.0005$; diastolic: $r= 0.426$, $p< 0.0005$) and DDDs ($r= 0.727$, $p< 0.0005$). During CCT, only 1.5% of subjects had side effects.

Conclusions. In contrast to the high rate of side effects during SIT, CCT appears to be the safer test with a very low event rate.

OC22 - EXPRESSION OF CLOCK-RELATED GENES IN BENIGN AND MALIGNANT ADRENAL LESIONS

A. Angelousi [1], N. Nasiri-Ansari[2], A. Karapanagioti[2], C. Parianos[3], C. Aggeli[3], G. Zografos[4], G. Kyriakopoulos[2], T. Choreftaki[5], T. Kounadi[6], H. S. Randeva [7], G. Kaltsas[8], A. G. Papavassiliou [2], E. Kassi[2]

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Introduction: Although the function of the SCN in circadian rhythm regulation has been extensively studied, limited data exist on how peripheral tissue clock function contributes to this regulation. In this study we aimed to investigate the clock genes expression in the adrenocortical neoplasms.

Methods: mRNA and protein levels of 6 clock-related genes (CLOCK, BMAL1, PER1, CRY1, REV-erb, and ROR) were studied in 29 fresh frozen human adrenocortical neoplasms (benign, malignant) and their peritumoral-control tissue, through qPCR and Western Blot analysis, respectively. Tissues were collected immediately after surgery which was performed in the same time frame (07:00-10:00 am) for all patients. Twenty adrenal tissues were histologically confirmed as adenomas (8 cortisol-secreting (CSA), 5 aldosterone-secreting or Conn adenomas (CA) and 7 non-functional adenomas (NFA)), 3 samples were characterized as adrenal hyperplasia and 6 as adrenocortical carcinoma (ACC). Clock-related genes expression of the adrenal neoplasms was compared with the peritumoral tissue (expressed as ΔΔCTs) as well as among groups (expressed as fold ratio pathological:normal). Biochemical analysis confirmed the hormonal profile of the patients.

Results: CSA, NFA as well as hyperplasias showed down-regulation of mRNA levels in all 6 clock –related genes compared to their peritumoral normal tissues. CA showed up-regulation of mRNA levels in these 6 genes compared to peritumoral normal tissues as well as compared to CSA (statistical significant for BMAL1 and PER1) and NFA (statistical significant for all genes except ROR and CRY1). Protein expression showed the same pattern with mRNA levels. ACCs showed down-regulation of mRNA levels of CRY1 and PER1 compared to peritumoral normal tissues whereas CLOCK mRNA levels were up-regulated (however, differences were not statistical significant). BMAL1 mRNA levels had the same pattern of expression between ACC and peritumoral tissues. Western blot analysis confirmed these results.

Conclusions Our study demonstrated the expression of CLOCK, BMAL1, PER1, CRY1, REV-erb, and ROR in human adrenal tissues at mRNA and protein level with different pattern between the fasciculate and reticularis zone as well as between benign and malignant adrenal neoplasms. The role of clock-related genes in the adrenal tumorogenesis still need to be clarified as well as the 24h oscillation pattern of these genes in the adrenal tissue.

OC23 - UNTARGETED METABOLOMICS OF ADRENAL HYPERTENSION USING PLASMA NMR

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Background: The ENSAT-HT project aims to establish a signature for different forms of adrenal hypertension based on a multi-omics approach, including untargeted metabobolomics of plasma. Profiling the metabolome using 1H-Nuclear Magnetic Resonance Spectroscopy (NMR) has led to the discovery of biomarkers for many diseases, such as inborn errors of metabolism. Our aim within the scope of ENSAT-HT is to define the metabolic signature of patients suffering from Primary Aldosteronism (PA), Cushing's Syndrome (CS) and Pheochromocytoma and Paraganglioma (PPGL), and compare it to primary hypertensives (PHT) and healthy volunteers (HV).

Methods: Heparin plasma samples were collected from patients and distributed to all centers collaborating on ENSAT-HT. These samples were analyzed, along with Quality Control samples (QC) using NMR spectroscopy. The resulting spectra from both sample types were processed using signal alignment and peak picking routines, so as to convert the spectra to a readable table by multivariate statistical analysis (MVA) tools. The resulting datasets were subsequently normalized, batch corrected and transformed. As a first step in MVA, Principal Component Analysis (PCA) was applied as an unsupervised method to check for outliers and trends within the datasets.

Results: Spectra collected were of high quality. Diagnostic plots of data processing gave away the robustness of the resulting peak data that were subsequently used for statistical analysis. Upon inspection of the PCA score plots of NMR plasma data, a tight clustering of the QC samples based on order of analysis was observed. This confounder was dealt with by using batch correction methods, leading to a significantly smaller amount of technical variation within the complete dataset. Finally, as shown in the 3D PCA score plot which describes 39% of the variation present in the dataset (Figure), by coloring samples based on their respective groups, three clusters of samples can be observed as PHTs (blue, n=106), HVs (green, n=132) and Adrenal Hypertensives (PA, PPGL, CS, all in red, n=230).

Conclusions: We have established a reliable method for untargeted metabolomics using plasma NMR. The initial analysis suggests that patient classification based on whether there is adrenal or primary hypertension appears to be feasible. However, removing other sources of variation, such as sample origin, age of sampling and patient gender is a necessary

step before reaching any definitive conclusions concerning disease group differentiations. Future plans include, along with diminishing the effects of these confounders, the analysis of urine NMR and UHPLC-QTOF plasma data, and the application of supervised statistical methods to further understand the metabolism of patients with PA, PPGL and CS.
[Figure is uploaded separately]

OC24 - LEVOKETOCONAZOLE, THE SINGLE 2S,4R ENANTIOMER OF KETOCONAZOLE, IS A POTENTIAL NOVEL STEROID SYNTHESIS INHIBITOR FOR MEDICAL TREATMENT OF CUSHING'S SYNDROME

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Introduction: Racemic ketoconazole is used as steroid synthesis inihibitor for medical treatment of Cushing's syndrome (CS). Levoketoconazole (COR-003) is the single 2S,4R enantiomer and is thought to be more potent compared to racemic ketoconazole. Preclinical studies also suggest that it may have a favorable safety profile. In this study, we compared the in vitro effects of levoketoconazole and racemic ketoconazole on cortisol production and the adrenal steroid profile, as well as on ACTH secretion by corticotroph pituitary adenoma cells.
Materials and methods: HAC15 cells and 14 primary human adrenocortical cultures, with or without ACTH, and corticotroph AtT20 cells and primary human pituitary adenoma cultures were incubated with levoketoconazole or racemic ketoconazole (0.01 - 10 μM). Cortisol and ACTH were measured in the supernatant using a chemiluminescence immunoassay system (Immulite XPi). Steroid profiling was carried out by liquid chromatography-tandem mass spectrometry (LC-MS/MS).
Results: In HAC15 cells, levoketoconazole inhibited cortisol production at significantly lower concentrations (EC50: 0.300 μM) than racemic ketoconazole (0.611 μM; $P < 0.0001$) after a 3 day incubation. IC50values of levoketoconazole on basal cortisol production in primary adrenocortical cultures varied with a factor 24 difference (0.00578 μM to 0.140 μM). There was a trend towards higher sensitivity to levoketoconazole compared to racemic ketoconazole in primary adrenal cultures. Levoketoconazole had significantly stronger inhibitory effects on concentrations of the steroid profile in HAC15 cells and several adrenocortical cultures compared to racemic ketoconazole. In AtT20 cells, levoketoconazole inhibited cell

growth and ACTH secretion, and levoketoconazole (10 μM) inhibited cell growth in one of two primary human corticotroph pituitary adenoma cultures (-44%, $P < 0.001$).
Conclusion: Levoketoconazole is a potent inhibitor of cortisol production in human adrenocortical cells, but the degree of suppression is variable between tissue specimens of patients. Levoketoconazole inhibits steroid precursors more potently compared to racemic ketoconazole and might also inhibit ACTH secretion and growth of pituitary adenoma cells. Together with the previously reported potential advantages, these data indicate that levoketoconazole seems a promising novel treatment option for Cushing's syndrome.

OC25 - MEASUREMENT OF DEXAMETHASONE LEVELS WITH LC-MS/MS DURING 1-MG DEXAMETHASONE SUPPRESSION TEST IN ROUTINE CLINICAL PRACTICE

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Background: The first line diagnostic tests for Cushing's Syndrome (CS) are 24h urinary free cortisol (UFC), late-night salivary cortisol (LNSC) and 1 mg dexamethasone suppression test (DST). Regarding DST, it is generally considered that 1 mg of dexamethasone is able to suppress cortisol secretion, however its routine measurement is not available.
Aim: The aim of the study is to develop a threshold for dexamethasone level after DST in the screening of CS.
Patients, materials and methods: From December 2017 to July 2018 we studied 103 consecutive patients, referred to our Endocrine Unit for suspected CS. Among them, 84 were used in the retrospective part of the study (to calculate the cut-off) and 19 were considered in the prospective part (validation cohort). Dexamethasone was assumed at late night (between 23.00 and 24.00) and blood sample for serum cortisol was collected the next morning before 9.00. In the same sample dexamethasone level was measured with liquid chromatography with tandem mass spectrometry (LC-MS/MS). LNSC and UFC levels, assessed with LC-MS/MS, were used to exclude CS. Clinical parameters were collected and analyzed. The 2.5th percentile of dexamethasone level in patients with adequate cortisol suppression (<50 nmol/L) after DST was used as threshold.
Results: The cohort analyzed consisted in 16 patients with CS and 87 non-CS (50 with adrenal incidentaloma and 37 controls). The calculated dexamethasone threshold after DST is 6.3 nmol/L (consistent with previous study). Overall, DST confirmed high sensitivity (92% at 50nmol/L

as cut-off) and moderate-low specificity (67%, considering 30% of autonomous cortisol secretion in adrenal incidentaloma) to diagnose CS. Dexamethasone levels were adequate (>6.3 nmol/L) in all patients with CS. However, dexamethasone levels were <6.3 nmol/L in 7 out of non-CS patients (8%) that did not adequately suppress serum cortisol (LNSC and UFC were used to exclude CS in these 7 subjects). Dexamethasone levels were not affected by gender, smoke, age and serum creatinine. Dexamethasone levels were increased in obese patients (18.8 vs 10.2 nmol/L, p<0.05), thus leading to lower cortisol levels after DST.
Conclusions: Routine dexamethasone measurement after DST could be used to corroborate serum cortisol levels, enabling to discard up to 8% of false positive cortisol results (in patients with inadequate dexamethasone levels to suppress cortisol). In such cases, UFC and LNSC could be used to exclude CS.

OC26 - THE IMPACT OF AUTONOMOUS CORTISOL SECRETION ON BODY COMPOSITION

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Objective: Visceral fat and sarcopenia are two independent factors of increased cardiovascular risk. Limited data exist in performance of these markers in patients with autonomous cortisol excretion.
Methods: Patients were prospectively enrolled between 2014-2018. We included adults with adrenocortical adenomas on abdominal computed tomography (CT) imaging diagnosed with nonfunctioning adrenal tumor (NFAT), mild autonomous cortisol excess (MACE) and Cushing syndrome (CS).Age, sex and BMI 1:1 matched healthy controls were recruited. Body composition measurements (visceral fat [V], subcutaneous fat [S], visceral/total fat [V/T] and total abdominal muscle mass [M],) were calculated from CT imaging.
Results: Of 227 patients with adrenal adenomas, 20 were diagnosed with CS, 76 with MACE and 131 with NFAT. Median age of patients was 56 years (range, 18-89), and 67% were women. Median BMI was 31 kg/m2 (range, 18-58). Median size of the adrenal tumour was 2.4 (range, 0.5-13 cm) and median HU 6 (range, -27 to 36). Adrenalectomy was performed in 52 (23%) patients.
Prevalence of hypertension, diabetes mellitus type 2, and obesity was similar among patients with CS, MACE and NFAT, however, higher prevalence of osteoporosis and osteopenia was noted in patients with CS compared to MACE and NFAT (55% vs 32% vs 16%, P<0.001).
No difference in total fat was found in MACE vs NFAT, however, the proportion of visceral fat (V/T) was higher in MACE (0.43 vs 0.37 in NFAT,

P=0.005). After adjusting for age, sex and BMI, both mean M (P=0.03) as well as M to total fat ratio (P=0.02) were independently and inversely correlated with DST cortisol in patients with adrenal adenomas.
When compared to matched healthy controls, patients with adenomas demonstrated significant differences in muscle mass (30%, 25% and 20% lower in CS, MACE and NFAT when compared to controls, respectively, P<0.0001 for all). Similarly, V/M ratio was 117%, 77% and 51% higher in CS, MACE and NFAT, respectively, when compared to controls (P<0.0001 for all).
Conclusion: DST cortisol is positively correlated with visceral fat and negatively correlated with muscle mass. Patients with adenomas present with lower muscle mass and higher proportion of visceral fat when compared to controls, including patients with so called “nonfunctioning” adenomas. Even a subtle abnormality in cortisol secretion, not detectable by current standard of care testing may play a detrimental role in body composition of these patients.

OC27 - COULD ARMC5 STATUS BE PREDICTED FROM THE CLINICAL, HORMONAL AND RADIOLOGICAL INVESTIGATIONS IN PRIMARY BILATERAL MACRONODULAR ADRENAL HYPERPLASIA (PBMAH) ? ANALYSIS OF A LARGE EUROPEAN SERIES OF PATIENTS

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Primary bilateral macronodular adrenal hyperplasia (PBMAH) is an heterogenous disease from the clinical, radiological and hormonal point of view. It is likely that many bilateral benign adrenocortical incidentalomas are PBMAH but diagnostic criteria are difficult. Germline inactivating mutations of the tumor suppressor gene ARMC5 have been shown to be responsible for PBMAH. Among patients who underwent adrenalectomy for severe Cushing’s syndrome, 55 % carried ARMC5 mutations. According to the current literature, in clear familial cases of PBMAH more than 80 % of patients show ARMC5 mutations while in sporadic cases the

mutation rate is around 20 to 25 %.
ARMC5 has been sequenced in 453 PBMAH patients (index cases) from Europe (COMETE and ENSAT networks) by Sanger or targeted NGS in our research laboratory or in the oncogenetics unit of Cochin Hospital. In this unselected cohort the referring endocrinologist was free to decide the diagnostic criteria. Our purpose was to identify a subgroup of patients more susceptible to present ARMC5 mutations. We have analyzed clinical, biological and morphological data (central review) for the 128 index cases followed in Cochin Hospital, harboring bilateral adrenal nodules and/or Cushing‘s syndrome (at least subclinical, defined as plasma cortisol after 1 mg dexamethasone > 50 nmol/L).
In the total cohort 62 different ARMC5 mutations were identified in 71/453 patients (16 %). The alterations are spread all over the coding sequence : 49 % missense mutations, 33 % frameshift deletions-insertions, 18 % non-sense mutations. For missense variants, pathogenic nature is determined by bioinformatics predictions (SIFT and Polyphen2 softwares) and/or functional studies. Among the 128 patients from Cochin reviewed in detail, the mutation rate is similar (16 %). Among the 85 patients with both bilateral adrenal lesions AND cortisol autonomy, the mutation rate is 32 % (21 patients : 100 % sensitivity). This mutation rate increases to 64 % (18 patients) in the 28 patients with at least 6 adrenal nodules (86 % sensitivity).
All the index cases with ARMC5 mutation have the association of bilateral adrenal nodules AND cortisol autonomous secretion. The existence of at least 6 adrenal nodules predicts a high rate of ARMC5 mutation with a good sensitivity. This underlines the heterogeneity of PBMAH and its genetic causes. The analysis on the whole cohort needs to be done to confirm these results and define criteria for routine ARMC5 genotyping.

OC28 - DIFFERENTIATION OF ADRENAL MYELOLIPOMA FROM ADRENOCORTICAL ADENOMA AND CANCER BY MICRORNA EXPRESSION PROFILING

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Introduction: The differentiation of adrenal myelolipoma (AML) from adrenocortical carcinoma (ACC) might be difficult because of the variable proportion of fat and hematopoietic elements in AML. Both AML and ACC often grow large. There is no reliable blood-borne marker in the differential diagnostics of these two neoplasms to date. MicroRNAs have been identified as promising biomarkers in many tumors, including adrenal neoplasms, but the applicability in AML has not been investigated yet.

Aim: Our aim was to identify circulating microRNA markers in AML, ACC and adrenocortical adenoma (ACA) through large-scale microRNA expression profiling.

Methods: Next-generation sequencing (NGS) was performed on 30 formalin-fixed paraffin-embedded archived tissue samples (AML, ACC, ACA 10 samples each) by Illumina MiSeq. The validation of significantly over-expressed microRNAs was performed by real-time RT-qPCR in an independent validation cohort (15 AMLs, 14 ACAs and 12 ACCs). Moreover, 11 preoperative plasma samples from each tumor group were investigated for circulating microRNA.

Results: By NGS, relatively overexpressed levels of miR-451a, miR-486-5p, miR-363-3p and miR-150-5p were found in AML compared to ACC and ACA. We have found up-regulated miR-184, miR-483-5p, miR-483-3p and miR-183-5p in ACC samples compared to AML and ACA.

Validation confirmed the overexpression of all the 4 microRNAs in AML compared to ACC and ACA, whereas miR-184, miR-483-5p and miR-183-5p were found to be significantly overexpressed in ACC to ACA, but not to AML. Circulating miR-451a and miR-363-3p were significantly overexpressed in AML, whereas circulating miR-483-5p and miR-483-3p were only significantly overexpressed in ACC vs. ACA.

Conclusion: Up-regulated miR-451a might be a potential minimally invasive marker of adrenal myelolipoma. A remarkable result of our study is that the expression of miR-483-5p in AML and ACC was not significantly different. This finding is notable, as miR-483-5p is considered to be the best marker of adrenocortical carcinoma so far and thus its applicability as a biomarker of ACC might be limited.

PO01 - STEROID HORMONES AND CANCER IMMUNITY – INSIGHT INTO ADRENOCORTICAL CARCINOMA

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Context

Adrenocortical carcinoma (ACC) are endocrine malignant neoplasms associated with severe aggressiveness. By applying a “multiple omics” approach, we recently categorized ACC patients based on genetic alterations into two distinct subgroups; an “immune” phenotype with good and a “steroid” phenotype with bad outcome.

Hypothesis

Our central hypothesis is that the steroid phenotype is associated with glucocorticoid-induced T-cell anergy that can be rescued by steroid inhibitors and by reactivating the immune system. In a first step, we investigate tumor-infiltrating immune cells and try to identify tumor-specific mutant antigens (TSMA).

Methods

We performed immunofluorescence analysis targeting tumor-infiltrating CD4+ and CD8+ T-cells, regulatory T-cells, B-cells and macrophages/dendritic cells in 58 primary ACC. Additionally, we quantified the expression of the immune checkpoint markers programmed-death 1 (PD1) and its ligand PD-L1 using IHC. Furthermore, ACC-associated somatic mutations were analyzed in silico. The binding affinity (BA) to MHC receptors of mutant peptide and wild type was predicted using netMHCpan.

Results

Most ACCs show infiltrates by T-cells (80%, 37±65 cells/HPF), both cytotoxic (72%, 24±53) and helper cells (57%, 19±16 cells/HPF), Tregs (48%, 4±4) and M□/DC (73%, 6±4) and an intra-tumoral expression of PD1 (36%, 15±30) and PD-L1 (83%, 34±82), while B-cells were absent. Interestingly, the only ACC-infiltrating immune cells associated with

overall survival are T-helper-cells (HR for death: 0.34, 95%CI 0.12-0.95, p=0.005). The in silico analyses revealed more than 30 potentially relevant TSMA (e.g. RPL22, CTNNB1, ATRX) and in some of them very strong BA was detected (RPL22: in HLA A*03:02: 30.3 nM mut. vs. 2556.4 nM in wt).
Conclusion
First, tumors of ACC patients are characteristically infiltrated by CD3+ CD4+ T-helper-cells that positively influence patients' overall survival suggesting prognostic relevance. Second, mutated peptides change their BA to HLAs presenting tumor specific neoantigens that might be targetable via immunotherapeutic approaches. Prospectively, these generated data will be further investigated and verified in vitro.

PO02 - MORBIDITY, AND MORTALITY OF BONE METASTASES IN ADVANCED ADRENOCORTICAL CARCINOMA. A MULTICENTER RETROSPECTIVE STUDY

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Abstract
INTRODUCTION:
Adrenocortical carcinoma (ACC) is a rare disease that commonly spreads to the liver, lungs and lymph nodes. Bone metastases are infrequent.
OBJECTIVE:
The aim of this report was to describe the clinical characteristics, survival perspective, prognostic factors and frequency of adverse skeletal related events (SREs) in patients with ACC who developed bone metastasis.
METHODS:

This is a retrospective, observational, multicenter, multinational study of patients diagnosed with bone metastases from ACC who were treated and followed up in referral centers in three European countries (France, Italy and The Netherlands) and at The University of Texas MD Anderson Cancer Center in USA.
RESULTS:
One hundred fifty-six patients were enrolled. The median overall survival was 11 months. In 91% of patients bone involvement was associated with other metastatic sites, mainly visceral. Seventy out of 148 evaluable patients (47%) developed SREs: 26/148 (17%) only bone fractures, 1/148 (1%) only hypercalcemia, 26/148 (17%) only spinal cord compression. Seventeen patients out of 148 (12%) presented more than one SRE. In multivariate analysis glucocorticoid hypersecretion was the only prognostic factor significantly associated with a higher mortality risk (Hazard Ratio [HR] 2.24, 95% Confidence Interval [CI]: 1.19-4.23, p=0.013) and with the development of a SREs (of border line significance).
The administration of antiresorptive therapies (bisphosphonates and denosumab) was associated with a lower risk of death (but not significant) and their survival benefit appeared confined in patients attaining serum mitotane levels within the therapeutic range.
CONCLUSION:
Bone metastases in ACC patients are associated with poor prognosis and high risk of SREs. The therapeutic role of bone resorption inhibitors (bisphosphonate or denosumab) administration to improve patients outcome deserves to be tested in a prospective clinical trial.

PO03 - EDP PLUS MITOTANE FOLLOWED BY CYTOREDUCTIVE SURGERY IN THE MANAGEMENT OF PATIENTS WITH LOCALLY ADVANCED OR OLIGOMETASTATIC ADRENOCORTICAL CARCINOMA

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Introduction: Chemotherapy with Etoposide, Doxorubicin, Cisplatin plus Mitotane (EDP-M) is the standard approach in adrenocortical cancer

(ACC) patients with locally advanced or metastatic disease. The role of additional cytoreductive surgery in case of oligometastatic disease is controversial. The aim of this study was to evaluate the outcome of a consecutive series of patients with locally advanced/oligo metastatic ACC addressed to EDP-M followed by cytoreductive surgery.
Methods: This is a monocentric retrospective study. We collected clinical and pathological data of patients initially treated with EDP-M for 4-6 months and subsequently addressed to cytoreductive surgery in case of disease response or stabilization. The primary endpoint was to evaluate the prognostic role on progression free survival (PFS) of surgery. Secondary endpoints: the prognostic role of surgery on overall survival (OS), the prognostic role on PFS and OS of post-surgical Ki67. Finally, as ancillary studies, we explored the prognostic impact on PFS and OS of radiologic response obtained after chemotherapy in CT and PET scan according to RECIST 1.1 and CHOI criteria, volume variation and SUVmax variation of target lesion.
Results: 30 eligible patients were included in the study with both primary lesion or disease relapse. 18 patients underwent cytoreductive surgery after chemotherapy. Median PFS was 15.5 months in these patients and 6.1 months in those who did not have a surgical approach (p=0.028). The corresponding median OS was not reached vs 10.7 months (p=0.010). Lower post-surgical Ki67 expression (below the median) was associated with longer PFS just failing to attain the statistical significance (p=0.081). With respect to the prognostic role of radiologic response obtained after chemotherapy in CT and PET scan, a statistically significant correlation was seen between CHOI criteria and PFS (p=0.019), whereas disease response assessed by RECIST, volume variation and SUVmax variation after chemotherapy failed to be associated with patient outcome.
Conclusion: Cytoreductive surgery after chemotherapy has a prognostic role and can potentially increase the efficacy of chemotherapy in patients with locally advanced or oligometastatic ACC. Post-surgical Ki67 and disease response assessed with CHOI criteria could represent additional prognostic factors. The retrospective nature of the study, the limited number of patients included, and the short follow-up are the main limitations.

PO04 - VAV2 AND FASCIN-1 COORDINATELY PROMOTE ADRENOCORTICAL CARCINOMA CELL INVASION

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Adrenocortical carcinoma (ACC) is a rare endocrine malignancy with a high risk of relapse and metastasization. The molecular mechanisms underlying adrenocortical tumourigenesis remain to be fully elucidated and more light needs to be shed on the biological bases of ACC aggressive behaviour. We have previously identified the Rho small GTPases guanine nucleotide exchange factor (GEF) VAV2 as a novel Steroidogenic Factor 1 (SF-1) dosage-dependent target gene in ACC cells. Our previous studies revealed that VAV2 is an essential factor driving malignancy in ACC (1). Here we have studied the role of FASCIN-1, an actin-bundling protein recently identified as a potential marker of ACC malignancy (2), in the remodelling of actin cytoskeleton and the invasion of human ACC cells driven by an increased SF-1 dosage.Our results show that FASCIN-1 silencing suppressed increased filopodia formation and invasion through Matrigel occurring when SF-1/VAV2 are overexpressed. Similarly, FASCIN-1 pharmacological blockade inhibited cell invasion through Matrigel only in conditions of increased SF-1 dosage. The striking similarity to the phenotypes observed upon VAV2 knock-down prompted us to study the potential VAV2/FASCIN-1 interplay in promoting ACC cell invasiveness. Remarkably, transfected VAV2 could rescue the effect of FASCIN-1 knock-down on cell invasion, while FASCIN-1 overexpression could not fully compensate for the effect of VAV2 silencing. These results are consistent with a model where VAV2 by its GEF activity triggers activation of Cdc42 and Rac1, which promote cytoskeleton remodelling and filopodia - lamellipodia/ruffles formation. These structures are stabilized by FASCIN-1, leading to increased cell migration and invasion. Further studies are in progress to elucidate the exact mechanisms by which these two proteins functionally interact to support ACC invasive and metastatic phenotype.

1) Sci. Signal.10: eaal2464 (2017)
2) Oncotarget 6: 5695-5706 (2015)

PO05 - FASCIN-1 AS A NOVEL PROGNOSTIC BIOMARKER IMPLICATED IN INVASIVENESS OF ADRENOCORTICAL CARCINOMA

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Background: Adrenocortical carcinoma (ACC) is a rare endocrine tumour characterized by poor prognosis and lack of efficacious therapies. Novel prognostic markers are urgently needed to better stratify ACC patients and improve the current limited therapies.

Aim: This study aimed to assess the prognostic value of the actin-bundling protein fascin-1 (FSCN1) in ACC.

Study design & Methods: A local monocentric series of 37 ACC cases

and two independent validation ACC cohorts, from the European Network for the Study of Adrenal Tumours (ENS@T), were studied. FSCN1 expression was assessed in the local series by semi-quantitative immunohistochemistry (IHC) and Western Blot technique, while mRNA expression was first evaluated in the local series by quantitative RT-PCR analysis and then confirmed in the two independent validation cohorts. FSCN1 prognostic power was assessed by Kaplan-Meier analysis and compared to that of tumour stage, Weiss score and Ki67 labelling index (Ki67-LI).
Results: FSCN1 immunohistochemical expression was an independent prognostic factor, also improving results obtained with staging and Ki67-LI for prediction of disease-free (DFS) and overall (OS) survival. Stratifying patients into low and high expression levels of FSCN1 transcript significantly predicts DFS and OS (DFS: Log Rank p=0.013, HR=5.93[1.19-28.99], p=0.029; OS: Log Rank p=0.019, HR=8.60[0.99-74.20], p=0.049). Similar results were obtained in the validation cohorts. FSCN1 and Steroidogenic Factor-1 (SF-1) transcript levels were significantly higher in tumours displaying at least one of the three Weiss score parameters associated with invasion ("invasive" Weiss score parameters: sinusoidal, venous and capsular invasion), suggesting an association between FSCN1 and invasiveness in ACC. Finally, a positive correlation between FSCN1 and SF-1 was also found.
Conclusion: These findings demonstrate that FSCN1 is a promising independent prognostic marker in ACC and may serve as a potential therapeutic target to block tumour spread.
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PO06 - INVESTIGATING THE ROLE OF THE LIVER X RECEPTOR IN POTENTIATING MITOTANE THERAPY IN ADRENOCORTICAL CARCINOMA

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Introduction: Adrenocortical carcinoma (ACC) is a rare, aggressive malignancy which carries a poor prognosis due to limited treatments. Adjuvant mitotane following tumour resection improves survival but is limited by a narrow therapeutic window, severe adverse effects and escape from therapeutic efficacy. Mitotane is known to modulate its adrenolytic effect via SOAT1 inhibition causing toxic free cholesterol accumulation in adrenocortical cells. Liver X receptors (LXRs) are nuclear hormone receptors highly expressed in adrenal tissue. LXRs act

to maintain transcellular and intracellular cholesterol homeostasis. We hypothesis that lesioning LXRα can alter intracellular cholesterol levels and associated mediators in adrenocortical cells thus potentiating the adrenolytic effect of mitotane.

Methodology: ATCC-H295R ACC cells were pre-treated with the LXRα antagonist GSK2033 (5μM) or inverse agonist SR9243 (0.1μM) followed by mitotane treatment (20, 40, 50μM) for 6 hrs. Cell death was assessed by flow cytometry. Intracellular lipid droplets were evaluated using an Amnis Imagestream Flow Cytometery® to visualise and quantify neutral lipids using BODIPY®. Combined effects of mitotane and LXRα inhibition on cholesterol efflux proteins and SOAT1 were evaluated by qRT-PCR. Lipid droplet associated markers were assessed using western blotting.

Results: Co-treatment with GSK2033 and SR9243 potentiated mitotane killing at sub-therapeutic doses 20μM and 40μM (Fig1). ImageStream® analysis demonstrated a decrease in lipid droplet formation in mitotane treated groups compared to vehicle. GSK2033 treatment further decreased lipid droplet formation alone and with mitotane (Fig2). This was accompanied by an increase in free cholesterol and a decrease in cholesterol esters. Additionally cholesterol efflux receptors and perilipin expression were reduced. LXRα modulators GSK2033 and SR9243 boths significantly downregulated LXRα target genes ABCA1 and ABCG1 ($n=3$, $p>0.05$).

Conclusion: LXRα inhibition in combination with Mitotane increases killing in H295R ACC cells in vitro using both antagonist GSK2033 and inverse agonist SR9243. This is accompanied by diminished lipid droplets and associated proteins, increased free cholesterol and decreased cholesterol esters following GSK2033 treated versus mitotane alone. Targeting LXRα, its putative ligands, or associated cholesterol mediators may present a novel mechanism to broaden the therapeutic window for mitotane in the setting of ACC.

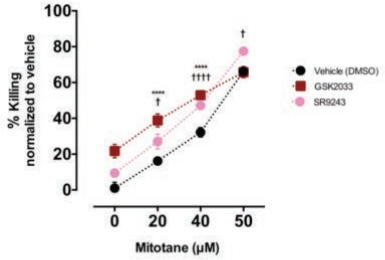

Figure 1. LXRα inhibition potentiates mitotane induced killing in H295R cells.

† SR9243 0.1 μM vs Mitotane (at equilivant dose) p>0.05 n=4
††† SR9243 0.1 μM vs Mitotane (at equilivant dose) p≤0.0001n=4
**** GSK2033 5 μM vs Mitotane (at equilivant dose) p≤0.0001n=4

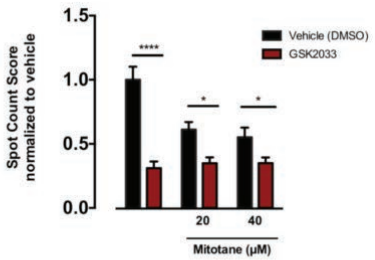

Figure 2. LXRα antagonist GSK2033 decreases lipid droplets alone and in combination with mitotane

* GSK2033 5 μM vs Mitotane (at equilivant dose) p>0.05 n=3

PO07 - ADJUVANT MITOTANE THERAPY OF ADRENOCORTICAL CARCINOMA IN ITALY: ANALYSIS OF THE DATABASE LYSOSAFE

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BACKGROUND AND OBJECTIVE: Mitotane is widely used as post-operative adjuvant treatment of adrenocortical carcinoma. However, the management of therapy is largely empirical. The aim of the study is to evaluate its use in expert Italian centers, analyzing data of LYSOSAFE database.

METHODS: Retrospective analysis of patients reported in the dabatase, treated with mitotane for ≥ 6 months and with at least 3 measurements of blood mitotane levels during follow-up. Data are expressed as median and range.

RESULTS: We identified 110 patients (F/M = 67/43, age 47, 16-80 years), treated with adjuvant mitotane for 48 months (7-215) with a 2.0 g/day (1.0-4.5) dose. In 94.1% of the cases, mitotane therapy was initiated within 3 months of surgery. Achievement of mitotane levels in range (14-20 mg/L) required 8 months (1-75) from the start of therapy. The adjuvant treatment was discontinued in 88 cases, of which 36 (40.9%) for end of treatment (after 58, 24-142 months), 29 (33.0%) for recurrence, 11 (12.5%) lost at follow-up/not available data, 5 (5.7%) toxicity, 4 (4.5%) patient's decision, 3 (3.4%) concomitant pathologies. Only 14 patients (12.7%) had no mitotane levels >14 mg/L, while the remaining 96 had mitotane in range in 40% (7-100%) of the measurements, and 40 patients (41.7%) had more than 50% of mitotane levels in range. In a multiple

regression model, variables correlating with given mitotane dose were sex (lower doses in women; β = -0.23, p=0.02) and BMI (higher doses with higher BMI; β= 0.22, p=0.02). On the contrary, the percentage of measurements in range did not correlate with gender, BMI, age or dose.
CONCLUSIONS: In Italy, adjuvant mitotane therapy is initiated early and is continued for a long time, with careful mitotane monitoring. A low-dose regimen is usually used and therapeutic range is often reached after several months. Toxicity of the drug is acceptable, requiring a permanent withdrawal of treatment in a minority of cases. Most patients achieve the therapeutic range of mitotane, although with significant fluctuations during follow-up. Sex and BMI influence the dosage of mitotane, but not the achievement of therapeutic range, which seems to be therefore dependent on individual factors, not yet identified.

PO08 - N-MYC DOWNSTREAM-REGULATED GENE MEMBER 4 (NDRG4) AND ITS REGULATORY MICRORNA MIR-139-5P ARE POTENT BIOMARKERS FOR ADRENOCORTICAL CARCINOMA DIAGNOSIS AND PROGNOSIS

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Introduction: MicroRNA (miRNA) landscape in adrenocortical carcinoma (ACC) suggests that dysregulated miRNA signatures are associated with ACC aggressiveness. We have previously shown that, among others, miR-139-5p is overexpressed in aggressive ACC. We further identified N-myc Downstrean-Regulated Gene 4 (NDRG4) as a major target of miR-139-5p. In this work, we investigated (1) miR-139-5p and NDRG4 expressions as well as their diagnostic/prognostic potential in three independent cohorts of ACC, (2) NDRG4 expression in adrenocortical tumors using immunohistochemistry and (3) the possible mechanisms underlying miR-139-5p and NGRG4 deregulations in ACC.
Methods: Diagnostic/prognostic potential of miR-139-5p/NDRG4 was assessed using public ACC (n=44) datasets or quantitative PCR (n=20). NDRG4 immunohistochemistry (IHC) was performed on a third cohort from the University Hospital of Grenoble (n=20). Regulation of miR-139-5p/NDRG4 expression was studied in the NCI H95R cell line.
Results: NDRG4 mRNA levels were inversely correlated with miR-139-5p levels in ACC samples. Upregulation of miR-139-5p and downregulation of NDRG4 demonstrated a striking prognostic value. Immunohistochemical analysis of NDRG4 revealed that its under-expression discriminated

between recurring/metastatic and non-recurring tumors with a sensitivity of 100% and a specificity of 75%. A strong correlation between the IHC score of NDRG4 and its transcript or protein expression was observed. Finally, silencing b-catenin expression in NCI H295R cells led to a dramatic decrease in miR-139-5p expression and cell invasiveness with a concomitant increase in NDRG4 expression. Conclusion: our study reveals high diagnostic and prognostic value for NDRG4 and miR-139-5p in aggressive ACC. Moreover, immunohistochemical detection of NDRG4 appears as a promising routine complementary histopathological tool. Our ongoing investigations suggest a crosstalk between miR-139-5p/NDRG4 axis and β-catenin signalling cascade in aggressive ACC.

PO09 - PROGNOSTIC FACTORS IN PATIENTS WITH ADVANCED/METASTATIC ADRENOCORTICAL CARCINOMA RECEIVING II-LINE GEMCITABINE-BASED CHEMOTHERAPY

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Background: Etoposide, Doxorubicin and Cisplatin plus Mitotane (EDP-M) combination chemotherapy (CT) is the gold standard in the management of advanced/metastatic adrenocortical carcinoma (ACC). However, after EDP-M failure very few effective options exist. Gemcitabine plus Capecitabine (Gem/Cape) is a frequently adopted II line CT, but only a minority of patients (pts) will obtain substantial benefit from this regimen. The identification of prognostic parameters can help to select potentially responsive pts.

Patients and methods: We retrospectively analyzed pts with metastatic progressing ACC treated between 2011 and 2018 at our Institution. Pts received 800 mg/sqm Gemcitabine on days 1, 8 and 1500 mg/day Capecitabine continuously. Previous mitotane therapy was maintained.

Results: Clinical data from 39 consecutive pts were collected. Median age at Gem/Cape start was 50 years (16-68). More than 90% of pts had previously received at least 1 line of CT and the Charlson's Comorbidity Index revealed the presence of multiple comorbidities (score ≥5) in >50% of pts. A median number of 3 (1-17) cycles was administered. Toxicity was overall mild with G3-G4 hematological toxicities in 9 (23%) pts. ORR (partial response + stable disease) at 4 months was 33% and median progression-free survival (PFS) was 3.25 months. After Gem/Cape failure, 49% of pts received ≥1 line of further CT and median overall survival (OS) from Gem/Cape start was 8 months. Among clinical variables, the

presence of baseline anemia (HR 2.48 95% confidence interval [CI] 1.06-5.8, p .036), lung metastases (HR 2.23 95%CI 1.03-4.82, p .04) and liver metastases (HR 2.18 95%CI 1.07-4.45, p .003) were associated with a shorter PFS at univariate analysis. Only lung metastases maintained independent predictive value at multivariate analysis. The presence of baseline anemia (HR 4.414 95%CI 1.598-10.73 p .003), lung metastases (HR 2.728 95%CI 1.146-6.493 p .023), liver metastases (HR 2.75 95%CI 1.231-6.142 p .014) were associated with a shorter OS at univariate analysis. Baseline anemia remained significant at multivariate analysis.
Conclusions: This study confirms that in clinical practice the Gem/Cape schedule is moderately active and is well tolerated in advanced, heavily pretreated ACC pts. Analysis of activity data shows superior results than historical controls. Analysis of clinical characteristics identified the metastatic pattern and baseline anemia as potential negative predictive factors.

PO10 - PHARMACOKINETIC DRUG-DRUG INTERACTION BETWEEN MITOTANE AND ETOPOSIDE IN THE TREATMENT OF ADRENOCORTICAL CARCINOMA

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Background:
Adrenocortical carcinoma (ACC) is a rare malignancy with a poor prognosis. The combination of mitotane and platinum-etoposide chemotherapy is the front-line treatment in advanced ACC, although this regimen shows limited efficacy. Drug-drug interaction between mitotane -which is a strong pharmacokinetic inductor of CYP3A4 and ATP-binding cassette (ABC) multidrug transporters- and etoposide –which is a substrate of these proteins- may contribute to chemoresistance in ACC. The aim of this study was to evaluate the pharmacokinetic interaction between mitotane and etoposide.
Methods:
From December 2016 to October 2017, this observational study included 5 consecutive ACC patients treated with platinum-etoposide (120 to 150 mg/m2 on days 1-2-3 at cycle 1) chemotherapy in referral center for rare adrenal diseases and oncology department of Cochin hospital, Paris. Plasma etoposide concentrations were measured using liquid chromatography at 0, 4 and 24 hours after each etoposide infusion. In the absence of dose-limiting toxicity, a dose escalation of etoposide was proposed since cycle 2.

Results:
Patients received 2 to 6 chemotherapy cycles, in association with mitotane (4 patients, median mitotane plasma concentration of 14.2 mg/L) or after mitotane discontinuation (1 patient, plasma concentration 1 mg/L). Etoposide clearance was higher in association with mitotane (4.95 L/h (range 2.67 to 6.20)) than after discontinuation (2.53 L/h (2.02 to 2.78), Wilcoxon p=0.014) or than in a reference population not treated with mitotane (1.81 L/h). Etoposide dose escalation was performed in 4 patients treated with mitotane, resulting in 2 minor tumor response and 1 febrile neutropenia, both at etoposide dose of 300 mg/m2.
Conclusion:
Drug-drug interaction between mitotane and etoposide may partly explain the low efficacy of platinum-etoposide chemotherapy in ACC. This observation suggests further a potential benefit of increasing etoposide dosage in ACC patients receiving mitotane.
All authors declare that they have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

PO11 - A SERUM MICRORNA SIGNATURE ASSOCIATED WITH RECURRENCE IN ADRENOCORTICAL CANCER

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Introduction: Investigation on circulating microRNAs (miRNAs) as biomarkers for adrenocortical carcinoma (ACC) is still an emerging field. Circulating miR-483-5p has been identified as pre-operative and post-operative diagnostic and prognostic biomarker for ACC. Nevertheless, combinatorial use of multiple miRNAs is expected to improve the sensitivity and specificity of biomarker panels. Our aim was to determine whether ACC recurrence was accompanied by increases in serum levels of selected miRNAs.
Methods: We conducted a single center retrospective analysis using post-operative serum samples from the COMETE-TACTIC cohort (Cochin Hospital, Paris). Absolute copy numbers of serum miRNAs were determined using real-time quantitative PCR in 10 patients with adenomas (ACA), 20 patients with non-recurring ACC (median follow-up 7 years, range [3-24]) and 17 patients with recurring ACC (median time to recurrence 18.9 months, range [1.4-150]).
Results: Significantly higher levels of circulating miR-483-5p, miR-139-5p, miR-210 and miR-503 were found in patients with recurring ACC as

compared to those without recurrence. ROC analyses allowed us to define miRNA cut-off values associated with recurrence. An expression level of a given miRNA greater than the optimal cutoff value of the corresponding miRNA was correlated with poor prognosis.
Conclusion: Our data demonstrate that serum miRNAs levels in post-operative samples are correlated with recurrence and support further testing of miRNAs to guide ACC patient surveillance after surgery. Prospective and longitudinal studies are needed to confirm these observations.

PO12 - EFFECTIVENESS OF HIGH DOSE PROTOCOL MITOTANE THERAPY IN THE MANAGEMENT OF ADVANCED ADRENOCORTICAL CANCER: A SINGLE CENTRE EXPERIENCE

B. Bakhit[1], B. C. Whitelaw[1], J. Gilbert[1], P. Coskeran[1], S. Diaz-Cano[2], D. R. Taylor[3], N. F. Taylor[3], L. Ghatore[3], D. Lewis[4], G. Vivian[5], D. Sarker[6], P. Ross[6], L. Davis[1], J. Clough[1], J. Hubbard[7], G. Galata[7], A. Prachalias[8], K. M. Schulte[7], S. J. B. Aylwin[1]

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Introduction: The recently published European Society of Endocrinology guidelines on the management of adrenocortical carcinoma (ACC) suggests the use of mitotane in patients with advanced ACC (stage III and IV) and high risk of recurrence. Achieving and maintaining therapeutic mitotane level (≥14 mg/L) has been linked with improved outcomes.
Aim: To evaluate the effectiveness of a defined* high dose protocol mitotane therapy in patients with advanced ACC.
Methods: Review of patients presenting to King's College Hospital (KCH) with advanced ACC, and the mitotane concentration achieved through the Lysosafe monitoring service.
Results: N = 44 patients with stage III (n=22) and IV (n=22) ACC were first diagnosed, and actively managed with surgery and/or mitotane therapy at our centre between 2008-2017,. 40/44 patients underwent surgical resection of the primary tumour, with 11 patients receiving systemic chemotherapy. 41/44 patients were initiated on mitotane. The median

overall survival of patients with stage IV was 25.3 months, median survival for stage III has not been reached.
A total of 47 patients were included in the mitotane pharmacokinetic analysis: 41 patients in our cohort, plus 6 patients who had prior management, including surgery, elsewhere and were referred for mitotane initiation. Six patients were excluded: 2 patients died shortly after mitotane initiation, 1 patient had no samples sent, 1 patient withdrew due to a severe reaction; and 2 patients have not reached 12 weeks of therapy at the time of writing. Of the remaining 41 patients, 33 were initiated on the 'high dose' protocol and 8 on the 'low does' protocol. For patients on the high dose protocol, 25/33 (76%) reached mitotane concentration of ≥14mg/L within 12 weeks of initiation of therapy, compared to 3/8 (37.5%) patients from the low dose protocol group (p=0.037). In the high dose protocol group, 20 patients (80%) maintained therapeutic drug concentration in ≥50% of the subsequent follow-up samples and 12 (48%) maintained therapeutic drug concentration in ≥75% of samples.
Discussion and conclusion: High dose mitotane protocol is an effective strategy to achieve and maintain therapeutic drug concentration. When combined with an assertive surgical approach and, in some cases, chemotherapy, this has resulted in better outcomes (median OS 25.5 months in stage IV) than previously published series suggesting median OS of <12 months.
* Kerkhofs JCEM 2013

PO13 - ACTIVITY AND SAFETY OF TEMOZOLOMIDE AS SECOND/ THIRD CHEMOTHERAPY LINE IN PATIENTS WITH ADVANCED ADRENOCORTICAL CARCINOMA

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Background: Adrenocortical carcinoma (ACC) is a rare and aggressive malignant disease. The efficacy of standard systemic therapy- Etoposide, Doxorubicin, Cisplatin plus mitotane (EDP-M)- in the management of patients with locally advanced or metastatic disease is limited. Preclinical data showed that temozolomide has a significant anti-proliferative activity

on ACC cells in vitro. The aim of this study is to retrospectively evaluate the efficacy and safety of temozolomide as second/third line approach after progression to the standard chemotherapy.
Methods: This is an Italian multicentric retrospective study. We collected anagraphic, clinical and pathological data of patients treated with temozolomide at the dose of 150-250 mg/m2 die (g: 1 -> 5 q28). The primary end-point was clinical benefit, defined as objective response or disease stabilization after 4 months. Secondary endpoints were overall survival, progression free survival and drug safety.
Results: 26 patients have been included in the study recruited over four Italian Institutions. Ten patients (38.5%) obtained a clinical benefit from temozolomide treatment. In particular, 4 patients had a stable disease and 5 of them had a partial response. Median PFS was 3.5 months and median OS was 7 months. Toxicities were limited to grade G1-2 confirming temozolomide safety.
Conclusions: Temozolomide appeared to be active in the management of advanced ACC patients with disease progression to first line EDP-M. The efficacy however was limited as demonstrated by the short PFS and OS of the treated patients.

PO14 - PEPTIDE RECEPTOR RADIONUCLIDE THERAPY IN THE MANAGEMENT OF LOCALLY ADVANCED OR METASTATIC ADRENOCORTICAL CARCINOMA

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Background: Adrenocortical carcinoma (ACC) is a rare and aggressive disease. Mitotane plus/minus Etoposide Adriamycin and Cisplatin (EDP-M) are the only efficacious systemic therapies actually available in patients with locally advanced or metastatic disease. Gallium-68-Dotatate ((68)Ga-Dotatoc) peptides positron emission tomography/computerized tomography (PET/CT) is widely used in neuroendocrine cancer diagnosis and to select patients for peptide receptor radionuclide therapy (PRRT). In this study, we explored the proportion of advanced ACC patients with uptake at PET/CT and the possible therapeutic role of PRRT.
Methods: This is a retrospective study in which we included patients with locally advanced or metastatic disease who performed (68)Ga-Dotatoc PET/CT after progression to at least one line of chemotherapy. We

collected anagraphic, clinical and radiological data in order to assess the presence of tumor uptake and to evaluate response to PRRT in case of positive disease.
Results: 11 consecutive ACC patients were referred for a (68)Ga-Dotatoc PET/CT scan. All patients had metastatic disease, the sites of diseases were locoregional in 6 patients, liver in 6 patients, lung in 6 patients, and bone in 2 patients. Significant tumor uptake was seen in 2 cases with a SUVmax of 31.8 and 10.5 respectively. Both patients underwent PRRT. In the first case, 4 cycles were performed obtaining a partial response (SUVmax after therapy 4.8) which lasted 12 months. Instead, 2 cycles were performed in the second case leading to a disease stabilization lasting 4 months. The treatment was well tolerated, only one patient suffered from mild back pain and decreased lymphocyte blood count.
Conclusion: PRRT is feasible in a minority of ACC patients, due to limited uptake of the disease at (68)Ga-Dotatoc PET/CT. However in the few cases of significant tumor uptake, this treatment modality can be active.

PO15 - PRE-OPERATIVE USE OF METYRAPONE AND MITOTANE COMBINATION THERAPY IN THE MANAGEMENT OF ADRENOCORTICAL CARCINOMA ASSOCIATED WITH CUSHING'S SYNDROME

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Introduction: Patients with adrenocortical carcinoma (ACC) and Cushing's syndrome (CS) are reported to have a worse overall survival in comparison with patients with non-secretory tumours. Metyrapone blocks cortisol synthesis by inhibiting 11β-hydroxylase enzyme. Mitotane is an adrenolytic agent used as an adjuvant therapy in advanced ACC that blocks steroidogenesis at multiple levels. We describe a strategy to use metyrapone to rapidly control cortisol, in parallel with initiation of mitotane therapy and then to withdraw metyrapone once biochemical control has been achieved by mitotane.
Aim: 1) To evaluate the effectiveness of the use of metyrapone and

mitotane combination therapy preoperatively in patients with ACC and CS, and 2) To evaluate the use of liquid chromatography tandem-mass spectrometry (LC-MS/MS) in the biochemical monitoring of these patients.
Methods: Review of patients with ACC and CS who were treated with metyrapone and mitotane prior to surgical intervention. Metyrapone/mitotane day curve (cortisol levels at 0, 120, 240 and 360 minutes using LC-MS/MS) was done within 2 weeks after initiation of therapy, and regularly thereafter. Mean levels of cortisol and 11-deoxycortisol (S) were calculated. Suppression of cortisol was used as a marker of effective metyrapone blockade, while suppression of both cortisol and S was used as a marker of effective mitotane blockade.
Results: N=7 patients with advanced ACC and CS were treated preoperatively with metyrapone and mitotane in our centre between 2016 and 2018. One patient was excluded as metyrapone was discontinued early due to drug intolerance. The median starting dose of metyrapone was 1500 mg per day. Mean cortisol level prior to initiation of therapy was 888 nmol/L, compared to 151.7 nmol/L on the first metyrapone/mitotane day curve within 2 weeks. Metyrapone was discontinued once suppression of S was achieved. The mean duration of metyrapone treatment was 13 weeks. The median overall survival has not been reached; progression-free survival was 12.4 months.
Discussion and conclusion: Effective pre-operative medical management of cortisol excess results in a better outcome in patients with ACC and CS. Metyrapone in combination with mitotane quickly and effectively achieves biochemical cortisol blockade. The use of LC-MS/MS provides an effective method of monitoring, which allows discontinuation of metyrapone once the therapeutic effect of mitotane is reached.

PO16 - STEROL-O-ACYL TRANSFERASE 1 PROTEIN EXPRESSION ALONE IS NOT SUFFICIENT TO PREDICT RESPONSE TO MITOTANE TREATMENT IN ADRENOCORTICAL CARCINOMA

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Background: Objective response rate to mitotane in advanced ACC is only ~20% and adverse drug effects are frequent and potentially serious. Markers of treatment response are not established. We have previously discovered that mitotane is an inhibitor of sterol-O-Acyl transferase 1 (SOAT1) which leads to depletion of cholesterol esters, endoplasmic reticulum stress and cell death in the ACC cell line NCI-H295. Data from a small cohort of patients with advanced ACC treated with mitotane monotherapy suggested longer progression-free survival in patients with high SOAT1 expression in ACC tissue.

Aim: To investigate SOAT1 protein expression as a marker of treatment response to mitotane.

Methods: SOAT1 protein expression was semiquantitatively determined by immunohistochemistry in full sections of 223 ACC treated with mitotane monotherapy in an adjuvant (n=151) and palliative setting (n=72) from eleven ENSAT centers. Expression was classified as high (H-score ≥2) and low (H-score <2) and correlated with recurrence-free (RFS) and progression-free survival (PFS), overall survival (OS), and mitotane levels after three and six months.

Results: After multivariate adjustment for sex, ENSAT stage and Ki67 index, RFS (HR=1.3, log rank p=0.09) and OS (HR=1.6, log rank p=0.14) in adjuvantly treated ACC patients did not differ significantly between tumours with high and low SOAT1 expression. Similarly in the palliative setting, OS (HR=1.0, p=0.47) and PFS (HR=0.6, p=0.66) were not different. In line, high SOAT1 expression did not predict if patients reach the therapeutic window of mitotane.

Conclusion: SOAT1 expression alone was not correlated with clinically significantly RFS, PFS, and OS in ACC patients neither in the adjuvant nor in the palliative setting.

PO17 - UNWANTED EFFECTS OF POST-OPERATIVE ADJUVANT MITOTANE TREATMENT IN PATIENTS WITH ADRENOCORTICAL CARCINOMA: THE EXPERIENCE OF THE SAN LUIGI HOSPITAL

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Mitotane is the milestone of pharmacological treatment of adrenocortical carcinoma (ACC). Aim of the present study was to investigate the endocrine and metabolic effects of post-operative adjuvant mitotane treatment and their management.

We did a retrospective analysis on 74 ACC patients treated with adjuvant mitotane following radical surgery at our center since 2000. We evaluated TSH, FT4, PRA, aldosterone, total testosterone, SHBG, LH, FSH, total cholesterol, HDL cholesterol and triglycerides during the first two years of mitotane treatment (baseline, +6, +12, +24 months). In patients treated with a specific therapy, we evaluated levels at the time of initiation of therapy and after 6 months.

Our 74 ACC patients (35M/39F), median age 46 years, were treated with adjuvant mitotane for 40 months. We observed the following trends: a) significant decrease in cortisol (13.2-3.2-31.6mg/dl $p<0.001$) but a minimal increase of ACTH (42-99-49-71 pg/ml; $p=0.047$); b) increase in PRA values (0.75-1.50-1.45-2.15 ng/ml/h; $p<0.001$); c) no change in TSH values (1.57-1.64-1.78-1.60 mUI/l; $p=0.27$); d) significant reduction of FT4 (1.04-0.76-0.78-0.76 ng/dl; $p<0.001$); e) increase of total testosterone in men (5.4-7.7-7.5-11.5 ng/ml; $p=0.018$); f) increase in SHBG (48-180-180-180 nmol/l; $p<0.001$); g) reduction of calculated free testosterone (11.3-5.3-5.3-9.4 ng/dl; $p=0.07$); h) no significant change in FSH (9.2-6.5-11-10.8 UI/l, $p=0.11$) and LH (5.0-27.1-35.4-18.1 UI/l; $p=0.11$); i) increase of total cholesterol (207-254-253-241 mg/dl; $p=0.02$) and HDL cholesterol (51-69-80-76 mg/dl; $p=0.07$); l) no change in LDL cholesterol and triglycerides.

This study shows that mitotane treatment induces, beyond the well-known hypoadrenalism, central hypothyroidism and primary hypogonadism in men. In women, gonadic function is generally preserved, although we recorded menstrual irregularities in 30% and occurrence of ovarian cysts in 68% of fertile women. Mitotane induces an early alteration of lipids, with a prevalent increase of total cholesterol. During follow up, the following replacement therapies were instituted: fludrocortisone in 32% of patients (after a median of 15 months from mitotane start), levothyroxine in 38%

(8.5 months), testosterone in 34% of men (33 months). Lipid lowering drugs have been started in 50% of patients after 5 months.
In conclusion, mitotane determinates a variety of endocrine and metabolic alterations that need to be promptly corrected in order to achieve a better tolerability and adherence to therapy.

PO18 - IN VITRO CYTOTOXIC ACTIVITY OF CABAZITAXEL IN ADRENOCORTICAL CARCINOMA

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Background: One important mechanism limiting the efficacy of chemotherapeutic drugs in AdrenoCortical Carcinoma (ACC) cells is the expression of the MDR1 gene, encoding the P-glycoprotein (P-gp). Cabazitaxel (Caba), a novel taxane used for advanced metastatic prostate cancer, displayed poor affinity for P-gp and was found efficacious in MDR-1 expressing tumors.
The aim of this study was to evaluate in vitro the cytotoxic effect of Caba in experimental cell models of ACC.
Methods: NCI-H295R, HAC15 and SW-13 cell lines and six ACC primary cell cultures were used and cell viability was analyzed using MTT assay. The involvement of MDR1 gene in the cytotoxic effect of Caba was evaluated both by silencing the gene expression with the interfering RNA technique and by pharmacological inhibition using a selective inhibitor of P-gp, tariquidar. A commercially available Apoptosis Array was carried out to study the intracellular pathways activated in Caba-exposed NCI-H295R cells. Drug combination experiments were performed to study the possible pharmacological interaction between Caba and mitotane.
Results: Caba induced a greater reduction of cell viability in primary cell cultures compared to the old-generation taxanes paclitaxel and docetaxel, with some individual differences. The efficacy of Caba was demonstrated as well in NCI-H295R cell line, with an order of potency, based on the respective IC50 value, that was: Caba (15nM IC95%: 10.7-23.2nM)> docetaxel (35nM IC95%:20.6-58.9nM)> paclitaxel (55nM IC95%: 27.9-107.1nM), although these differences did not reach a statistical significance. HAC-15 cells, derived from NCI-H295R cells,

displayed a similar response, while in SW-13 cells, that are devoid of P-gp, no differences in the taxane activity was observed. The role of P-gp in mediating the Caba effect was confirmed by results obtained whit the inhibition of P-gp activity, both by siRNA or by 10 nM of tariquidar. Finally, Caba exposure significantly increased the expression of proteins involved in both the intrinsic and extrinsic apoptotic pathway and in the cell cycle regulation. Combination experiments evaluated by the Chou-Talalay method revealed a synergistic effect of Caba and mitotane.
Conclusions: Taken together these results demonstrated for the first time that Caba is active against ACC in vitro and provide the rational to design a clinical trial (CabACC study, EUDRACT2017-001591) that is currently recruiting.

PO19 - IN VITRO ANTITUMOR ACTIVITY OF PROGESTERONE IN HUMAN ADRENOCORTICAL CARCINOMA CELLS

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Systemic therapies: Mitotane and chemotherapy, currently in used in the management of patients with adrenocortical carcinoma (ACC), show limited efficacy, and there is need to develop new therapeutic approaches. Here we investigated the antitumor activity of progesterone (Pg) in the NCI-H295R cells and in primary cell cultures derived from ACC patients.
Methods: Cell viability was evaluated by MTT assay. In NCI-H295R cells, cell cycle analysis was conducted by flow cytometry. Apoptosis was studied by double staining AO/EtBr and by cleavage of caspase 3. The expression and activity of membrane Pg receptors (mPRs) were evaluated by western blot. The ability of Pg to affect the Wnt/β-catenin pathway was studied by immunofluorescence. Pg and mitotane combination experiments were also performed, to evaluate their effect on

ACC cell viability.
Results: Pg exposure for 4 days exerted a concentration-dependent inhibition of cell viability in NCI-H295R. Cortisol-secreting ACC primary cell cultures (n=3) displayed a concentration-dependent cytotoxicity induced by Pg, while non-secreting primary tumors (n=2) were less sensitive. Interestingly, at least 40% of neoplastic cells belonging to cortisol-secreting tumors expressed nuclear Pg receptor. The number of apoptotic NCI-H295R cells exposed to Pg at its IC50 of 25 μM for 4 days was increased up to 39 ± 2%; while necrotic and living cells were 2 ± 2% and 59 ± 3% respectively (apoptotic cells: untreated vs treated cells: $P < 0.001$), with no effect in the cell cycle distribution. Apoptosis was also demonstrated by the increase of cleaved caspase 3 levels after Pg treatment. Furthermore, the cytotoxic effect of Pg seemed to involve as well a reduction of b-catenin nuclear localization. Finally, a contribution of a non-genomic effect mediated by mPRs cannot be excluded, as we demonstrated that NCI-H295R cells expressed functionally active mPRs as well as PGRMC1. The Pg antineoplastic activity was synergically increased when mitotane was added to the cell culture medium, measured by the Chou-Talalay method.
Conclusions: Pg exerted a cytotoxic activity in ACC cells, inducing apoptosis via activation of Pg receptors. Both the genomic and non-genomic effects of progesterone seemed to mediate the cytotoxicity, although this point is still under investigation. The synergistic cytotoxic activity of progesterone with mitotane provides the rationale for testing this combination in a prospective clinical study.

PO20 - THE MOLECULAR CHAPERONE HEAT SHOCK PROTEIN 90 (HSP90) AS NOVEL THERAPEUTIC TARGET FOR THE TREATMENT OF ADRENOCORTICAL CARCINOMA (ACC) AND CUSHING SYNDROME

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The molecular chaperone Hsp90 contributes to different oncogenic signaling pathways. Accordingly, active agents targeting Hsp90 function have shown therapeutic efficacy in several cancer types. ACC, a rare

tumor with restricted therapeutic opportunities and Hsp90 clients' key role in various cellular processes make therapeutic investigations of Hsp90 inhibitors in ACC a desirable approach. We studied effects of Hsp90 inhibitors in single as well as in combined treatment with the clinical used EDP-M scheme (Etoposid (E), Doxorubicin (Dx), Cisplatin, Mitotane). Viability of NCI-H295R and MUC-1 cells at different time points were investigated using various inhibitor concentrations. N-terminal Hsp90 inhibitors demonstrated varying effects on cell viability in single treatment (Luminespib (L):13.6±2.1, Ganetespib (G):11.3±2.6 vs. untreated NCI-H295R:100.0±0.0 and L: 40.8±2.7, G: 45.5±3.9 vs. untreated MUC-1:100±0.0). Additionally, we observed additive, concentration-dependent effects in combination with Dx or E together with G or L in both cell lines. NCI-H295R cells responded to a higher extent (NCI-H295R: L+Dx 28.6±5.0 and MUC-1: L+Dx: 53.5±2.3 vs. 100% of basal). Furthermore, we identified a pronounced decrease of pAKT in both cell lines upon treatment with L and G. However, only in MUC-1 cells we observed a reduction of pERK. We detected a modulation of cortisol secretion, but no distinct variation of CYP11B1 expression in NCI-H295R while no such effects were detectable in MUC-1. Preliminary experiments demonstrated time dependent effects on the Wnt/β-catenin pathway in both cell lines upon treatment with Hsp90 inhibitors. Taken together, our results suggest that inhibition of Hsp90 could be a potent therapeutic target option in ACC.

PO21 - IMMUNOHISTOCHEMICAL ASSESSMENT OF INTRATUMORAL CELLS EXPRESSING CD68 AND CD8 IN ADRENOCORTICAL TUMORS: A COMPUTERIZED MORPHOMETRIC ANALYSIS

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We have recently proposed a computerized morphometric model to quantify Ki67 proliferation index in the workup of adrenocortical carcinomas and adenomas (ACCs and ACAs). The inflammatory infiltrate and milieu play a critical role in the pathogenesis and progression of many cancers. The study was aimed to assess, by computerized morphometry, the inflammatory pattern in adrenocortical neoplasms.

Thirteen ACAs and 19 ACCs samples (4 consecutive 4 μm sections for each case) were analyzed. Specimens were immunostained for Ki67, CD8 T-lymphocytes, CD15 granulocytes and reticulin. Volume fractions of Ki67 positive and negative cells, vascular and inflammatory compartments,

CD15 granulocytes, CD8 T-lymphocytes and surface fraction of reticulin were assessed with a computerized morphometric model.
On Ki67 stained sections, volume fraction of nuclei were highest in ACCs in Ki67 negative (ACCs .12251, ACAs .06652) and positive cells (ACCs .01296, ACAs .00101). ACCs showed the highest values of nuclear/cytoplasmic ratio in Ki67 negative (ACCs .20991, ACAs .09247) and positive cells (ACCs .69691, ACAs .26423). Surface fraction of reticulin was lower in ACCs (ACCs 10.60, ACAs 34.70). Volume fractions of CD15 (ACCs .00350, ACAs .00112) and CD8 T-cells (ACCs .00723, ACAs .00345) were significantly higher in ACCs and positively correlated with Ki67 expression.
Our data show an association between volume fractions of Ki67 positive cells and inflammatory cells in adrenal neoplasms.
Further studies are needed to investigate the functional significance of different inflammatory cell subsets in ACCs and if the assessment of inflammatory infiltrate may find a place in the diagnostic algorithm of adrenocortical tumors.

PO22 - ANALYSIS OF PROTEIN EXPRESSION OF PD-L1 IN PATIENTS WITH ADRENOCORTICAL CARCINOMA

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Introduction. Adrenocortical cancer (ACC) is a rare and aggressive malignant tumor with poor prognosis. There are no effective chemotherapeutic approaches in advanced ACC. Prognostic and predictive factors are unclear. The expression of PD-L1 as a potential prognostic marker and therapeutic target for anti-PD-L/PD-L1 therapy seems timely and relevant.
Aim: The explore PD-L1 protein expression in tumor samples of patients with ACC.
Materials and methods. To identify the expression of PD-L1 receptors, an immunohistochemical (IHC) study of postoperative tumor tissue samples from 20 patients with ACC was performed. Immunohistochemical analysis was performed on sections embedded in paraffin on an automatic immunohistostainer Ventana. The antibody PD-L1 (28-8) was used in the study. According to the manufacturer's recommendations, PD-L1 antibody (28-8), Abcam was optimized for use at automatic immunohistostainer Ventana, BenchMark GX, in conjunction with the Ventana detection kits.
Results. Overall, the median age was 48 +/–11.1 years ranging from 27-68 years. Men dominated the study. At the time of analysis, 19 patients

(95%) had metastatic disease. Most of patients (85%) received one or more chemotherapy regimens.
The negative IHC status of PD-L1 was defined in 16 patients (80%). PD-L1 expression was determined in 5% of tumor cells in 1 patient (5%). PD-L1 was expressed of 40% of tumor cells in one patient (5%). A high level of PD-L1 expression (≥50%) was detected in 2 patients (10%). Thus, the positive IHC status of PD-L1 was defined in 4 patients (20%).
Conclusion. Thus, as in other solid tumors, PD-1 expression is detected in tumor cells in ACC. Further research on a larger population of patients will determine the prognostic and predictive value of PDL-1 protein expression in ACC.

PO23 - ADJUVANT SURGERY IN THE MANAGEMENT OF STAGE IV ADRENOCORTICAL CARCINOMA

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Introduction: we explored the role of adjuvant surgery (AS) in the management of advanced stage ACC deemed inoperable at diagnosis but converted to surgery due to tumor regression after chemotherapy.
Methods: retrospective analysis of a prospectively maintained database. We analyzed data concerning the clinical course of stage IV patients from diagnosis onward. Survival of cases submitted to AS was compared to survival of those who did not reach surgery.
Results: from June 2015 to August 2018, 8/13 patients deemed inoperable at diagnosis, were operated on after chemotherapy (EDP, 4 to 7 cycles) and Mitotane.
At diagnosis, 1 patient was asymptomatic while the others had hormonal syndrome (cortisol in 5 cases, aldosterone in 1 and the two in 1) and mass effect (3 cases).
At operation symptoms had disappeared.
Surgery was indicated on the basis of restaging CT which demonstrated partial response, not only in terms of size but also in terms of radiological features and biological behaviour at PET scan.
Surgery did never achieve oncological radicality: 7/8 patients had pulmonary metastases and the remaining 1 had bone and subclavian nodes metastases. Abdominal radicality was achieved in 7/8 cases, R1 resection in 1. In 5 cases citoreductive surgery was completed by HIPEC with cisplatin and adriamycin.
Ninety-days postoperative mortality was nil. Major morbidity was observed in 2 cases (25%): 1 pancreatic fistula, managed conservatively, and 1 case of septic shock complicated by acute haemorrhage requiring

re-intervention and open abdomen management of a compartmental syndrome; ICU stay was 103 days.
Excluding these complicated cases, the mean LOS was 12,7 days.
All patients continued Mitotane after surgery.
On September 15, 2018 two patients were died after 17 and 20.6 months from diagnosis. The remaining 6 patients are alive after 11, 13, 14, 16, 22, and 38 months from diagnosis, respectively.
Mean OS and PFS were respectively 20 [13.17 – 38.1] and 15 months [7.98-25.58] from the diagnosis. Mean OS from surgery was 12 months [5 – 26]. Median survival had not been reached in the AS group yet. Mean OS of the 5 patients who were not operated on was 7.6 months [1-14,4]; none of them is alive.
Conclusions: this initial experience highlights a possible role of AS in the multimodal management of stage IV ACC.

PO24 - METALLOTHIONEINS AND MINICHROMOSOME MAINTENANCE PROTEIN-2 EXPRESSION IN ADRENOCORTICAL TUMORS

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Background: Some totally resected adrenal-confined adrenocortical carcinomas (ACCs) may metastasize and others may not, a fact that challenges clinicians in selecting patients who may benefit from adjuvant therapy. Ki-67 has been shown to be positively correlated with tumor aggressiveness. Nevertheless, some tumors with low Ki-67 index may metastasize, while other high index tumors may not. In order to efficiently identify patients with high recurrence risk, several studies have been held to address tumor aggressiveness using different histopathological proliferative markers. In a study completed on six ACC patients, investigators demonstrated that expression of the immunohistochemistry markers Metallothioneins (MT) and Minichromosome maintenance protein-2 (MCM-2) was significantly higher in ACCs vs. adrenocortical adenomas (ACAs). However, the correlation between their expression and tumor stage was not studied. Consequently, the present study was designed to compare MT and Mcm-2 expression in ACCs and ACAs, as well as to correlate between their expression and the different stages of ACC.
Methods: The study comprised 14 patients operated on for ACC and 15 patients operated on for ACA. Hematoxylin/eosin staining was used for histological evaluation under light microscope and sequential sections were used for MCM-2 and MT staining. The evaluation of Mcm-2 staining

was recorded as numeric percentage of cells with a nuclear color reaction on selected hot spots, and MT nuclear staining was recorded on a 4-point scale, where 0, 1, 2 and 3 reflected: no nuclear-cell reaction; 1% - 10%; 11% to 50%; and more than 50% positive nuclear-cell reaction, respectively. Data are presented as mean ± SD.
Results: One of the ACC patients was with ENSAT stage I, 6 with stage II, 2 with stage III and 5 with stage IV. MT and MCM-2 expression was significantly higher in ACCs (2.3 ± 1.2 and 24% ± 17, respectively) than in ACAs (1.2 ± 0.6 and 0.8% ± 0.7, p=0.008 and p<0.001, respectively). In ACCs, significant positive correlation was found between MCM-2 expression and Weiss revisited score (WRS) (p=0.022) but not with Weiss score (WS); no correlation was found between MT neither with WS nor with WRS. High positive correlation was found between MCM-2 expression and the presence of the histologic feature “presence of mitotic rate greater than 5 per 50 high-power fields” (p=0.033). In ACCs, MCM-2 but not MT expression was shown to be highly correlated with stage IV carcinoma (p=0.008 and p=0.165, respectively).
Conclusions: MCM-2 and MT are overexpressed in ACC, and MCM-2 expression is highly correlated with metastatic disease. More studies are needed to evaluate MCM-2 expression as a potential prognostic factor in ACCs.

PO25 - IGF-1EC EXPRESSION IN ADRENAL GLAND NEOPLASMS

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Introduction: Aberrant production of insulin growth factor 1 (IGF-1) has been associated with tumorigenesis. IGF-1Ec which is a splice variant of IGF-1 has been associated with survival and proliferation of tumor cells, as well as with their ability to metastasize by promoting their epithelial–mesenchymal differentiation. Although the expression of IGF-1Ec has been investigated in various cancers, data on adrenal neoplasms are missing. Herein, we aimed to examine the expression of IGF-1Ec at mRNA and protein level in adrenocortical carcinomas (ACCs), adrenocortical adenomas (ACAs) and pheochromocytomas (Pheo) human tissues. Additionally, we investigated the role of IGF1-Ec in proliferation and migration of adrenal cortex carcinoma cells.

Methods: The mRNA levels of IGF-1Ec were evaluated by qPCR in adrenal gland fresh frozen tissues (ACA=8, ACC=6, Pheo=11). Immunohistochemical (IHC) staining of paraffin-embedded tissues (ACA=15, ACC=13, Pheo=8) was performed using anti-IGF-1Ec antibody. IHC staining was scored with the immune-reactive scoring system (IRS). SW-13 cells were incubated for 24 and 48 hour with IGF-1Ec peptide at concentrations 10, 20, 40, 50 and 100ng/ml and XTT cell proliferation assay and scratching assay were performed.

Results: The mRNA levels of IGF-1Ec were significantly increased in adrenal cortex neoplasms (ACC+ACA) compared to medulla (Pheo) and significantly higher in ACCs compared to ACAs. These finding were also confirmed at protein level, since 100% of ACCs and 73.3% of ACAs were positively stained, showing a more intense staining in ACCs (IRS=6.62) as compared to ACAs (IRS=3.6). Interestingly, no IGF-1Ec protein was detected in the Pheo. Incubation of SW-13 cells with 100ng/ml IGF-1Ec for 48h resulted in a significant increased cell proliferation and cell migration capability compared to the untreated cells.

Conclusion: In the present study, we demonstrated for the first time the expression of IGF-1Ec spice variant in human adrenal neoplasms. Our ex-vivo data revealed its relation with the biological behavior of the adrenal cortex neoplasms, as there was a higher expression of IGF-1Ec in carcinoma compared to adenoma, while the in vitro results imply that this relation may be causal since IGF-1Ec peptide promoted a more aggressive behavior of adrenal cancer cells. Further studies are warranted in order to address its possible prognostic role and/or its use as therapeutic target.

PO26 - EVALUATION OF URINARY MIR-483-5P IN ADRENOCORTICAL CANCER PATIENTS

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Introduction: Minimally invasive blood-borne circulating microRNAs might be used for the preoperative differentiation of adrenocortical carcinoma (ACC) and adrenocortical adenoma (ACA). Circulating hsa-miR-483-5p is so far the best microRNA biomarker of ACC. To the best of our knowledge, there have been no studies concerning the potential applicability of urinary has-miR-483-5p as a non-invasive biomarker of ACC and its correlation with plasma hsa-miR-483-5p.

Aim: Our aim was to investigate the expression of urinary hsa-miR-483-5p and its correlation with its plasma counterpart.

Methods: Plasma and urinary samples from 23 ACC and 23 ACA patients were analysed using real-time RT-qPCR. To evaluate the diagnostic applicability of hsa-miR-483-5p, ROC-analysis was performed.

Results: Significant overexpression of hsa-miR-483-5p was observed in carcinoma patients' plasma samples compared to adenoma patients' ($p<0.0001$, sensitivity: 87 %, specificity: 78.3 %). In urinary samples, however, no significant difference could be detected between ACC and ACA patients.

Conclusions: Plasma hsa-miR-483-5p has been confirmed as significantly overexpressed in adrenocortical cancer patients and thus might be exploited as a minimally invasive preoperative marker of malignancy. The applicability of urinary hsa-miR-483-5p for the diagnosis of adrenocortical malignancy could not be confirmed.

PO27 - ANTIPROLIFERATIVE EFFECTS OF EF24, A CURCUMIN DERIVATIVE, IN ADRENOCORTICAL TUMOR CELL LINES

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Background: Curcumin is a polyphenol extracted from the plant Curcuma longa L. It has numerous properties and is used in many preclinical

conditions, including cancer. Curcumin has been tested in colorectal, lung, breast, liver and many others tumor cell lines. It is known that curcumin has low bioavailability, while its derivative EF24 showed enhanced solubility. However, its effects have been never explored in adrenocortical tumor cell models.
Aim: This work analyzed the efficacy of EF24, a curcumin derivative, in 2 adrenocortical tumor cell line models, SW13 and H295R.
Results: EF24 reduced cell viability by MTT with IC50 of 6.5 ± 2.4 μM and 4.9 ± 2.8 μM for SW13 and H295R cells, respectively. Combination index (EF24 associated with mitotane) suggested an additivity effect in both cell lines. Cell cycle analysis revealed an increase of subG0/G1 phase, while motility assay showed a decrease in migratory cell capacity after drug treatment and similarly clonogenic assay indicated that EF24 (alone or combined with mitotane) could reduce colonies number. Also Wnt/β-catenin, NF-κB, MAPK and PI3k/Akt pathways were modulated by Western blot analysis when treating cells with EF24 alone or combined with mitotane.
Conclusions: This work analyzed for the first time a derivative of curcumin, EF24, in adrenocortical tumor cell lines. These results suggest that EF24 could potentially impact on adrenocortical tumors, laying the foundation for further research.

PO28 - ANTINEOPLASTIC ACTIVITY OF ARTEMISIN IN A PATIENT WITH HEAVILY PRETREATED METASTATIC ADRENOCORTICAL CARCINOMA

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Background Adrenocortical carcinoma (ACC) is a rare and aggressive disease. Etoposide, doxorubicin and cisplatin plus mitotane (EDP-M) is the standard systemic therapy for patients with locally advanced/ metastatic disease. No effective therapies are available in patients with disease progression to EDP-M. Several pre-clinical studies have shown an antitumor activity of the herb artemisin on a variety of malignancies. The main mechanism of the antineoplastic activity is not fully elucidated, however the observed inhibition of p53 and wnt-beta catenin pathways, makes this drug potentially useful in the management of ACC. We present

a patient with heavily pre treated metastatic ACC who obtained a long lasting disease response after the assumption of artemisin.
Case Report A male patient, 61 yr old, had a long clinic history of advanced ACC: the disease was diagnosed in May 2008 when the patient underwent left adrenalectomy. Surgery was complicated by capsular rupture. The histological examination showed: adrenal lesion of 7x5.5x4.5 cm, with eosinophilic cytoplasm cells, 10 mitoses/10, Ki67 25%, Weiss Score 6, Van Slooten score:18.5. No adjuvant mitotane was prescribed.
At disease relapse, the patients received six cycles of EDP from December 2009 to June 2012, 2° line chemotherapy (cht) with carboplatin and taxol from June 2012 to August 2012, 3° line cht with metronomic capecitabine and gemcitabine from April 2013 to June 2013. Mitotane treatment was introduced in December 2009 and it was maintained during all chemotherapy lines. In January 2017, systemic therapies, including mitotane, were interrupted. In May 2017 the disease showed a further progression with the appearance of 3 minute liver focal lesions of 8 mm, 6 mm and 7 mm at magnetic resonance imaging (MRI).
After an herbalist's consultation the patient started taking capsule of Artemisin Annua (600mg x 2/die for 5 days followed by 5 days off). In July 2017, MRI showed disease response with reduction of two liver lesions (8 vs 5 mm, and 7 vs 5 mm while the third lesion remained stable). The treatment was well tolerated. The patient didn't suffer from any particular symptoms, no signs of toxicity were observed at clinical examination. The disease remained stable at the following MRI (January 2018).
In February 2018, due to a herpes zoster on the left side of abdomen, artemisin assumption was interrupted. In May 2018 MRI showed dimensional increase of the liver lesions (5 vs 8.2 mm and 5 vs 6 mm). Artemisin was then re-introduced but at the last MRI evaluation (August 2018), the disease showed further liver progression (11 vs 8.2 mm and 8 vs 6 mm).
Conclusion: Artemisin was an active and well tolerated drug in the management of a patient with advanced, heavily pretreated, ACC. The long lasting disease response observed (12 months) is noteworthy. The drug deserves to be further explored in pre-clinical studies and prospective clinical trials.

PO29 - MITOTANE: RAPID SUPRATHERAPEUTIC LEVELS AND NEPHROTOXICITY- A ROLE FOR CYP2D6?

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A 24 year old gentleman presented with severe abdominal pain. Cross-sectional imaging revealed a 9.6x11.7x10.6cm adrenal mass with hepatic IVC invasion and thrombus in the distal right main pulmonary artery. There was no biochemical evidence of hormone secretion.

He underwent an extensive operation to resect the mass, including cavotomy and vessel repair. Renal artery compromise led to a right nephrectomy. Histology revealed a completely excised adrenocortical carcinoma with Ki67 of 50%. He proceeded to have 44 Gy of external beam radiotherapy to the adrenal bedin 22 fractions.

He was started on mitotane (rapid titration protocol). After only two weeks, his mitotane levels were measured and were supratherapeutic at 21.6mg/L. Mitotane was initially omitted and subsequently titrated to achieve levels just within the therapeutic range as higher levels caused debilitating nausea.

Given his rapid attainment of therapeutic levels, he was consented for genetic testing for cytochrome enzyme variants. This revealed a homozygous variant in CYP2D6 consistent with a poor metaboliser phenotype and a heterozygous variant in CYP2B6. Although various cytochrome P450 enzymes have been implicated in mitotane metabolism (including CYP2B6), CYP2D6 has not. It is possible that it may have a role and perhaps in combination these variants have a profound effect on mitotane metabolism in this individual.

However, after one year, his renal function started to deteriorate. He was found to have nephrotic range proteinuria with a normal serum albumin. Full nephritic screen was negative. He underwent a renal biopsy and was found to have thinned basement membranes. His renal function continued to deteriorate and with other pharmacological culprits having been stopped, a decision was made to trial a break from the mitotane. This caused improvement in his renal function and reduction in proteinuria over a period of 4-6 weeks and he has reached a new baseline renal function which is stable. There is no evidence of disease recurrence off mitotane six months later.

We present a case of a young man with an aggressive adrenocortical carcinoma who developed supratherapeutic levels of mitotane in a very short period of time. Investigation of this may have elucidated a novel cytochrome P450 enzyme involved in metabolism of this drug. He developed worsening renal function, which with all other causes excluded appears to be secondary to mitotane and we hypothesise that this may be secondary to a different pathway of mitotane metabolism.

PO30 - LIVING WITH METASTATIC ADRENOCORTICAL CARCINOMA: 21 YEARS OF SUCCESSFUL TREATMENT

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In 1997, a 24 year old University student presented to her local hospital with symptoms of androgen excess and left loin pain. Cross sectional imaging identified a 10cm calcified adrenal mass. Unilateral left adrenalectomy was performed and histology revealed a completely excised adrenocortical carcinoma. She was referred to our unit for follow up and post-operatively had no biochemical or radiological evidence of disease recurrence.

In 2001, her testosterone levels started to rise and abdominal MRI revealed a 5x4cm adrenal bed mass plus multiple sub-centimetre metastatic pulmonary nodules. She had repeat surgery with adrenal bed clearance, left nephrectomy, splenectomy and distal pancreatectomy. Post-operatively she commenced mitotane and had complete radiological resolution of lung nodules with an undetectable testosterone

In 2004, imaging revealed new pulmonary nodules which progressed through mitotane treatment. She went to NIH, Bethesda and had bilateral laser assisted resection of her pulmonary metastases. There was no other evidence of disease. Mitotane was stopped and she returned home.

From 2006-2009 she was monitored as an outpatient and identified to have new pulmonary nodules which were slow growing but not increasing in number. She remained well in herself with no other radiological or biochemical evidence of disease and was able to stop her adrenal replacement therapy. The pulmonary nodules were treated recurrently with radiofrequency ablation. In 2010 there was no evidence of active disease post ablation and she went on to conceive naturally and have a successful pregnancy.

Post-partum, a PET scan in 2012 revealed a single focus of disease at the site of the last RFA. This was deemed unsuitable for further RFA due to its proximity to vessels and she proceeded to have a right upper lobectomy; histology was consistent with adrenocortical carcinoma.

From 2012-2017 she remained well with no evidence of disease recurrence. Surveillance imaging in 2017 revealed a new left sided diaphragmatic mass in keeping with recurrence at the original tumour site. She underwent left VATS resection of the mass with reconstruction of the diaphragm and remains well post-operatively.

We present an unusual case of metastatic secretory adrenocortical carcinoma that has been treated over a 21 year period with multiple surgeries, mitotane and radiofrequency ablation. The patient is currently well with two healthy children and no clinical, biochemical or radiological evidence of disease.

PO31 - HEALTH-RELATED QUALITY OF LIFE IN ADRENOCORTICAL CARCINOMA: A QUALITATIVE STUDY IN PATIENTS, PARTNERS AND HEALTHCARE PROFESSIONALS

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Purpose: Adrenocortical carcinoma (ACC) is a rare disease with a poor prognosis and highly toxic treatment options. Little is known about how ACC impacts health-related quality of life (HRQoL) and no research on patient-reported outcomes has been conducted. This qualitative study explores the patient, partner and healthcare professional perspectives on the HRQoL impact of ACC.

Method: In focus groups interviews, we investigate HRQoL in order to identify patient concerns in living with ACC and its treatments. Two focus groups will be held (October 2018) with 6 patients on mitotane treatment and 4 patients who received surgery alone, and their partners. In addition, 5 healthcare professionals involved in the treatment of ACC will be interviewed to find additional factors influencing HRQoL. Qualitative content analysis will be used to analyze the interviews.

Results: Results are expected in October 2018. We will produce a list of symptoms, adverse effects and functional limitations that patients with ACC, their partners and healthcare professionals find influential to HRQoL.

Conclusions: We expect ACC to have a large impact on HRQoL. We plan to use our findings to generate a disease-specific questionnaire to measure HRQoL in ACC patients, to be used in addition to the EORTC-QLQ-C30 questionnaire. This will benefit future ACC research, treatment decisions and patient monitoring.

We would like to use this opportunity to present the patient and partner reported findings of the focus groups with regard to their perspectives on HRQoL in ACC. We think that these unique initial findings are of great interest to the audience of the ENS@T meeting in Florence.

PO32 - CHARACTERIZATION OF CELL DEATH INDUCED BY SOAT1 INHIBITION IN ADRENOCORTICAL CARCINOMA CELLS

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Background: Mitotane is the only drug approved for treatment of adrenocortical carcinoma (ACC). We have found that mitotane inhibits Sterol-O-Acyl transferase 1 (SOAT1) which leads to depletion of cholesterol esters and increase of free cholesterol in the ACC cell line NCI-H295. We demonstrated activation of endoplasmic reticulum response pathway that resulted in decreased cell viability.

Aim: To characterize the mechanisms underlying cell death resulting from SOAT1 inhibition with inhibitors mitotane, ATR101, AZD3988 and Sandoz58-035.

Methods: SOAT1 inhibition was quantified in NCI-H295 cells , ER-stress marker expression by qPCR, cell viability by WST1-assay, lipid composition by GC-MS, and mitotane-induced ROS with carboxy-H2DCFDA. Cell death induced by mitotane was determined by mitotane treatment alone and in combination with either the pan caspase inhibitor zvad-fmk, necroptose inhibitor necrostatin-1, ferroptosis inhibitor liproxstatin or ferroptosis activators erastin and RSL-3 by cell titer glo viability assay..

Results: Mitotane induced ROS in a concentration and time-dependent manner which was blocked by reduced glutathione. ATR101, AZD3988 and Sandoz58-035 were inhibitors of SOAT1 in NCI-H295 cells with IC50 of 3 nM, 13 nM and 0.9 nM. Correspondingly, expression of ER-stress marker CHOP and splicing of X-box protein 1 mRNA was activated by mitotane, ATR101 and poorly by AZD3988. Mitotane and ATR101 but not AZD3988 and Sandoz58-035 treatment induced the accumulation of free cholesterol in NCI-H295 cells after 6h. Cell viability was impaired with mitotane, ATR101 and AZD3988 but not Sandoz58-035. None of the inhibitors could reverse the effects of mitotane while synergistic effects were observed for RSL3. Erastin was shown to reverse the effects of mitotane treatment.

Conclusion: Although SOAT1 inhibition was confirmed for all compounds, downstream effects on ER-stress markers and cell viability exhibit

marked differences. Hence it is likely that targets different from SOAT1 are relevant for in vitro cytotoxic activity of mitotane and ATR101. Caspases do not seem to be predominantly involved in mitotane-induced cell death. Although we observed a synergistic effect of mitotane with RSL3, ferroptosis inhibitors were not able to block mitotane-induced cell death. The precise mechanism of cell death and immunogenic cell death pathways require further investigation.

PPGL 33-51 POSTER

PO33 - INVOLVEMENT OF PROTOCADHERIN GAMMA C3 IN THE INVASIVE BEHAVIOR OF PARAGANGLIOMAS/ PHEOCHROMOCYTOMAS

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Paragangliomas (PGLs) and pheochromocytomas (PCCs) are rare tumors that derived from neuroendocrine cells. Most are benign but up to 25% are malignant (distant metastases). In fact, metastasis is one of the most important causes of death in these cases.

About 40% patients with PCC/PGL have germline mutations in well-characterized genes such as those encoding the SDH (succinate dehydrogenase) complex. SDHB mutations has been associated with metastatic PCC/PGLs but mechanisms are not defined yet. Nowadays, markers of metastatic disease are limited. Thus, there is a need to find biomarkers to identify patients at high-risk of metastasis.

Global DNA hypermethylation are present in PCC/PGLs developed in patients with mutations in SDH genes. However, none epigenetic traits have been associated with metastatic behavior of SDHB-PCC/PGLs. We found that the protocadherin gene, PCDHGC3, was methylated in primary SDHB-related PCC/PGLs with metastatic behavior. This significantly correlated with downregulation of PCDHGC3 mRNA and a decreased patients' overall survival.

Here, we analyzed the functional significance of PCDHGC3 loss in cancer cell phenotypes using 786-O (+) and RCC4 (+) cell lines in which PCDHGC3 gene expression was partially abolished by shRNA lentiviral transfection. Cell-based assays showed that shRNA-induced silencing of PCDHGC3 resulted in significant increase of cell proliferation. Cell proliferation was also promoted in control cells when incubated under hypoxic conditions (1% O2) and this was further enhanced following treatment with PCDHGC3-shRNAs. Moreover, repression of PCDHGC3 led to a significant increase in clonogenic growth, cell migration and collective cell invasion suggesting that PCDHGC3 acts as a tumor

suppressor gene. Thus, we propose that epigenetic silencing of PCDHGC3 gene is critical for the tumorigenicity of SDHB-related PCC/PGLs being putatively involved in the metastatic transformation of tumor cells.
In conclusion, PCDHGC3 might be a potential biomarker for prediction of metastasis development in patients with SDHB-PCC/PGLs.

PO34 - PROGRAMMED CELL DEATH - LIGAND 1 EXPRESSION IN METASTATIC PHAEOCHROMOCYTOMAS AND PARAGANGLIOMAS

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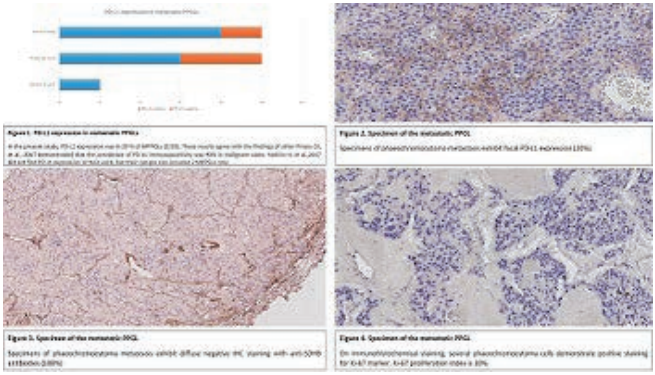
Introduction
Metastatic phaeochromocytoma/paraganglioma (MPPGL), in particular, associated with mutations of any subunit of succinate dehydrogenase complex (SDHx), is characterized by a pseudohypoxia, which causes an activation of immune-suppressive mechanisms, including increased expression of the immune checkpoint protein programmed death-ligand 1 (PD-L1) and its receptor (PD1).
Objectives
The aim of the study is to evaluate PD-L1 expression in metastatic PPGL in correlation with SDHx-mutation status.
Material and Methods
Formalin-fixed paraffin-embedded tissues from 10 patients with metastatic PPGL were investigated for the expression of PD-L1 and SDHB. Immunohistochemistry (IHC) staining was performed on 3µm sections using the Leica Bond Max stainer. The primary antibodies anti-PD-L1 (SP142; Spring) and anti-SDHB (21A11AE7;Abcam) were incubated at the concentration 1:100. Cases displaying ≥5% of tumor cells with a PD-L1 expression were considered positive.
Results and Discussion
We identify PD-L1 expression in 20 % of MPPGLs (2/10). These results agree with the findings of other studies. Pinato DJ, et al., 2017 demonstrated that the prevalence of PD-L1 immunopositivity was 40% in malignant cases. Yashiro H, et al.,2017 did not find PD-L1 expression in their MPPGLs, but the sample size included 2 MPPGLs only.
In our study, both PD-L1 positive cases are negative for SDHB IHC (40% of cells in one case and 100% of cell in another one do not bind anti-SDHB antibodies).
Conclusion
According to recent studies, PPGL demonstrate low PD-L1 expression in both malignant cases and benign ones. However, anti-PD-L1 immunotherapy is considered to be a potential medication in PD-L1 positive MPPGL. Pembrolizumab is currently being investigated in a phase II clinical trial (NCT02721732) for MPPGL.

To date, PD/PD-L1 inhibition is approved for several tumors, including melanoma, kidney cancer, and non-small cell lung cancer. Resistance to anti-PD-1/PD-L1 therapy is observed in about 60% of patients. Identification of underling mechanisms for PD-L1 expression is essential for better predicting the response to immunotherapy and discovering of novel treatment strategies.

In our study, PD-L1 expression is accompanied by negative IHC for SDHB meaning absence of B,C or D subunit of succinate dehydrogenase and pseudohypoxia as a key mechanism in MPPGL pathogenesis. The current investigation is limited by the sample size. Future studies on the current topic are therefore recommended.



PO35 - SDHD KNOCKOUT CELLS, A NEW MODEL TO EXPLAIN SDHB-RELATED MALIGNANCY IN PARAGANGLIOMA

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Background: Pheochromocytoma and Paraganglioma (PPGL) are rare neuroendocrine tumours, characterized by a strong genetic component. Among the 15 PPGL susceptibility genes, SDHB mutations are associated with increased malignancy and poor prognosis. However, the exact causes of SDHB-related invasiveness remain unexplained.

Objective: To evaluate the differences between SDHB and other SDHx genes mutations, in order to elucidate the mechanisms behind SDHB-dependent malignancy.

Material and method: A new chromaffin cell (imCC) line mutated on the Sdhd gene was generated by a CRISPR-Cas9 approach. We compared

its phenotype to that of the Sdhb-/- imCC previously generated in the lab. We characterized both cell types in terms of proliferation, migration and adhesion and established their methylation, transcriptomic and metabolic profiles. The extracellular matrix (ECM) was also examined thanks to matrisome analyses and matrix-swap experiments were performed.

Results: Interestingly Sdhb-/- cells display a much more aggressive phenotype than Sdhd-/- cells, as highlighted by increased adhesive and migratory properties, in accordance with the different clinical behaviour of tumours observed in patients carrying these mutations. Sdhd-/- cells therefore appear as a good model to explain the mechanisms behind SDHB-dependent malignancy. Methylome analysis revealed different methylation profiles in Sdhd vs Sdhb mutated cells, leading to a specific regulation of genes involved in hypoxia and genes encoding proteins of the ECM. In vitro, matrix-swap experiments confirmed the important role of the ECM in the acquisition of metastatic properties: the ECM secreted by Sdhb-/- cells increased the adhesive and migratory abilities of Sdhd-/- cells.

Conclusion: These data suggest that this new Sdhd-/- cell line is a promising model that will provide new findings to understand the mechanisms involved in SDHB-related PPGL malignancy and highlight the role of ECM reprogramming in the acquisition of the metastatic phenotype.

PO36 - A DEVELOPMENTAL MODEL OF PARAGANGLIAR TUMORIGENESIS HIGHLIGHTS THERAPEUTIC TARGETS

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To identify genes and pathways commonly deregulated in head and neck paragangliomas (PGLs), we integrated genome-wide copy number variation (CNV) analysis with microRNA and immunomorphological studies. Gene-centric CNV analysis of 24 cases identified 104 genes most significantly targeted by PGL-associated alterations. A “NOTCH signaling pathway” implicated in vasculoangiogenesis was the most significantly enriched term (P = 0.002). Expression of the relevant proteins was confirmed in 47/47 head and neck PGLs, with no relationships to germline SDHx mutation status or SDHB immunostaining (1). Five microRNAs that control cell differentiation (miR-200a,b,c and miR-34b,c) were among those most downregulated in the PGLs. To understand the origin of PGLs, we developed a biobank comprising 77 cases, 18 primary cultures, 4 derived cell lines, 80 patient-derived xenografts and 11 cell-derived xenografts. We investigated these unique complementary materials using morphofunctional, ultrastructural and flow cytometric assays accompanied by microRNA studies (2). We found that PGLs contain stem-like cells with hybrid mesenchymal/vasculoneural phenotype, stabilized and expanded in the derived cultures. The cultures depended on the downregulation of the miR-200 and miR-34 families, which allowed high NOTCH1, PDGFRA and ZEB1 protein levels. PGL xenografts, both tissue- and cell culture-derived, recapitulated the typical vasculoneural tumor structure and arose from mesenchymal-like cells through a fixed developmental sequence. First, vasculoangiogenesis organized the microenvironment, building a perivascular niche that in turn supported neurogenesis. Neuroepithelial differentiation was associated with severe mitochondrial dysfunction, not present in cultured PGL cells, but acquired in vivo during xenograft formation. Imatinib, that targets endothelial-mural signalling, blocked PGL xenograft formation (11 xenografts from 12 cell transplants in the control group versus 2 out of 10 in the treated group, P = 0.0015). Overall our results were unaffected by the SDHx gene carrier status of the donor patient, characterized for 70 out of 77 cases. In conclusion, we explain the biphasic vasculoneural structure of PGLs and identify an early and pharmacologically actionable phase of PGL organization (2).

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1) Cama et al Acta Neuropathol 2013 doi: 10.1007/s00401-013-1165-y.
2) Verginelli et al Acta Neuropathol. 2018 doi: 10.1007/s00401-017-1799-2.

PO37 - DIFFUSION WEIGHTED IMAGING (DWI) HIGHLIGHTS SDHB-RELATED TUMOURS – A RADIATION FREE ALTERNATIVE FOR SURVEILLANCE IMAGING

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Background

There is general consensus that asymptomatic carriers of SDHB mutations should undergo periodic surveillance imaging. It is established that 68Ga-DOTATATE PET/CT is superior for localisation of primary head & neck paragangliomas and SDHB-associated metastatic phaeochromocytoma and paragangliomas (PPGLs). For surveillance, MRI has the major advantage of avoiding radiation exposure. Its sensitivity and specificity for detecting PPGL will be dependent on sequences obtained and expertise of the reporting radiologist. For MRI diffusion weighted imaging (DWI) sequences to compete with PET in terms of sensitivity and specificity they need to be able to identify small PPGLs at all the possible body sites in which they can occur and ideally metastatic deposits.

Method

Our SDHB surveillance screening scans include DWI sequences. We reviewed 18 patients with 28 SDHB-related tumours. All these scans had already been comprehensively double reported. We presented the scans to a radiologist, expert in reporting PPGL screening scans, without specifying the location of the tumour (if present) and asked them to use DWI sequences to guide a more focused review. If DWI identified an area of interest, this was then compared with the standard MRI sequences. The site and size of the tumour was recorded, as well as the b values and ADC values for the sequences obtained.

Results

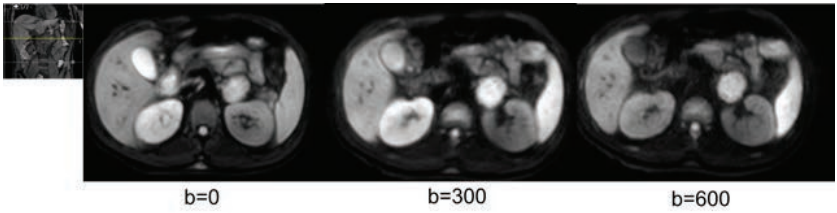
The DWI sequence identified all 28 tumours. Many false positive areas of focus were noted and excluded. The tumours detected by DWI ranged in size from 5-52mm. Primaries were identified on DWI in the abdomen (n=13), adrenal gland (n=1), thorax (n=3), neck (n=2), and bladder (n=2). Additionally one SDHB-related GIST and two SDH-related RCCs were also highlighted by DWI, as were metastatic deposits in the liver and bone. The smallest metastatic deposits visualised were 5mm. In addition we will present examples of MR images that demonstrate the value of DWI sequences.

Conclusion

We would recommend, for units not already adding DWI sequences to their MRI screening scans, that these sequences are considered so that a wider experience can be reported on the sensitivity and specificity of the size and location of tumours and metastatic deposits that can be

identified. If the sensitivity is confirmed in a larger series and for all SDH subunits, this will provide added reassurance about identifying small SDH-related tumours, without the consequences of exposing the patients to radiation based imaging techniques.

Figure 1: An example MR imaging panels showing an 18-year-old male with a 46mm phaeochromocytoma clearly visible. The panel shows an example of the different characteristics of the phaeochromocytoma across different b-values.



PO38 - DIAGNOSTIC ACCURACY OF CT IMAGING TO EXCLUDE PHEOCHROMOCYTOMA: A SYSTEMATIC REVIEW, META-ANALYSIS AND COST ANALYSIS

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Introduction: It is usually recommended to measure metanephrines in case of an incidentally discovered adrenal tumor, in order to rule out a pheochromocytoma as underlying etiology. It has been suggested that measurement of metanephrines could be obviated in case of an adrenal tumor with an attenuation value ≤10 Hounsfield Units (HU) on unenhanced CT. In order to assess the potential clinical applicability of this alternative diagnostic strategy we performed a meta-analysis on the diagnostic accuracy of the 10 HU threshold and estimated the associated reduction in diagnostic costs.

Methods: MEDLINE and EMBASE were searched and studies reporting the proportion of pheochromocytomas on either side of the 10 HU threshold at unenhanced CT were included. The pooled proportion of patients with attenuation >10 HU was determined as well as the modelled financial costs of the current and alternative diagnostic approach.

Results: 2636 studies were identified. 30 studies were included reporting on a total of 1191 pheochromocytomas. The overall risk of bias and

concern for applicability were considered to be low for the majority of the included studies according to the QUADAS-2 tool. Heterogeneity was not observed between studies (Q=11.7, P=0.99, I2 = 0.0%). The pooled proportion of patients with attenuation >10 HU was 0.99% (95% CI: 0.984-0.995). Using the new diagnostic approach, determination of metanephrines can be obviated in 69% (SEM 10%) of patients with an adrenal incidentaloma, which could save an estimated €74 ± 11 on a per patient basis.
Conclusion: Pheochromocytomas can be reliably ruled out in case of an adrenal lesion with an unenhanced CT attenuation value ≤10 HU. Therefore, determination of metanephrines could be restricted to adrenal tumors demonstrating an unenhanced CT attenuation value >10 HU. Implementing this novel diagnostic strategy is likely to be more patient friendly as well as cost-saving.

PO39 - PREVALENCE OF AN ASSOCIATED TUMOR IN PATIENTS AFFECTED BY NON-SYNDROMIC PHEOCHROMOCYTOMA/ PARAGANGLIOMA

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Background. Pheochromocytoma/Paraganglioma (PCC/PGL) is a rare tumor, genetically inherited in up to 35% of cases. Recently, the

occurrence of other tumors in patients affected by PCC/PGL due to SDHxmutations has attracted interest. Design. The aim of the present study was to evaluate if patients affected by non-syndromic PCC/PGL have an increased risk to develop an associated tumor. Data were collected retrospectively in 10 Italian centers. Methods. Patients affected by VHL, MEN2 and NF1 were excluded. We analyzed data of 652 patients, 291 M (44.6%), with a median age at diagnosis of 50 years [37-61] and a median follow-up of 44.50 months [12-101.75]. The genetic analysis was performed in 466 patients. 138 (29.6%) had a mutation in the following genes: 71 in SDHD (51.4%), 34 in SDHB (24.7%), 12 in MAX (8.7%), 9 in TMEM127 (6.6%), 7 in SDHC (5.0%), 4 in SDHA (2.9%) and 1 in SDHAF2 (0.8%), respectively. Results. We investigated if there were differences regarding results of genetic analysis and positive family cancer history. Smoking, alcohol intake and toxic exposure have been evaluated as possible risk factors for associated tumors.Eighty-four out of the 118 patients with an associated neoplasia were genotyped and 17 (14.4%) were mutated: 6 in SDHD (7.14%), 4 in TMEN127 (4.8%), 3 in MAX (3.6%), 2 in SDHB (2.4%), 1 in SDHC (1.19%) and 1 in SDHA (1.19%), respectively. Seventy-six (64.4%) out of 118 second tumors were malignant. Mutated patients showed the same risk of developing another tumor compared with patients without pathogenetic variants. A positive family cancer history was found in 44.9% (53/118) of patients presenting an associated tumor vs. 32.3% (173/534) of patients with a negative history (p=0.007). No differences were found in terms of smoking, alcohol intake and toxic exposure. Conclusions. This study is still ongoing. We are currently comparing our data with those of the general population (AIRTUM database). The results of this study could influence the clinical management of patients affected by non-syndromicPCC/PGL.

PO40 - POTENTIAL PROGNOSTIC BIOMARKER OF METASTATIC DISEASE IN SDHB RELATED PARAGANGLIOMA AND PHEOCHROMOCYTOMA

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Paragangliomas and pheochromocytomas (PPGLs) are rare neuroendocrine neoplasia whose clinical spectrum ranges from benign, in most cases, to progression toward a metastatic disease. This clinical diversity is not fully explained by known genetic aberrations; germline mutations in the SDHB gene (coding for the core subunit of the succinate dehydrogenase complex), which leads to succinate accumulation, is the only known trait that predispose to metastasis. However, there are not reliable biomarkers to identify SDHB-carriers that are prone to develop metastasis. Thus, there is a critical need to develop biomarkers to identify patients at high-risk of metastatic disease.

We aimed at identifying epigenetic biomarkers of metastatic disease. Genome-wide profiling of DNA methylation in diverse clinical and genetic PPGLs subtypes revealed that metastatic SDHB-PPGLs carry long-range de novo methylation of the PCDHA, PCDHB and PCDHG gene clusters (PCDHs) which is not present in benign SDHB-PPGLs. High levels of PCDHGC3 promoter methylation were validated in primary metastatic SDHB-PPGLs, it was found amplified in the corresponding metastases and it was significantly correlated with PCDHGC3 reduced expression. Moreover, hypermethylation of PCDHGC3 correlated with shorter overall survival in metastatic PPGL patients.

In conclusion, we provide first evidences that PCDHGC3 could be a promising diagnostic factor of malignancy, a prognostic marker for metastatic PPGLs and could also be a useful target for therapy of metastatic PPGLs.

PO41 - POSITIVE IMPACT OF GENETIC TEST ON THE MANAGEMENT AND OUTCOME OF PATIENTS WITH PARAGANGLIOMA AND/OR PHEOCHROMOCYTOMA

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Context

Paragangliomas and pheochromocytomas (PPGL) are characterized by a strong genetic component with up to 40 % of patients carrying a germline mutation in a PPGL susceptibility gene. The current clinical practice guidelines endorsed by the Endocrine Society and the European Society of Endocrinology both recommend that genetic screening should be proposed to all patients with PPGL. However, it has never been shown whether the identification of a germline mutation in one PPGL susceptibility gene changes the outcome of mutation-carriers.

Our objective was to evaluate how a positive genetic test impacts the management and outcome of propositus which carrying a germline mutation in one of the four major PPGL susceptibility genes (SDHB, SDHD, VHL and SDHC).

Methods and Patients

We performed a multicentric retrospective study on 221 propositus carrying an SDHB, SDHD, SDHC or VHL germline mutation. Patients were divided into two groups:

- Genetic patients, who were informed of their genetic status within the year following the first PPGL diagnosis, and whose mean follow up was 7 years (range 1-17),
- Historic patients who only benefited from the genetic test at least seven years after initial PPGL diagnosis.

Results

Compared to Historic patients, Genetic patients had a better follow-up, with a higher number of examinations and a reduced number of patients lost to follow-up (9.6% versus 72 %, $p<0.0001$). During follow-up, smaller (18.7 mm versus 27.6, $p=0.0128$) new PPGL and metastases as well as lower metastatic spread were observed in Genetic patients. Importantly, these differences were reversed in the Historic cohort after genetic testing. Genetic patients who developed metachronous metastases had a better 5-year survival than Historic ones ($p=0.0127$).

Conclusion

Altogether our data suggest that the early knowledge of the genetic status has a positive impact on the management and on the clinical outcome of the patients with a germline SDHx or VHL mutation and therefore, strengthen the international guidelines recommending the practice of the genetic test at the time of PPGL diagnosis.

PO42 - REGULATION OF CATECHOLAMINE BIOSYNTHESIS UNDER HYPOXIA AND PSEUDOHYPOXIA IN PHEOCHROMOCYTOMAS AND PARAGANGLIOMAS

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Pheochromocytomas and paragangliomas (PPGLs) are rare catecholamine-producing neural crest-derived tumors with variable aggressiveness. PPGLs with an activation in pseudohypoxic pathways (cluster 1) are associated with an immature catecholamine phenotype and carry a higher risk for metastatic spread, in comparison with cluster 2 PPGLs with an activation of kinase-signaling pathways. Our present study investigates the role of hypoxia and pseudohypoxia on catecholamine biosynthesis in PPGLs.

Pheochromocytoma cell lines naturally lacking Hif2α (MPC and MTT) or expressing both Hif1α and Hif2α (PC12) were cultivated under extrinsic hypoxia (1% O2) or in spheroid culture (intrinsic hypoxia) and cellular catecholamines were analyzed. Cultivation under spheroid conditions as well as extrinsic hypoxia enhanced cellular levels of dopamine (DA) in Hif2α deficient MPC and MTT cells. PC12 cells were not able to form spheroids, but also showed elevated DA under extrinsic hypoxia. In all cell lines, norepinephrine (NE) was mildly increased after exposure to hypoxia. To distinguish between Hif1α and Hif2α driven effects we re-expressed Hif2α in MTT cells (MTT HIF2α). MTT HIF2α and respective control showed an up-regulation of DA and NE under extrinsic and intrinsic hypoxia, correlating with an increased expression level of tyrosine hydroxylase (Th).

Pseudohypoxic conditions, simulated by the re-expression of Hif2α under normoxia did not influence cellular DA and NE levels. Patient tumors carrying a somatic gain-of-function mutation in HIF2α (n=5) showed decreased HIF1α gene expression in comparison to other cluster 1 (5 SDHB, 3 SDHD, 5 VHL) and cluster 2 (4 NF1, 7 MEN2) PPGLs. Elevated expression of HIF2α in all cluster 1 tumors compared to cluster 2 confirms previous results in the present cohort. All three groups (HIF2α vs. cluster 1 vs. 2) showed a similar TH expression, but a significant correlation ($p = 0.0027$) between HIF2α and TH expression could be observed, indicating a regulation of TH via HIF2α under continuous stabilization of HIF2α (pseudohypoxia).

In conclusion, catecholamine biosynthesis mediated through increased expression of the rate-limiting enzyme TH is elevated by extrinsic and

intrinsic hypoxia and regulated by HIF1α in a short-term response. In contrast, continuous activation of hypoxia-related genes under pseudohypoxia leads to a HIF2α-mediated impact on TH expression (long-term response).

PO43 - COMPARISON OF MOLECULAR CHARACTERISTICS BETWEEN SDHB AND VHL MUTATED PPGLS

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Recent studies have revealed that pheochromocytomas and paragangliomas (PPGLs) can generally be divided into two major clusters: Cluster 1 includes the tumors with mutations in the VHL gene and those in the subunits of the succinate dehydrogenase (SDHx) that lead to dysregulation of Krebs cycle and activation of hypoxia signaling pathway. However, it is known that only SDHB mutations are associated with malignant tumor behavior. Therefore, the comparison of molecular mechanisms between SDHB and VHL mutated PPGLs might provide insights into the pathophysiology of malignant PPGLs.

Immunohistochemistry (IHC) of SDHA and SDHB, which is recommended for the diagnosis of SDHx mutations in PPGLs in combination with digital image analysis was performed in human PPGL samples (n=193). Staining intensities of SDHA in SDHB mutated tumor (n=15) was significantly higher in comparison to those with VHL mutations (n=18, $p<0.01$). To explore SDHA expression levels at the molecular level in vitro, knockdown of Sdhb and Vhl genes were achieved in rat pheochromocytoma (PC12)

cells. Both, knockdown of Sdhb or Vhl resulted in maintained Sdha protein expression, while higher expression was not observed in the context of Sdhb knockdown. Vhl knockdown further induced spindle-shaped morphology of PC12 cells and apoptosis via activation of caspase-3/7 at serum starved conditions. In addition, Vhl knockdown suppressed mTOR pathway activation and resulted in upregulation of protein levels of Bnip3. Conversely, combined knockdown of Vhl and Bnip3 rescued PC12 cells from apoptosis. Based on these findings, expression of Bnip3 may contribute to benign features of cells with inactivating Vhl mutations. In addition, knockdown of Sdhb and Vhl gene affected mitochondrial morphology of PC12 cells as quantified by increased mitochondrial fusion. Because inhibition of mitochondrial fusion through knockdown of Mfn1 gene decreased cell numbers in Vhl knockdown cells, mitochondrial fusion might contribute to cell survival in the context of inactivated Vhl gene. In summary, we demonstrate differences in the molecular characteristics between SDHB and VHL mutated PPGLs at the level of in vitro and IHC analysis, which may contribute to understandings of the malignant behavior of PPGLs.

PO44 - LAPAROSCOPIC AND ROBOTIC ASSISTED PARTIAL ADRENALECTOMY IN THE TREATMENT OF PHEOCHROMOCYTOMA: SYSTEMATIC REVIEW

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Laparoscopic and robotic assisted partial adrenalectomy in the treatment of pheochromocytoma: Systematic Review

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Traditionally total adrenalectomy has been the standard procedure for the treatment of adrenal tumors irrespective of size and location. However, recently, there is growing interest for the performance of partial adrenalectomy in order to avoid the side effects associated with the need for lifelong exogenous steroid replacement. Aims: This systematic review aimed to investigate the role of laparoscopic and robotic assisted partial adrenalectomy in the treatment of pheochromocytoma.

Methods: Electronic databases were searched with the appropriate search terms for the time period up to and including December 2017. Full publications including clinical trials randomized or not, prospective studies, retrospective studies, case series, case reports, that provided relevant data, met inclusion criteria.

Results: Seventy five possibly relevant studies were identified. Abstracts

were reviewed and finally fourty one studies were retrieved in full text and were included in the systematic review. The majority of the data came form retrospective studies, case series and case reports. Partial adrenalectomy has been described in both hereditary and sporadic pheochromocytoma. The use of partial adrenalectomy is highly debated in the case of unilateral pheochromocytoma due to the difficulty in excluding malignancy. The majority of literature data focus on herediatry pheochromocytoma, especially patients with RET ot VHL mutations. The low risk of malignancy and high risk of bilateral tumors are obvious in the above genetic syndromes especially in MEN2. Recurrence rate is estimated at about 10% for pheochromocytoma. Overall steroid dependence rate is estimated at 90%. Long term follow up of the patients has not been standardized. The surgical technique has not been standardized and open questions remain regarding the tumor margin, the adrenal vein preservation, the means of hemostasis.
Conclusion: Laparoscopic and robotic assisted partial adrenalectomy is an option especially for patients with bilateral pheochromocytomas. However, open questions remain regarding the indications in the case of unilateral pheochromocytoma, In addition standardization of the technique is needed.

PO45 - IMPAIRED THROMBIN GENERATION IN PATIENTS WITH PHEOCHROMOCYTOMA

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Objective: The aim of the ongoing study is to investigate the impact of excess of catecholamines on plasma fibrin clot properties, thrombin generation and fibrinolytic activity in patients with arterial hypertension.
Design and methods: In our ongoing project we evaluated 11 patients with pheochromocytoma and 15 age, gender, body mass index, glycemic status, blood pressure levels and number of medication ($p>0.05$) matched patients with essential hypertension (EHT) (Table 1). The assessment of secondary causes of hypertension was performed according to the current guidelines.
Fibrin clot properties including clot permeability (Ks), clot lysis time (CLT), and turbidimetric parameters of clot formation were determined.
Plasma thrombogenic potential was assessed using calibrated automated thrombograph (CAT) and following parameters have been determined: (1) lag time - time from the beginning of the reaction to the beginning of thrombin generation, (2) peak height -maximum amount of thrombin, (3) time to peak (ttpeak) - time from the beginning of the reaction to

peak height and (4) endogenous thrombin potential (ETP) - area under the thrombin-generation curve, reflecting the total amount of thrombin generated during the test.

Hemostatic markers such as plasminogen activator inhibitor (PAI-1), an activity of thrombin activatable fibrinolysis inhibitor (TAFI), tissue plasminogen activator (tPA), prothrombin fragment 1 + 2 (F1+2) and complexes of plasmin-alpha(2)-antiplasmin (PAP) were assessed with enzyme-linked immunosorbent assays (ELISAs)

The study is supported by the National Science Centre 2013/11/N/NZ5/03740.

Results:

Patient with pheochromocytoma were characterized by impaired thrombin generation (lower ETP and lower thrombin peak) as compared to patients with EHT. There was no significant differences between patients with pheochromocytoma and EHT in regards to fibrin clot properties, nor in the other assessed hemostatic parameters (Table 1).

Table 1. Plasma fibrin clot properties and thrombin generation parameters in hypertensive patients with pheochromocytoma and patients with essential hypertension

	Pheochromocytoma n=11	Essential hypertension n=15	P
Age, ys	46.7±14.02	46.5±17.7	0.98
Women, n(%)	5(45.5)	8(53.3)	0.69
Ks, 10^{-9}cm^2	5.31±1.48	4.69±1.15	0.26
CLT, min	112.7±29.9	122.4±21.9	0.38
Lagtime, min	3.46±0.83	3.25±0.78	0.54
ETP, nM•min	1767.15±457.86	2345.56±238.14	**0.002**
Thrombin Peak, nM	269.38±55.83	373.77±68.38	**<0.001**
ttpeak, min	7.03±1.35	6.5±1.19	0.31

Ks – clot permeability, CLT-clot lysis time, ETP-endogenous thrombin potential, ttpeak – time to peak

Ks – clot permeability, CLT-clot lysis time, ETP-endogenous thrombin potential, ttpeak – time to peak

Conclusions: The very preliminary results of the ongoing study may suggest that patients with pheochromocytoma are characterized by impaired thrombin generation as compared to matched patients with essential hypertension.

PO46 - A RARE CASE OF HYPERTROPHIC CARDIOMYOPATHY INDUCED BY CATECHOLAMINE-PRODUCING TUMOR

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Background: Hypertrophic cardiomyopathy (HCM) can be inherited or secondary to uncontrolled hypertension or metabolic changes. Pheochromocytoma (PHEO) is rare cause of secondary hypertension (<2%), but characterized by severe effects on the cardiovascular system, as well as many types of cardiomyopathies, including peri-partum, hypertrophic, dilated and Takotsubo. HCM associated with PHEO can be reversible after removal of catecholamine-secreting tumor.

Case presentation: A 37-year old woman presented to our Centre with an history of chest pain, palpitations and paroxysmal hypertension. ECG showed left ventricular hypertrophy (LVH); subsequent transthoracic echocardiogram and cardiac magnetic resonance confirmed a severe LVH with a prevalent involvement of the anterior portion of interventricular septum. A diagnosis of HCM was made and confirmed by endomyocardial biopsy (Figure 1A). Repeated hormone investigations showed high values of 24-h urinary metanephrines (396 and 421 μg/24h; n.v. <350 μg/24h). Abdominal computed tomography scan showed an enlarged left adrenal gland with concordant strong uptake of 123I–metaiodobenzylguanidine (MIBG) at the scintigraphy. The patient received a diagnosis of "Pheochromocytoma complicated by catecholamines–induced hypertrophic cardiomyopathy"; so, patient underwent to surgically removal of adrenal tumor. Unexpectedly, the histological examination showed an adrenal adenoma mixed with nodules enriched in epinephrine–types secretory granules at electron microscopy (Figure 1B). At six-months follow-up examination, the patient's 24-h urinary metanephrines levels were normalized and the transthoracic echocardiogram showed a reduction of LVH indexes.

Discussion: Catecholamine-induced cardiomyopathy is defined by a reversible left ventricular dysfunction without coronary artery stenosis. Treatment of PHEO-associated cardiomyopathy consists in curative surgical resection of catecholamine-producing tumor. This is a unique case of a catecholamine-induced HCM caused by a cortical adrenal adenoma with epinephrine-neurosecretory granules inside. The presence of these metabolites, stored into nodules enriched in multiple secretory granules, explains the strong uptake of the left adrenal lesion at 123I-MIBG scintigraphy. After the appropriate excision of PHEO, cardiomyopathy

may definitely reverse.

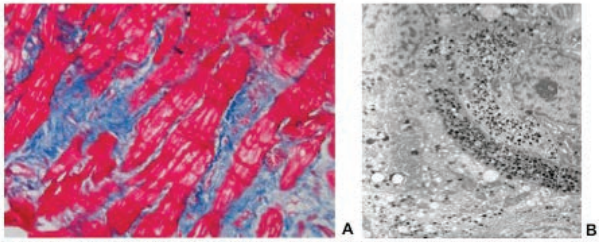

Figure 1A: Microscopic findings of biopsy specimen showing hypertrophic fiber, contraction bands and fibrosis. **1B:** Electron microscopy shows an adrenal adenoma mixed with nodules enriched in epinephrine-types secretory granules.

Conclusions: The diagnosis of uncommon case of PHEO in HCM has been very important to avoid future cardiovascular complications.

PO47 - EVIDENCE FOR THE EFFICACY OF COMBINATION TARGETED THERAPY AND A ROLE FOR GSK3 BLOCKADE IN MURINE PHAEOCHROMOCYTOMA CELL LINES AND HUMAN PHAEOCHROMOCYTOMA PRIMARY CULTURE

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Background

Treatment options for metastatic phaeochromocytomas/paragangliomas (PCC/PGL) are limited, and progression after the use of radionuclides (MIBG and PRRT) or chemotherapy is frequent. We have, therefore, investigated novel molecular-targeted therapy options alone and in combination in two murine PCC cell lines and 4 different human primary cultures in order to explore new therapeutic possibilities.

Methods

Two mouse PCC cell lines - MPC (more benign) and MTT (more aggressive) - generated from heterozygous Nf1 knockout mice, and four different human primary PCC/PGL cultures from 3 different patients (uterus and lymph node metastases of a metastatic PGL plus two adrenal PCCs), were treated with sunitinib, everolimus, BYL719 (PI3Kainhibitor), cabozantinib (TKI), entinostat (HER2 antagonist), octreotide, temozolomide and niraparib (PARPi). Cell viability was assessed by the „Cell Titer Blue®" assay. Protein expression was measured by western blot technology.

Results

5 μM BYL719 and 10 nM everolimus alone moderately decreased MPC and MTT cell viability (BYL719 by 49%, everolimus by 25% and 30%, respectively) but the BYL719/everolimus combination synergistically reduced MPC cell viability (by 68%), was additive in MTT cells (cell viability reduction by 65%) and synergistically inhibited GSK3. We have found synergistic effects of 10 nM everolimus plus 2 μM sunitinib on MPC and MTT cell viability and on GSK3 inhibition: sunitinib alone reduced cell viability by 19% in MPC and MTT cells, while the sunitinib/everolimus combination decreased MPC and MTT cell viability by 53% and 51%, respectively. However, the combination of cabozantinib plus everolimus was not synergistic. We have confirmed the efficacy of sunitinib (1 μM-8 μM) and BYL719 (2,5 μM-20 μM) in human primary PCC/PGL cultures, and also verified in human primary culture the synergism of BYL719 plus everolimus. Furthermore, entinostat, cabozantinib and niraparib alone reduced cell viability in human primary cultures. In contrast, octreotide and temozolomide had no significant effects on cell viability.

Conclusions

We have shown the synergistic anti-tumour potential of BYL719/everolimus and of sunitinib/everolimus combination treatment in MPC/MTT cells and confirmed synergism of BYL719/everolimus in human primary cultures. Thus, molecular targeted agents in combination may offer a superior approach to treating metastatic PCC/PGL, and blockade of the GSK3 pathway is a novel therapeutic possibility.

PO48 - METHOD DEVELOPMENT FOR UNTARGETED HIGH-THROUGHPUT PLASMA NMR METABOLOMICS OF ADRENAL HYPERTENSION

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Nuclear Magnetic Resonance Spectroscopy (NMR) has proven to be a valuable tool in the discovery and quantitation of disease-related

biomarkers. In order to apply NMR as part of ENSAT-Hypertension, a multi-omics approach to discern different forms of adrenal hypertension, a reform of the traditionally used protocol was necessary to enable NMR metabolomics on a large set of plasma samples. The new protocol consists of a novel combination of a NMR experiment, a neglected internal standard for metabolite quantitation, and a data processing routine.

Plasma was collected from four patients, two of which suffered from primary aldosteronism (PA) and the other two from pheochromocytoma/paraganglioma (PPGL). PA was pooled and aliquoted separately from PPGL plasma resulting in the creation of two sets of 10 replicate samples. These were either subjected to the traditional ultrafiltration approach to remove proteins and lipids altogether, or left unfiltered for analysis with two established NMR pulse algorithms for removing only the signals originating from the macromolecules. The CPMG pulse algorithm suppresses signals based on relaxation time, whereas the LED based on diffusion rate. A new internal standard, Maleic Acid (MA), was added to the buffer solution for absolute quantitation of metabolites, and its peak compared to that of the commonly used internal standard,TSP, which is known to broaden in the presence of macromolecules, thus hindering quantitation. Spectra were recorded on a Bruker Avance III, operating at 500 MHz, and next underwent data reduction using the SPEAQ R package for peak picking and alignment. Results were assessed using the Bruker Topspin software for spectral inspection and the SIMCA-P software for Principal Component Analysis (PCA).

Macromolecule signals were adequately suppressed in spectra from both NMR pulse algorithms for signal filtering, indicating the feasibility of the approach. The MA peak was not broadened, in contrast to the traditional quantitation standard. PCA models show the metabolic signature of the LED method being closer than CPMG to the ultrafiltration. The LED approach was also able to successfully differentiate the groups of samples, with an adequately low within group variance and a high between group variance.

Overall, our results indicate that our high-throughput approach, with its speed, affordability and ease of use, is well suited for large scale metabolomics studies in group differentiation, sample stratification, and putative metabolite quantitation. The LED experiment was capable of suppressing protein and lipid signals, indicating its applicability in plasma metabolomics. The fact that the MA peak was not broadened, shows that it is a reliable standard for quantitation. The established method is expected to prove valuable in providing the means for upgrading the diagnosis of the different forms of adrenal hypertension.

PO49 - MOLECULAR EXPRESSION PROFILING SHOWS PSEUDOHYPOXIA PHENOTYPE, VASCULOGENESIS AND MITOCHONDRIAL DYSFUNCTION IN HEAD AND NECK PARAGANGLIOMAS

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The present study aimed to characterize the molecular expression profiles of head and neck paragangliomas. Of the 22 cases investigated 10 carried germline class 3 to 5 sequence variants of the SDHA/B/C/D/AF2and TMEM127genes, 12 were non-carriers. Firstly, 14 HN-PGLs and 3 pools of 10 Jacobson's nerves were analysed by gene expression microarray analysis.Pathway enrichment (IPA) revealed oxidative phosphorylation and mitochondrial dysfunction as top canonical pathways enriched in paragangliomas, with 13 and 15 upregulated genes respectively. These genes encoded proteins mainly localized in complex I of the mitochondrial electron transport chain. Upstream regulator analysis highlighted activation of a hypoxia transcriptional program peculiar to the carotid body. RNA expression and/or immunohistochemistry analysis of matched samples confirmed the most important hits modulated in paragangliomas (DLK1, NDUFA4L2, ADM, RGS4 and RGS5). High expression of the protein products of these genes was independently confirmed by immunofluorescence, immunohistochemistry and western blot in an another group of head and neck paragangliomas and in 2 out of 5 paraganglioma-derived cell cultures. In conclusion, DLK1, NDUFA4L2, ADM, RGS4 and RGS5 are expressed in both SDHx-related and unrelated paragangliomas. The EGF-like transmembrane protein DLK1 is a regulator of cell growth and differentiation widely expressed during

the fetal period in the adrenal medulla and in the organ of Zuckerkandl (1). NDUFA4L2 links the pseudohypoxia phenotype to mitochondrial complex I dysfunction, whereas RGS4 and RGS5 are G-protein signaling regulators of vascular mural cells (2). Our data suggest that pseudohypoxia, mitochondrial dysfunction and aberrant vasculogenesis are constitutive features of paragangliomas.

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2) Manzur M, Hamzah J, Ganss R. Cancer Res. 2009 Jan 15;69(2):396-9. doi: 10.1158/0008-5472.CAN-08-2842.

PO50 - INCREASED IMMUNOEXPRESSION OF CD34 IN VON HIPPEL-LINDAU DISEASE-ASSOCIATED PHEOCROMOCYTOMAS

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Introduction: Von Hippel-Lindau (VHL) disease is a rare syndrome with an autosomal dominant inheritance, characterized by predisposition to multiple tumors due to mutations in the VHL gene, including pheochromocytomas (PHEO). The VHL protein resulting from mutated gene impairs the correct metabolization of hypoxia-inducible factor by proteasomes, resulting in a pseudo-hypoxia state, activating genes related to the angiogenesis. VHL-PHEO compared to sporadic cases are usually diagnosed at an earlier age, are more frequently multiple, secreting mainly noradrenaline and presenting low risk of malignancy. Objective: To correlate clinical and biomarker characteristics of PHEO in the VHL syndrome and to establish a comparison to sporadic cases of PHEO. Material and method: Clinical and laboratory data from 9 patients, 6 of them with sporadic PHEO and the other 3 patients with VHL-PHEO, were collected from medical records. Microscopic characteristics of the tumors and PASS criteria were evaluated in histological sections in the paraffin blocks of surgically removed PHEO. Immunohistochemical analysis was performed to confirm the diagnoses of PHEO (chromogranin A) and to evaluate apoptosis, cellular proliferation index and angiogenesis (caspase-3, Ki-67 and CD34 antibodies, respectively). Results: At the time of the diagnoses, the mean age of the patients was lower in the VHL group than in sporadic PHEO (Table 1). The classical triad of symptoms of PHEO (sweeting, palpitation and headache) was present only in sporadic cases and these patients exhibited higher urinary metanephrine values. None of the PHEO-VHL patients presented elevated metanephrine or catecholamines excretion in urine. The tumor size was smaller in VHL group than sporadic PHEO. Only one patient with VHL-PHEO presented

bilateral tumor. All cases of PHEO presented PASS criteria lower than 4. PHEO found in VHL syndrome exhibited significant increased vascular density as assessed by CD34 immunostaining and decreased expression of caspase-3 proteins in comparison to PHEO sporadic cases (Figure 1). There were no statistical differences of Ki-67 in both groups. Conclusion: PHEO in patients with VHL disease presented high level of vascularization with low apoptosis rate. There are no reports in the literature evaluating the immunoexpression of such proteins comparing sporadic and VHL-PHEO. The analysis of the vascular density with immunohistochemistry can contribute for the identification of VHL-PHEO in early stages of the disease. This study will soon be extended by the addition of new patients with both PHEO-VHL and PHEO sporadic cases resulting from multicenter collaboration. The authors declare no conflicts of interest.

Table 1. Comparison of clinical, biochemical and tumor characteristics between VHL and sporadic PHEO.

	Sporadic (n=6)	VHL (n=3)
Gender (M/F)	3/3	3/0
Age at Diagnosis: Mean (95% IC)	57,5 (50 - 64)	34,6 (33 - 36) *
Classical triad of symptoms	5 (83,3%)	0
Chronic hypertension	6 (100%)	0
Paroxistic hypertension	3 (37%)	0
Elevated urinary catecholamines	1 (16,6%)	0
Elevated urinary metanephrines	6 (100%)	0
Unilateral tumor	6 (100%)	2 (66,6%)
Bilateral tumor	0	1 (33,3%)
Extra adrenal	0	0
Largest tumor diameter (cm)	6,6 (5,8 - 8,0)	2,4 (0,8 - 4,3) #
PASS < 4 (benign)	6 (100%)	3 (100%)

*p=0,0003; #p=0,0007 (Student's t-test)

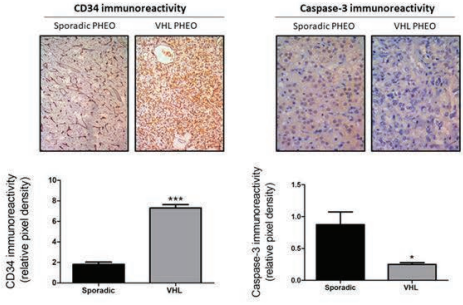

Figure 1. Tissue expression of CD34 and caspase-3 by immunohistochemistry. Representative images of VHL and sporadic PHEO (CD34 original magnification X 100; caspase-3 original magnification X 400; brown area, polymer peroxidase-based method). Each bar represents mean ± SEM of 3 stained tissue sections/tumor; Student's t-test.

PO51 - SYSTEMATIC REVIEW OF 20 YEARS OF GANGLIONEUROMAS

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Ganglioneuromas (GN) are rare, well-differentiated, usually benign chromaffin cell tumors arising from sympathetic ganglia. Information on GN is currently based on small case series and two larger studies (146 and 49 patients <26 years). Discrepancies in gender distribution, most frequent localization, median age of onset, and secretory behavior are evident.

We performed a systematic review of case reports and small series.

Articles listed in PubMed within the past 20 years in English concerning humans and containing “ganglioneuroma” were selected. Initial search generated 533 results. Upon careful review, 227 articles had to be excluded e.g. due to lack of relevant data or because the articles dealt with different tumor-types, leaving 305 articles representing 366 GN patients for evaluation.
Of the reported patients 61.7% were female. Median age at diagnosis was 28 years (0.16-93 years), with 35.5% of patients being younger than 18 years at diagnosis. On average, women were diagnosed 5.2 years earlier than men. GN were incidentally found in 24.3% of cases. There was no association between tumor size and presence of symptoms. Median tumor diameter was 6.0 cm (0.2-33.0 cm). I MIBG-scans were positive in 54.5% of cases. All MIBG-positive cases produced catecholamines and/or had a composite component.
Histologic examination revealed composite components in 17% of all GN. Hormone secretion was evident in 14% of cases (catecholamines 36/47, VIP 5/47, androgen 2/47, ACTH 1/47, ADH 1/47, prolactin (1/47), or gastrin (1/47). GN have been surgically removed in 93% of cases. Recurrence and metastatic spread were very rare.
To our knowledge, this comprehensive review summarizes characteristics of the largest number of GN patients over all age groups reported to date.

APA 52-56 POSTER

PO52 - CHARACTERIZATION OF THE GENETIC, CELLULAR AND MOLECULAR HETEROGENEITY IN ALDOSTERONE PRODUCING ADENOMA

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Background: Aldosterone-producing adenoma (APA) is the major cause of primary aldosteronism (PA), the most frequent form of secondary arterial hypertension. PA is characterized clinically by hypertension, often hypokalemia, increased plasma aldosterone and suppressed renin levels. Several recurrent somatic mutations in genes coding for ion channels (KCNJ5 and CACNA1D) and ATPases (ATP1A1 and ATP2B3) are found in APA. These mutations lead to increased intracellular calcium concentrations and activation of calcium signaling, resulting in increased CYP11B2 mRNA expression and aldosterone production. Previous data

support a two-hit model for APA development where the first hit drives increased cell proliferation while the second hit specifies the pattern of hormonal secretion.
Objective: To characterize the genetic, cellular and molecular heterogeneity of APA.
Results: Adrenal glands from 56 patients with APA were analyzed using DNA sequencing, histology and immunohistochemistry. Of 48 APA with DNA available, 32 carried recurrent mutations in KCNJ5, CACNA1D, ATP1A1 and ATP2B3. KCNJ5 mutations were more frequent in females and in young patients. Of the 48 samples, 16 were sequenced in a pilot study with a targeted NGS kit developed in the laboratory for sequencing all coding exons of all genes described in sporadic and familial PA. This technique allowed the identification of numerous additional variants, the rare and potentially deleterious variants of which were subsequently validated by Sanger sequencing. 56 APA exhibit heterogeneity in their cellular composition with an admixture of ZF-like cells and ZG-like cells of variable proportion. Distribution and intensity of immunostaining for aldosterone synthase and 11β-hydroxylase were variable in APA. APA carrying KCNJ5 mutations had a higher percentage of ZF-like and 11β-hydroxylase positive cells and lower percentage of aldosterone synthase positive cells. Multiplex immunofluorescent staining and image quantification is currently ongoing to allow correlation of genetic, histological and hormonal phenotypes.
Conclusion: APA in patients with PA show clear heterogeneity in cellular composition and aldosterone synthase and 11β-hydroxylase expression. Somatic mutations in driver genes for aldosterone production are more frequent than previously reported and correlated to peculiar molecular features.

PO53 - PARATHYROID HORMONE REGULATION BY ALDOSTERONE AND ANGIOTENSIN II: AN IN VIVO AND IN VITRO STUDY

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Context: Recent evidences suggest a pathophysiological link between the adrenocortical zona glomerulosa and the parathyroid glands, but the precise role role of aldosterone and angiotensin (Ang) II remains unknown.
Objectives: This study was designed to investigate: 1) the acute in vivo effect of Ang II on PTH secretion in patients with primary aldosteronism (PA) and essential hypertension (PH); 2) the in vitro effect of aldosterone and Ang II on PTH secretion in primary cultures of human parathyroid cells.

Design and setting: we investigated in vivo the acute effect of captopril (50 mg p.o.) on plasma PTH levels in 46 patients with PA, of which 27 with aldosterone producing adenoma (APA) before and after adrenalectomy, 19 with bilateral adrenal hyperplasia and in 73 patients with PH. For in vitro studies we used primary cultures of human parathyroid cells ex vivo, which were exposed to AngII [10-7M] and/or aldosterone [10-7M] in the presence and absence of specific antagonists.
Results: captopril lowered PTH levels (in ng/L) in PH patients (25.3±6.3 baseline vs 23.6±5.8 post captopril, p<0.0001) and in APA patients (26.3±11.6 vs 24.0±9.7 p=0.021) after PA correction with adrenalectomy, but had no effects in APA patients before adrenalectomy (34.6±14.2 vs 34.7±16.3 p=0.300) and in BAH patients (31.6±12.1 vs 30.3±12.2, p=0.337). In human parathyroid cells that maintained their ability to produce PTH in primary culture up to 7 days and express both the mineralocorticoid and the AT-1 receptor, aldosterone (+271±179% vs control, p<0.001) and angiotensin II (+267±226%, p=0.002) increased PTH secretion, albeit with no additive effect of the two stimuli. These secretagogue effects were abolished by canrenone and irbesartan, respectively.
Conclusion: The acute lowering of PTH after ACE inhibition in PH patients and in APA patients after cure of PA, along with the response of human primary parathyroid cells to Ang II and aldosterone, collectively implicate the renin-angiotensin-aldosterone system in the regulation of PTH secretion in humans

PO54 - CLASSIFICATION OF MICROADENOMAS IN PATIENTS WITH PRIMARY ALDOSTERONISM BY STEROID PROFILING

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Background: In primary aldosteronism (PA) the differentiation of unilateral aldosterone-producing adenomas (APA) from bilateral adrenal hyperplasia (BAH) is usually performed by adrenal venous sampling (AVS) or computed tomography (CT). CT often lacks the sensitivity to identify micro-APAs (adenoma diameter < 10 mm) that can be below the detection limit of CT. Our objectives were to establish if steroid profiling could identify patients with micro-APAs and determine if a diagnostic algorithm that integrates steroid profiling could minimize AVS procedures.
Methods: The study included patients with PA (n = 197) from Munich (n = 124) and Torino (n = 73) and comprised 33 patients with micro-APAs, 95 with macro-APAs, and 69 with BAH. Subtype differentiation was by AVS, and micro- and macro-APAs were classified according to pathology reports. Steroid concentrations in peripheral venous plasma were measured by liquid chromatography-tandem mass spectrometry.
Results: Steroid profiles of the micro-APA group were highly different to those of the macro-APA group. Lower concentrations of 18-hydroxycortisol (adjusted OR 0.36 per ng/mL, 95% CI 0.19-0.68, P = 0.001), pregnenolone (adjusted OR 0.67 per ng/mL, 95% CI 0.48-0.94, P = 0.019) and higher concentrations of dehydroepiandrosterone sulphate (adjusted OR 1.01 per ng/mL, 95% CI 1.01-1.02, P = 0.014) were associated with micro-APAs compared with macro-APAs. A random forest model classified micro-APA, macro-APA and BAH with 83.2% accuracy albeit with low specificity of 33.3% for micro-APA. A diagnostic algorithm was developed that integrated steroid profiling, CT scanning and AVS procedures limited to patients with discordant steroid and CT results. This would have increased the correct classification of micro-APAs to 67.9%, and improved the predictive accuracy of the 3 groups to 92.4% whilst reducing AVS procedures by 82.7%.
Conclusion: Steroid profiling of peripheral venous plasma has a potential utility for the selection of patients with micro-APAs in whom AVS should be considered mandatory.

PO55 - PRIMARY ALDOSTERONISM IS ASSOCIATED WITH DECREASED LDL AND HDL PARTICLE CONCENTRATIONS AND INCREASED GLYCA, A PRO-INFLAMMATORY GLYCOPROTEIN BIOMARKER

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Background: Primary aldosteronism (PA) may confer increased cardiovascular risk beyond effects on systemic blood pressure, but contributing mechanisms remain incompletely understood. We compared plasma (apo)lipoproteins and lipoprotein particle characteristics, GlycA, a pro-inflammatory glycoprotein biomarker of enhanced chronic inflammation, and plasma total branched chain amino acids (BCAA), measured using nuclear magnetic resonance (NMR) spectroscopy, between patients with PA, control subjects without hypertension, subjects with untreated hypertension and subjects with treated hypertension.

Methods: 20 PA patients were individually matched with 2819 control subjects without hypertension, 501 subjects with untreated hypertension and 878 subjects with treated hypertension participating in the PREVEND (Prevention of Renal and Vascular End-Stage Disease) cohort study with respect to age, sex, body mass index, smoking and statin use. The Vantera® Clinical Analyzer was used to determine NMR-based laboratory parameters.

Results: Total cholesterol, LDL cholesterol, HDL cholesterol, apolipoprotein (apo) B, apoA-I, LDL particle and HDL particle concentrations were all decreased in PA subjects vs. control subjects and subjects with untreated hypertension ($P<0.016$). Triglycerides and triglyceride-rich lipoprotein (TRL) concentrations were lower in PA subjects vs. subjects with (untreated) hypertension. GlycA was increased in PA vs. the three comparator groups ($P<0.016$). Total BCAA concentrations were unaltered in PA.

Conclusions: PA is associated with lower concentrations of LDL and HDL particles and to some extent also with lower TG and TRL particle concentrations. PA is also characterized by increased GlycA levels, indicating enhanced low grade chronic inflammation. Low HDL particle concentrations and increased GlycA could contribute to accelerated cardiovascular disease development in PA.

PO56 - IMMUNOHISTOPATHOLOGY AND STEROID PROFILES ASSOCIATED WITH BIOCHEMICAL OUTCOMES AFTER ADRENALECTOMY FOR UNILATERAL PRIMARY ALDOSTERONISM

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Unilateral primary aldosteronism (PA) is the most common surgically curable form of hypertension that must be accurately differentiated from bilateral PA for therapeutic management (surgical versus medical). Adrenalectomy results in biochemical cure (complete biochemical success) in almost all patients diagnosed with unilateral PA; the remaining patients with partial or absent biochemical success comprise those with persisting aldosteronism who were misdiagnosed as unilateral PA preoperatively. To identify determinants of postsurgical biochemical outcomes, we

compared the adrenal histopathology and the peripheral venous steroid profiles of patients with partial and absent or complete biochemical success after adrenalectomy for unilateral PA. A large multicentre cohort of adrenals from patients with absent and partial biochemical success (n=43) displayed a higher prevalence of hyperplasia (49% versus 21%, P=0.004) and a lower prevalence of solitary functional adenoma (44% versus 79%, P<0.001) compared with adrenals from age- and sex-matched patients with PA with complete biochemical success (n=52). We measured the peripheral plasma steroid concentrations in a subgroup of these patients (n=43) and in a group of patients with bilateral PA (n=27). Steroid profiling was associated with histopathological phenotypes (solitary functional adenoma, hyperplasia and aldosterone-producing cell clusters) and classified patients according to biochemical outcome or diagnosis of bilateral PA. If validated, peripheral venous steroid profiling may be a useful tool to guide the decision to perform surgery based on expectations of biochemical outcome after the procedure.

NAPACA 57-65 POSTER

PO57 - SAFETY AND EFFICACY OF LEVOKETOCONAZOLE IN CUSHING SYNDROME: INITIAL RESULTS FROM THE PHASE 3 SONICS STUDY

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Background: Levoketoconazole is a ketoconazole stereoisomer in clinical trials for endogenous Cushing syndrome (CS) treatment.

Methods: SONICS is a prospective, open-label, phase 3 maintenance-of-benefit study. Adults with confirmed CS and mean urinary free cortisol (mUFC) ≥1.5x the upper limit of normal (ULN) were treated in 3 phases: dose-titration (DT; goal of normalizing mUFC; 2-21 weeks), maintenance (M; 6 mo), and extended evaluation (6 mo; ongoing). Participants needed ≥2 adequate urine samples at each of baseline and end-of-maintenance (EoM). Primary endpoint was normalization of mUFC at EoM without a preceding dose increase during M; key secondary endpoints included cardiovascular risk markers. Prespecified adverse events (AEs) of

special interest were potential liver toxicity, QTc prolongation, and adrenal insufficiency (AI).

Results: Of 201 individuals screened, 94 enrolled and constituted the intention-to-treat primary endpoint analysis; 77 completed DT and advanced into M; 61 completed M. At baseline, median (range) age was 44 (18-75) years; 82% were female; 85% had pituitary CS and 9% had adrenal-dependent CS. Median (range) baseline UFC was 3x (1.2x-30x) ULN. Of those who advanced into M, 62 (81%) achieved mUFC normalization at end of DT. At EoM, 30% met the primary endpoint definition of mUFC normalization (95-percent CI, 21%-40%; P<0.025 vs null hypothesis of ≤20%). Including those who required M dose increase, 36/94 (38%; 95-percent CI, 28%-49%) normalized mUFC, and 45/94 (48%; 95-percent CI, 37%-58%) normalized or reduced mUFC by ≥50% from baseline. A total of 29/55 (53%) completing M with reported data had mUFC normalization at mo 6. Most (66%) M completers were receiving ≤600 mg/d. Fasting glucose, hemoglobin A1c, total and LDL-cholesterol, and weight decreased significantly (P<0.0001) from baseline. Four participants experienced a serious AE probably/definitely related to study drug (1 case of elevated liver function tests, 2 cases of prolonged QTc, and 1 case of AI). Overall, 3% had AI. No drug-related deaths occurred. AEs led to study drug discontinuation in 13% of subjects, 6% with liver-related AEs. Alanine aminotransferase was reversibly elevated >3x ULN in 11%, of which 3% were >5x ULN. QTc interval prolongation >500 msec occurred in 2%. The most common AEs, mostly mild in severity, were nausea (32%) and headache (28%).

Conclusions: In this large international prospective trial, levoketoconazole monotherapy normalized mUFC initially in 81% of CS patients who advanced into M. In ~30% of the intention-to-treat population, mUFC was normal without a preceding dose increase following 6 months of therapy, with a concomitant significant improvement in cardiovascular risk markers.

PO58 - CULLIN 3 IS A PARTNER OF ARMADILLO REPEAT CONTAINING 5 (ARMC5), THE PRODUCT OF THE GENE RESPONSIBLE FOR PRIMARY BILATERAL MACRONODULAR ADRENAL HYPERPLASIA

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Background: ARMC5 (Armadillo Repeat Containing 5) was identified as the gene responsible for PBMAH (Primary Bilateral Macronodular Adrenal Hyperplasia), with inactivating mutations reported in ~25% of PBMAH patients. The discovery of germline ARMC5 alterations established the first direct genetic link to PBMAH. Specific second events in tumors cause ARMC5 biallelic inactivation, leading to the classification of ARMC5 as a putative tumor suppressor gene. ARMC5 overexpression increased cell apoptosis but the mechanisms remain unknown. The structure of ARMC5 contains an ARM repeat and a BTB domain, important for protein-protein interactions, suggesting that the study of ARMC5 partners are important to understand its function. By co-immunoprecipitation coupled with mass spectrometry, we identified a potential interaction between ARMC5 and Cullin3 (CUL3), also suggested in online databases and Yeast-2-Hybrid assays. CUL3 is scaffold protein that mediates ubiquitination and subsequent degradation of specific substrates.
Methods: Therefore, our aim was to confirm the ARMC5/CUL3 interaction and investigate its roles. We performed immunoprecipitation experiments, bioluminescence resonance energy transfer (BRET) proximity assays and ubiquitination assays in order to investigate the interaction of ARMC5 with CUL3.
Results: ARMC5 co-immunoprecipitated with CUL3 and a hyperbolic BRET saturation curve was observed with YFP-CUL3 and ARMC5-Luc indicating a specific proximity between these proteins. We also observed that a missense mutation in the BTB domain (p.L754P) of ARMC5 disrupts the interaction with CUL3 and increases ARMC5 protein stability. Moreover, CUL3 increased ubiquitination of ARMC5, but did not modify the ubiquitination level of p.L754P ARMC5 mutant.
Conclusion: These data demonstrate that ARMC5 and CUL3 form a complex involving the BTB domain of ARMC5. Moreover, ARMC5 is ubiquitinated by the CUL3 complex and this mechanism of regulation can be altered by pathogenic ARMC5 mutations, suggesting new perspectives in the pathophysiology of PBMAH.

PO59 - WHOLE BLOOD METHYLOME ANALYSIS FOR THE IDENTIFICATION OF NOVEL BIOMARKERS OF CORTISOL EXCESS

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Cushing's syndrome and its associated comorbidities result from the chronic exposure to glucocorticoid excess. An accurate and early

identification is critical for effective surgical management and optimal prognosis. However, the current diagnostic approach based on hormonal assays not always allows a positive diagnosis, thus requiring novel, specific and easily measurable biomarkers of hypercortisolism. As already shown by several studies, stress-associated epigenetic marks can be measured at whole blood level analyzing the leukocyte DNA methylation profile.
Objectives: To analyze the leukocyte methylome of patients with Cushing's syndrome before and after hypercortisolism treatment in order to identify specific features related to DNA methylation.
Methods: Methylome analysis was performed on paired blood samples of twenty-five patients with diagnosed Cushing's syndrome, obtained by blood sampling before and several months after the treatment. Methylome data were generated by the Infinium®MethylationEPIC BeadChip chip and processed by using the GenomeStudio software version 2011.1 (Illumina,) in order to obtain the methylation level for the entire set of the analyzed CpGs. Array data analysis was performed using R version 3.4.4, by both unsupervised and supervised approaches.
Results: Unsupervised clustering of methylome data, carried out on the paired pre-/post-treatment samples of 8 out of 25 patients, showed a distribution of the 16 samples in 8 pairs, each corresponding to an individual. Moreover, we identified, in 7 out of 8 patients ($p = 0.035$), a methylation signature of hypercortisolism, corresponding to a differential methylation profile of a group of CpGs distributed along several regions of the genome.
A preliminary supervised comparison between pre- and post-treatment samples have been tested to identify the most discriminating probes allowing for predicting the group each sample belong to and to verify the hypercortisolism signature on the whole cohort: on 13 patients used as validation cohort, we obtained a good placement for 9 out 13 of the pre-treatment samples and for 8 out of 13 of the post-treatment ones.
Conclusions and perspectives: Our preliminary results show the existence of an epigenetic signature of hypercortisolism, detectable in whole blood samples of patients with Cushing's syndrome. A further optimization of the analysis method will allow us to more efficiently select the probes making the signature and to explore the genomic regions involved in such epigenetic changes as a consequence of excess of circulating cortisol. As perspective, it will be necessary to test the significant value of this signature on cases of moderate levels of hypercortisolism.

PO60 - MANAGEMENT OF SUBCLINICAL HYPERCORTISOLISM IN ADRENAL INCIDENTALOMA: AN ONGOING RANDOMIZED CLINICAL TRIAL

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Introduction. The adequate management of patients with unilateral adrenal incidentaloma (AI) and possible subclinical hypercortisolism (SH) is debated. A beneficial effect of adrenalectomy on cardiovascular risk factors in these patients have been suggested with a low-to-moderate quality evidence from heterogeneous studies. This randomized study was aimed to clarify the effects of adrenalectomy compared to conservative approach on the possible SH-related metabolic complications after a 24 months period.

Methods. We consecutively evaluated 394 AI patients (referred to 3 Italian Centres between June 2016 and June 2018). After exclusion criteria we have enrolled 18 SH patients (14 F; 64±7.4 yrs), without classical symptoms of overt hypercortisolism. SH was diagnosed in 18 patients on the basis of 1mg-dexamethasone suppression >1.8 μg/dl and low ACTH levels. Patients were randomized in two groups: surgery or conservative follow up (n=8:10 respectively). Urinary free cortisol, late night salivary cortisol, coagulation tests, and the presence of arterial hypertension (AH), type-2 diabetes (T2DM), dyslipidaemia (DL), psychiatric disorders (PD) and neurocognitive dysfunctions were evaluated at baseline, at 6 and 12 months after surgery or conservative follow-up. The evaluation of echocardiographic parameters, presence of vertebral fractures (VFx), osteoporosis (OP) and carotid atherosclerosis (CA), are scheduled at baseline and at 24 months.

Results. As expected, at baseline, age, diameter of the adenoma, parameters of adrenal function, and all the other parameters evaluated were comparable between the two groups. The overall prevalence of AH, OP, VFx, DL, T2DM, PD, CA were 66.7, 50, 27.8, 44.4, 16.7, 16.7, 11% respectively. So far, 13 patients have completed the 6 months (5 patients

operated and 8 patients followed up conservatively), 7 patients the 12 months and 2 patients the 24 months follow-up. In the operated group, 2/2 patients with AH showed an improvement in the blood pressure control after 6 months, that was maintained at 12 months. In 1 patient we observed a reduction of BMI>5% at the last available 6 months follow up. In the conservative group we did not observe any variation of these parameter or any worsening of cortisol secretion.
Conclusion: These preliminary data from a randomized study seems to confirm a beneficial role of adrenalectomy in SH hypertensive patients.
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PO61 - OVERNIGHT URINARY STEROID PROFILING: A PROPOSAL FOR A NEW TEST FOR THE DIAGNOSIS AND EVALUATION OF CUSHING'S SYNDROME

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BACKGROUND: Cushing's syndrome (CS) is caused by prolonged and inappropriate exposure to high levels of cortisol and is associated with significant morbidity and mortality. Twenty-four-hour urinary cortisol is one of the most useful tests to diagnose autonomous glucocorticoid secretion in CS although it has limitations, especially in "mild" and "subclinical" forms of cortisol excess. We hypothesized that given the diurnal rhythm of physiological cortisol secretion, the difference between autonomous and physiological glucocorticoid secretion should be higher at nighttime, hence overnight urine collections may provide improved detection of cortisol excess.
METHODS: Prospective collection of daytime and nighttime urines in patients with different degrees and aetiologies of cortisol excess and healthy controls. Urine samples were analysed by liquid chromatography-tandem mass spectrometry, quantifying 15 distinct adrenal steroids including glucocorticoid and androgens metabolites, with subsequent calculation of daytime, nighttime and 24-h urinary steroid excretion rates.
RESULTS: We included patients with overt CS (N=11), mild autonomous cortisol excess (MACE) in the context of adrenal incidentalomas (N=17), non-functioning adrenal incidentalomas (N=22), and healthy controls (N=28). Steroid excretion in controls reflected the diurnal pattern of adrenal steroid secretion, with lower nighttime than daytime excretion of glucocorticoid and adrenal androgen metabolites. Patients with overt CS showed significantly increased urinary excretion of cortisol

and its metabolites during both day- and nighttime; however, nighttime glucocorticoid excretion provided a better separation between patients and controls, increasing test specificity. While urinary cortisol excretion was of poor diagnostic value in MACE, both MACE and overt adrenal CS patients had significantly decreased nighttime androgen excretion in comparison to controls.
CONCLUSIONS: The secretion of urinary adrenal steroids in healthy subjects follows a circadian rhythm. Timed overnight urinary collection and subsequent steroid profiling indicates similar sensitivity and superior specificity of nighttime cortisol in comparison to 24-h cortisol. The simultaneous analysis of multiple adrenal steroids appears to be a promising tool for the stratification of patients with different degrees of cortisol excess and for the diagnosis and differential diagnosis of CS.

PO62 - ONSET OF AUTOIMMUNE DISEASES IN PATIENTS WITH CUSHING'S SYNDROME AFTER SUCCESSFULLY TREATMENT

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Introduction: Cushing's syndrome (CS) is a pathological condition characterized by chronic, excessive, and autonomous cortisol overproduction, associated with metabolic and cardiovascular complications, such as diabetes mellitus, metabolic syndrome, dyslipidemia, and hypertension.
It has also well established that plasma cortisol inhibits the effects of the immune system. As a result, patients with CS are protected from autoimmune and related diseases, but it is not known if the risk rises after their disease is resolved. Hence, nowadays, data about the occurrence of autoimmune diseases in CS is still lacking.
Aim: to evaluate the development of autoimmune diseases in patients with Cushing's syndrome diagnosis after successfully treatment.
Methods: we retrospectively analyzed data from 147 CS patients (91 with ACTH-independent and 54 with ACTH-dependent disease, 2 patients with ectopic ACTH production) referred to our ESH Excellence Hypertension Centre of University of Rome "Sapienza", Italy.
Results: 109 CS patients (74.1%) were surgically treated [67 ACTH-independent CS (61.5%) undergone to adrenalectomy, 42 ACTH-dependent CS (38.5%) to trans-sphenoidal surgery], and reviewed at 6, 12, and 24 months after clinical and biochemical remission of disease. In 9 (8.3%) patients (8 females and 1 male) of overall treated CS, during follow-up we observed the onset of one case of systemic lupus

erythematosus, one of rheumatoid arthritis, autoimmune thyroiditis (two cases of Basedow-Grave's disease and two with Hashimoto's thyroiditis), one patient with clinical characteritics of cutaneous psoriasis, one with myasthenia gravis, and, finally, one case presented giant-cell arteritis (Table 1). Furthermore, CS patients that developed autoimmune diseases after treatment, compared to the CS subgroup without autoimmune manifestations, showed at baseline, significantly higher levels of plasma cortisol (PC) and 24h-urinary free cortisol (UFC) (PC 459.5±160.3 vs 818.1±231.0 nmol/L, $p<0.001$; 24h-UFC 249.4±174.2 vs 514.5±173.8 nmol/24h, $p<0.001$), suggesting a probable immunomodulatory function of endogen cortisol overproduction on the onset of autoimmune complications.
Conclusions: our results demonstrate that patients with Cushing's syndrome after successfully treatment could develop autoimmune diseases and that increased plasma cortisol levels may contribute to more reactivity of the immune system. Therefore, after treatment, CS patients need to be strictly monitored in order to promptly diagnose the possible onset of autoimmune diseases.
Conflicts of interest: The authors report no relationships that could be construed as a conflict of interest.

PO63 - THE IMPACT OF AUTONOMOUS CORTISOL SECRETION ON BONE METABOLISM

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Background: Several studies reported on high prevalence of bone disease in patients with autonomous cortisol secretion (ACS), discordant to degree of bone density deterioration. Bone turnover marker measurement can potentially elucidate the underlying mechanism of ACS-related bone disease.
Objective: To describe the relationship between bone turnover markers in patients with adrenal adenomas and ACS.
Design: Prospective cohort study
Methods: Adults with adrenal adenoma and available biomaterial were enrolled over a 3.5 year period. Exclusion criteria were diagnosis of overt hormonal excess, malignancy, exogenous steroid use, treatment for osteoporosis. ACS was diagnosed based on DST cortisol (cortisol after overnight 1 mg dexamethasone) >1.8 mcg/dl. Osteocalcin, C-terminal telopeptide (CTX) (in all), and sclerostin (in 50% of cohort) were measured blinded to patient's diagnosis.
Results: One hundred ninety one patients (65% women) were diagnosed with adrenal adenoma (median tumor size 2.2 cm (0.5-14.4)) at a median

age of 59 years (28-93). ACS was diagnosed in 92 patients (DST cortisol of 3 mcg/dl (1.9-16)) and nonfunctioning adrenal tumor (NFAT) was diagnosed in 99 (DST cortisol of 1.2 mcg/dl (0.9-1.8). Prevalence of hypertension, diabetes mellitus, and dyslipidemia was similar between patients with ACS and NFAT (p=NS).
Bone disease was diagnosed in 42% and 23% of patients with ACS and NFAT (p=0.005) and included osteoporosis (13% vs 6%, p=0.1) and osteopenia (29% vs 17%, p=0.05), in ACS vs NFAT respectively. DST cortisol > 1.8 mcg/dl was a significant predictor of bone disease after adjusting for age and sex (OR 2.4, p=0.01).
Patients with ACS and NFAT demonstrated similar concentrations of osteocalcin and CTX. Sclerostin was significantly lower in patients with ACS (median 434 ng/L (111-1364) vs 589 ng/L (297-1273) in NFAT, p=0.006) in the univariate analysis, as well as when adjusted for age and sex (p=0.003). Age and sex adjusted sclerostin concentrations were inversely correlated with DST cortisol (p=0.04). Multivariate analysis (age, sex, BMI and sclerostin) identified sclerostin as a significant predictor of DST cortisol >1.8 mcg/dl (p=0.005). In addition, sclerostin was a significant predictor of bone disease in a multivariable model of age, sex, BMI, DST cortisol and sclerostin (OR 0.77 (CI95% 0.61-0.97) for each 100 ng/L of sclerostin increase, p=0.01)
Conclusion: DST cortisol >1.8 is a significant predictor of bone disease in patients with adrenal adenomas. Sclerostin was decreased in patients with ACS and predicted bone disease independently of DST cortisol. We conclude that even mild chronic hypercortisolism affects the osteocyte function, a possible underlying mechanism of bone disease in ACS. Further studies are needed to confirm these findings and to determine whether sclerostin can serve as a biomarker of the underlying bone disease related to ACS.

PO64 - THE DIAGNOSTIC EFFICACY AND USABILITY OF F-18 FDG PET-CT IN SCREENED PATIENTS WITH FUNCTIONAL AND NONFUNCTIONAL ADRENAL MASSES

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Backgrounds: Adrenal adenomas have been common clinical dilemma to evaluate functional or nonfunctional, benign or malign. We aim to determine if 18F-FDG PET/CT scan can differantiate functionality of adenomas and we compare SUVmax values of functional (Cushing Syndrome, Pheochromocytoma, Primary Hyperaldosteronism) and nonfunctional adrenal adenomas.
Methods: A study of PET CT reports over a 24 months period yielded 109 (73 females, 36 males, mean age 53.3± 10.02 year, range 24-70

year) patients who had been referred for 18F-FDG PET/CT imaging and for whom adrenal masses detected on CT or MRI imaging. All subjects underwent hormonal and biochemical analysis. Patients were analysed by a consensus of one experienced nuclear medicine physicians who were unaware of the patient data, except for side of the lesion. Adrenal lesions were objectively analyzed by measurement of the calculated standardized uptake value (SUV).
Results: Mean mass diameter of all the patients (n=109) was determined 2.1±4.3 (range 1-6.5 cm). Patients with cortisol secreting masses (n=4) had higher SUVmax of 10.1 compared to non-functional adenomas (n=96, average SUVmax 3.2) ,patients with pheochromcytomas (n=4, average SUVmax 8.7) and aldosterone secreting adenomas (n=1, SUVmax 3.30). A SUVmax cut-off of 4.135 demonstrated a sensitivity of %84.6 and specificity of 75.6% in identifying a cortisol-secreting mass.
Conclusions: In our study various FDG uptake was observed in patients with non-functioning, cortisol secreting adenomas or pheochromcytomas. The maximum SUVmax value, which is an index used to asses disease activity in FDG-PET imaging, was unexpectedly increased in cortisol secreting adrenal masses. 18F-FDG imaging may be practical technique to lateralize hormone secreting adenomas.

PO65 - THE IMPACT OF CORTISOL ON PLASMA FIBRIN CLOT PROPERTIES, THROMBIN GENERATION AND FIBRINOLYTIC ACTIVITY IN PATIENTS WITH CUSHING'S SYNDROME

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Objective: The aim of the ongoing study is to investigate the impact of excess of cortisol on plasma fibrin clot properties, thrombin generation and fibrinolytic activity in patients with Cushing's syndrome (CS)
Design and methods: In our ongoing project we evaluated 18 hypertensive patients with CS and 32 age, gender, body mass index, glycemic status, blood pressure levels and number of medication ($p>0.05$) matched patients with essential hypertension (EHT) (Table 1).
Fibrin clot properties including clot permeability (Ks), clot lysis time (CLT), and turbidimetric parameters of clot formation (the lag phase of fibrin formation and maximum absorbancy (ΔAbsmax) at 405 nm) were determined.
Plasma thrombogenic potential was assessed using calibrated automated thrombograph (CAT) and following parameters have been determined: (1) lag time - time from the beginning of the reaction to the beginning

of thrombin generation, (2) peak height -maximum amount of thrombin, (3) time to peak (ttpeak) - time from the beginning of the reaction to peak height and (4) endogenous thrombin potential (ETP) - area under the thrombin-generation curve, reflecting the total amount of thrombin generated during the test.
Hemostatic markers such as plasminogen activator inhibitor (PAI-1), an activity of thrombin activatable fibrinolysis inhibitor (TAFI), tissue plasminogen activator (tPA), prothrombin fragment 1 + 2 (F1+2) and complexes of plasmin-alpha(2)-antiplasmin (PAP) were assessed with enzyme-linked immunosorbent assays (ELISAs)
The study is supported by Institute of Cardiology, Warsaw, Poland (2.69/VII/16)
Results:
Patients with CS were characterized by less compact fibrin clot structure (higher Ks) as well as impaired thrombin generation (lower ETP and longer ttpeak) as compared to patients with EHT. There was no significant differences between patients with CS and EHT in other assessed hemostatic parameters. (Table 1).
Table 1. Plasma fibrin clot properties and thrombin generation parameters in hypertensive patients with Cushing's syndrome and essential hypertension

	Cushing's syndrome n=18	Essential hypertension n=32	P
Age, ys	43.9±10.9	45.8±13.6	0.61
Women, n(%)	14(57.8)	23(71.9)	0.65
Ks, $10^{-9}/cm^2$	7.89±3.93	5.09±1,45	**0.009**
CLT, min	115,4±26.2	115.5±27.5	0.98
Lagtime, min	3.46±0.71	3.09±0.66	0.085
ETP, nM•min	1892.31±516.72	2209.65±383.94	**0.031**
Thrombin peak, nM	298,59±111.08	350.06±81.44	0.096
Ttpeak, min	7.38±1.76	6.35±11.32	**0.038**

Ks – clot permeability, CLT-clot lysis time, ETP-endogenous thrombin potential, ttpeak – time to peakConclusions: The preliminary results of the ongoing study may suggest that patients with CS are characterized by less compact fibrin clot structure and impaired thrombin generation as compared to matched patients with EHT.

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