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COST – Harmonis@tion

1st Virtual Adrenal Tumor Masterclass

23-25 March, 2022

Zagreb, Croatia

Scientific Programme

and Abstract Book

Funded by the European Union

EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Scientific Programme

WEDNESDAY, March 23, 2022 – Clinical		
	Chair: Darko Kastelan (Croatia)	
16.00-16.05	Course opening and introduction to Day 1	
16.05-16.45	How to diagnose and treat adrenocortical carcinoma in 2022?	*Martin Fassnacht (Germany)*
16.45-17.25	Comorbidities in patients with adrenal incidentaloma	*Ljiljana Marina (Serbia)*
17.25-18.05	Diagnosis and subtype differentiation of primary aldosteronism	*Felix Beuschlein (Switzerland)*

THURSDAY, March 24, 2022 – Clinical/Translational		
	Chair: Judith Favier (France)	
16.00-16.05	Introduction to Day 2	
16.05-16.45	Management of pheochromocytoma and paraganglioma	*Henri Timmers (Netherland)*
16.45-17.25	How to deal with variants of unknown significance?	*Mercedes Robledo (Spain)*
17.25-18.05	PPGL cases with variants of unknown significance	*Joakim Crona (Sweden)*

FRIDAY, March 25, 2022 – Basic		
	Chair: Mercedes Robledo (Spain)	
16.00-16.05	Introduction to Day 3	
16.05-16.50	Meet the expert: Organoids in adrenal tumor research	*Michaela Luconi (Italy)*
16.50-17.35	Meet the expert: Omic platforms. What do you need to achieve robust results?	*Fatima Al-Shahrour (Spain)*

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Martin Fassnacht

How to diagnose and treat adrenocortical carcinoma in 2022?

How to diagnose and treat adrenocortical carcinoma in 2022?

Martin Fassnacht

University Hospital Würzburg, Germany

Adrenocortical carcinoma (ACC) is a rare and in most cases steroid hormone producing tumor with variable prognosis. Comprehensive endocrine and imaging work-up is crucial to detect all ACCs as early as possible. In this context, it is clearly recommended that all patients with suspected and proven ACC are discussed in a multidisciplinary expert team meeting.

Surgery still remains the single most important therapeutic option for non-metastatic ACC. However, even a state-of-the-art surgery cannot always prevent disease recurrence that is determined mainly by specific tumor characteristics. We consider that the concomitant presence of the following features characterizes a cohort of patients at low risk of recurrence, i) R0 resection, ii) stage I-II ACC, and iii) Ki-67 <10%. After the ADIUVO study, we do not recommend adjuvant mitotane as a routine measure for these patients, who can be managed with active surveillance thus sparing a potentially toxic treatment. However, patients at average risk of recurrence should be treated with adjuvant mitotane paying particular attention to supportive therapy to deal with possible unwanted effects of the drug. For patients at very high risk of recurrence that we arbitrarily define as the presence of at least one of the following features: Ki67 >30%, large tumor thrombus in the vena cava, R1 resection or complete resection in stage IV ACC, we encourage enrollment in the ADIUVO-2 study or increasingly recommend to combine mitotane treatment with four cycles of platinum-based chemotherapy.

For patients with advanced/metastatic disease, mitotane monotherapy or mitotane plus EDP are still treatments of choice. Monotherapy with checkpoint inhibitors is not the magic bullet, but is one of the second-line options (as it is still gemcitabine + capectiabine or streptozotocin). However, it is important that more patients will be treated within clinical trials (currently for instance CaboACC, Spencer).

Uniklinikum Würzburg

How to diagnose and treat adrenocortical carcinoma in 2022?

Martin Fassnacht

Dept. of Endocrinology and Diabetes

Medizinische Klinik und Poliklinik I

Lehrstuhl für Endokrinologie und Diabetologie

medeins MEDIZINISCHE KLINIK & POLIKLINIK I

Conflict of interest

- Investigator of trials run by
  - HRA Pharma
  - Enterome Pharma

EJE EUROPEAN JOURNAL OF ENDOCRINOLOGY

Clinical Practice Guideline | M Fassnacht and others | Management of adrenocortical carcinoma in adults | 179:4 | G1–G46

European Society of Endocrinology Clinical Practice Guidelines on the management of adrenocortical carcinoma in adults, in collaboration with the European Network for the Study of Adrenal Tumors

Martin Fassnacht[1,2], Olaf M Dekkers[3,4,5], Tobias Else[6], Eric Baudin[7,8], Alfredo Berruti[9], Ronald R de Krijger[10,11,12,13], Harm R Haak[14,15,16], Radu Mihai[17], Guillaume Assie[18,19] and Massimo Terzolo[20]

Eur J Endocrinol. 2018 Oct;179(4):G1-G46

European Society of Endocrinology

ens@t european network for the study of adrenal tumors

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ANNALS OF ONCOLOGY driving innovation in oncology

Volume 31 ■ Issue 11 ■ 2020

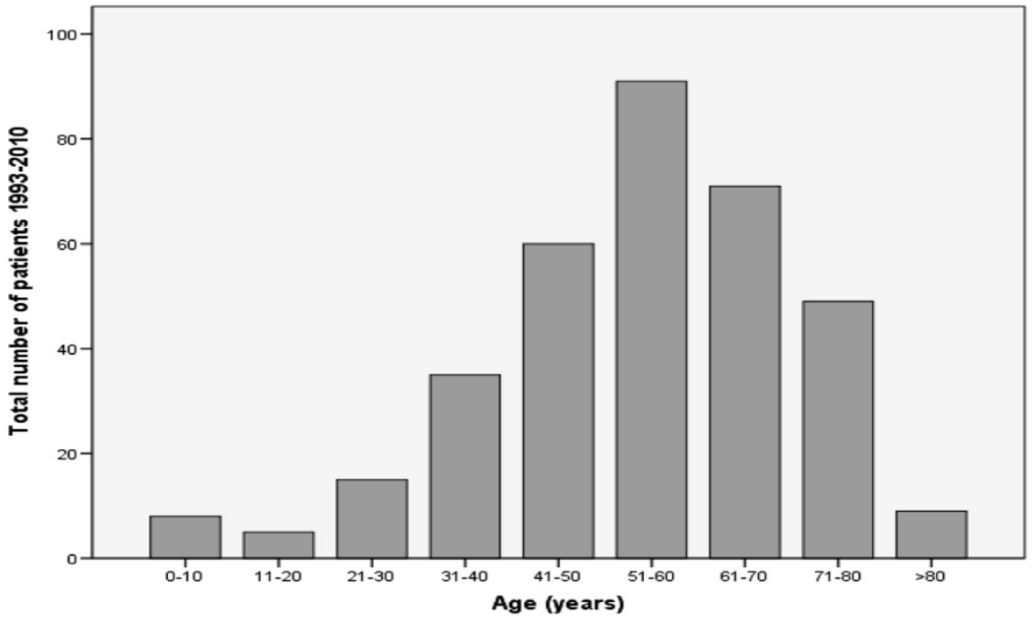
SPECIAL ARTICLE

Adrenocortical carcinomas and malignant phaeochromocytomas: ESMO—EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up†

M. Fassnacht[1,2], G. Assie[3,4], E. Baudin[5], G. Eisenhofer[6], C. de la Fouchardiere[7], H. R. Haak[8,9,10], R. de Krijger[11,12], F. Porpiglia[13,14], M. Terzolo[15] & A. Berruti[16], on behalf of the ESMO Guidelines Committee*

Epidemiology

- Data on the incidence are scarce
- Most reports suggest an incidence between 0.5 – 1.5 per million population (Kebebew et al. W J Surgery 2006, Goldon et al. JCEM 2009, Kerkhofs et al. Eur J Cancer 2013)
- All series describe a female dominance (ratio 1.5 : 1)



Kerkhofs et al. Eur J Cancer 2013

Our first recommendation

(not based on publications, but we feel strong about it)

R.1.1. We **recommend** that all patients with suspected / proven ACC are discussed in a multidisciplinary expert team meeting

- with adrenal expertise in :
  - Endocrinology
  - Oncology
  - Pathology
  - Radiology
  - Surgery

This team should have access to expertise in:

- Interventional radiology
- Radiation therapy
- Nuclear medicine
- Genetics
- Palliative care

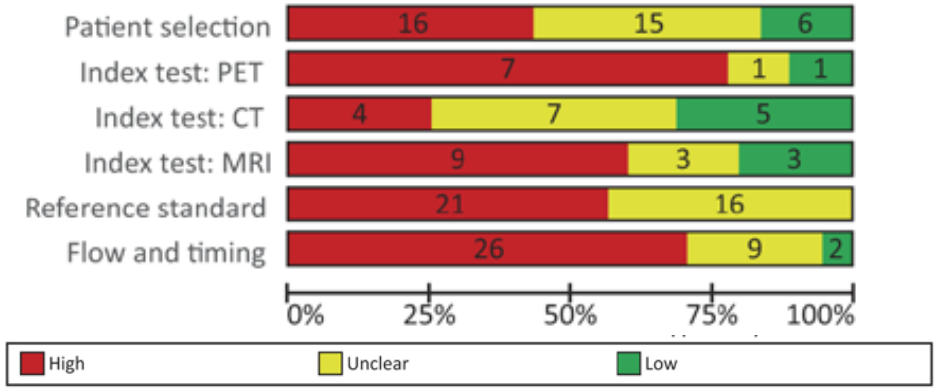
Diagnostic work-up

What are the best criteria to establish a benign tumor?

- Cochrane analysis (5.469 references, 525 full papers)

  => big disappointment: only very few studies were methodologically sound

  - CT: n = 16
  - MRI: n = 15
  - FDG-PET: n = 9



⇒only 19 studies could be included in the metaanalysis

⇒ Guideline conclusions:

The only reliable method is unenhanced CT:

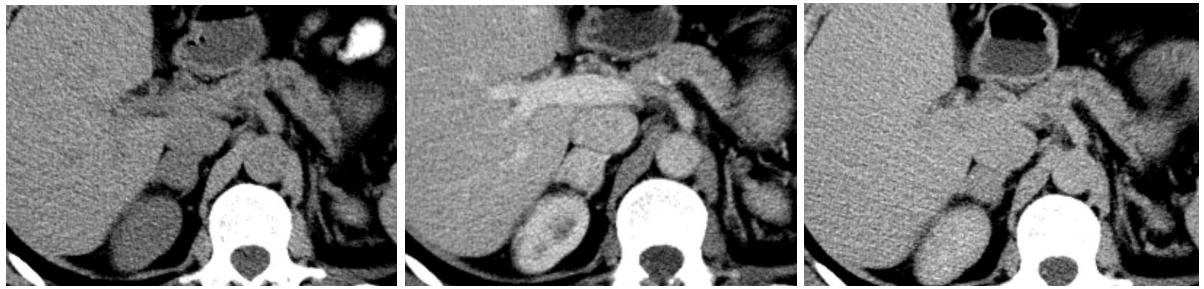
Homogeneous mass with Hounsfield Units ≤ 10

= benign mass (adenoma, lipoma, etc.) (1⊕OOO)

However, 30-40% of adenomas have HU > 10

New data on washout CT

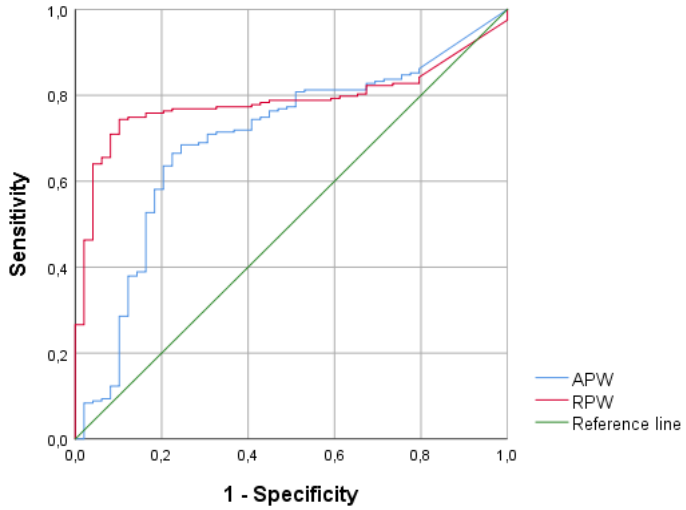
- Delayed washout CT in 252 adrenal masses
- Using „standard cutoffs“ we misdiagnose several patients
  - Relative percentage washout > 40% 4 of 49 malignant
  - Absolute percentage washout >60%: 11 of 49 malignant



0 min: 31 HU 1 min 115 HU 10 min: 49 HU

=> APW 78% RPW 57%

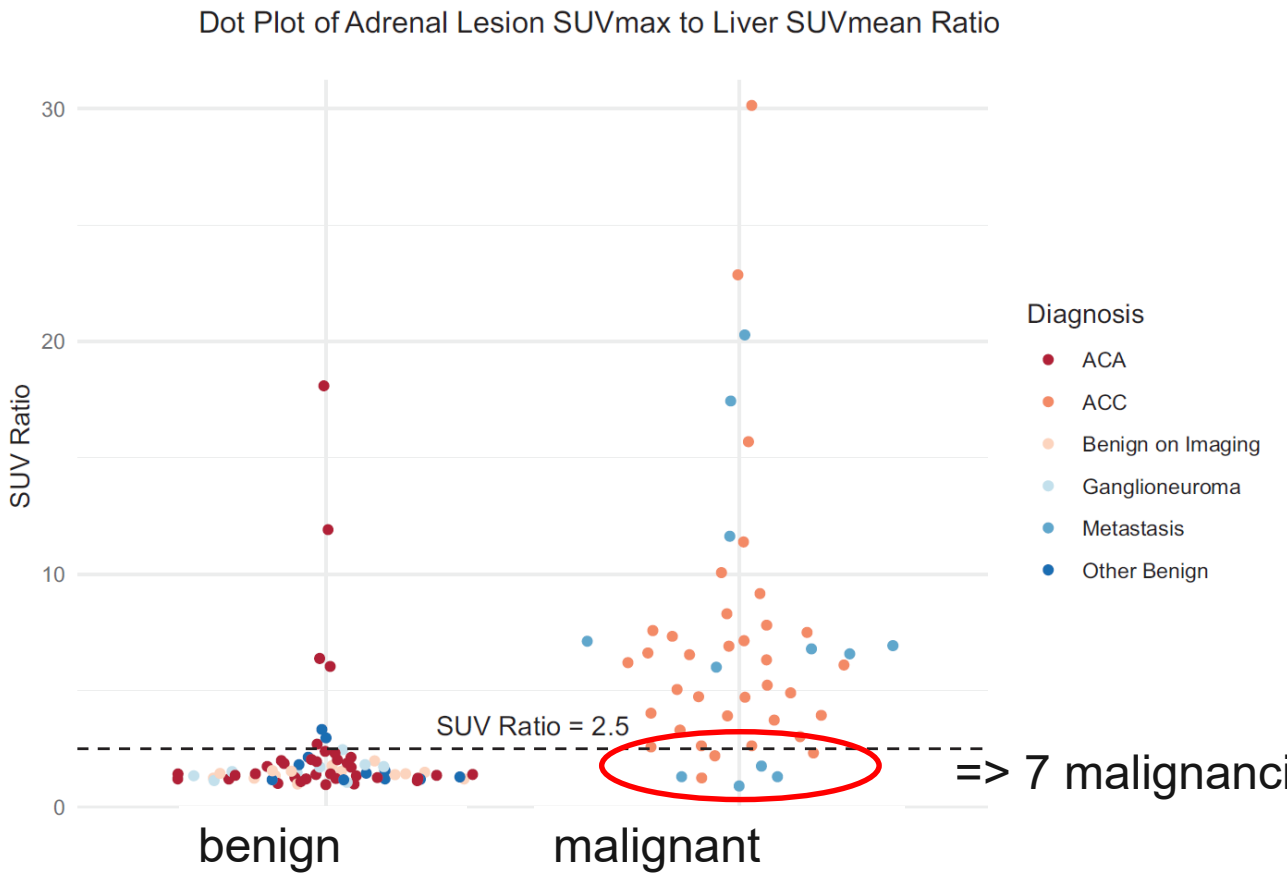
BUT histology: metastasis of renal cell cancer



- Adapting the cutoff to 58% for RPW
  - No false positive, but only 15% of benign tumors with unenhanced HU>10 could be identified

Update on FDG-PET/CT

- Retrospective analysis of 117 indeterminate adrenal masses by FDG-PET/CT (70 benign + 35 ACC + 12 metastases)

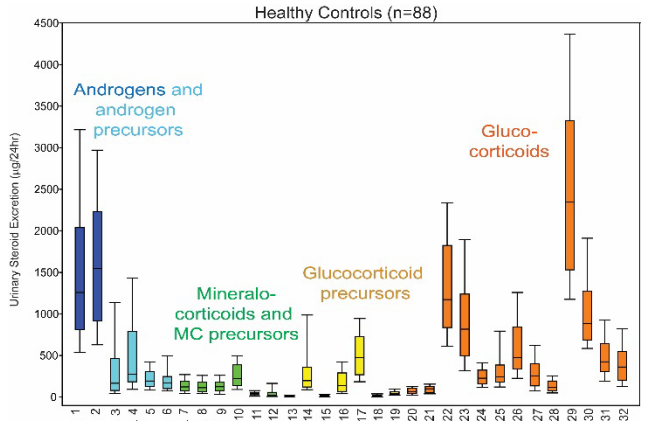


=> 7 malignancies (3ACC, 4 mets)

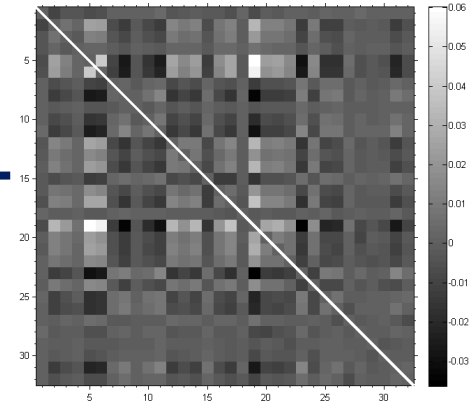
Diagnostic power of urinary steroid profiling using GC-MS technique

24-h urine

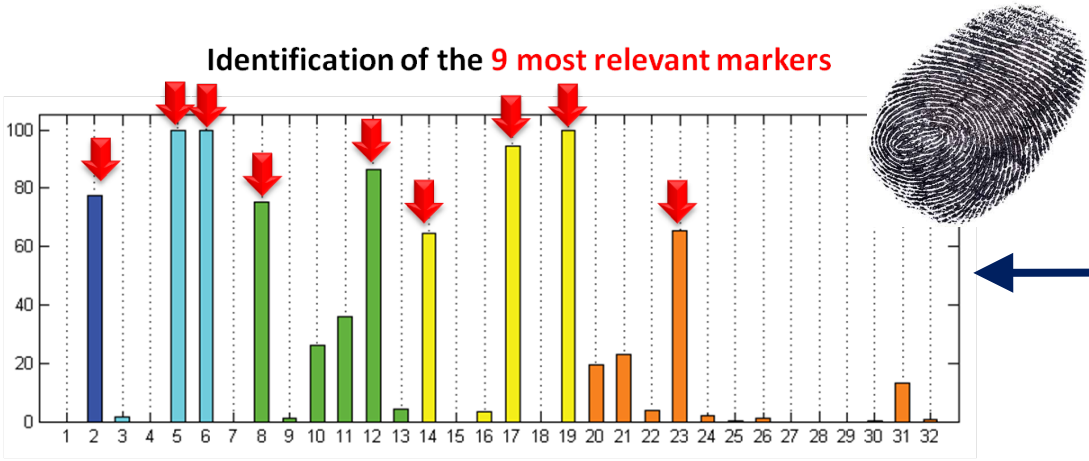
GC-MS



GMLVQ



102 ACA,
47 ACC



90% Sensitivity + 90% Specificity

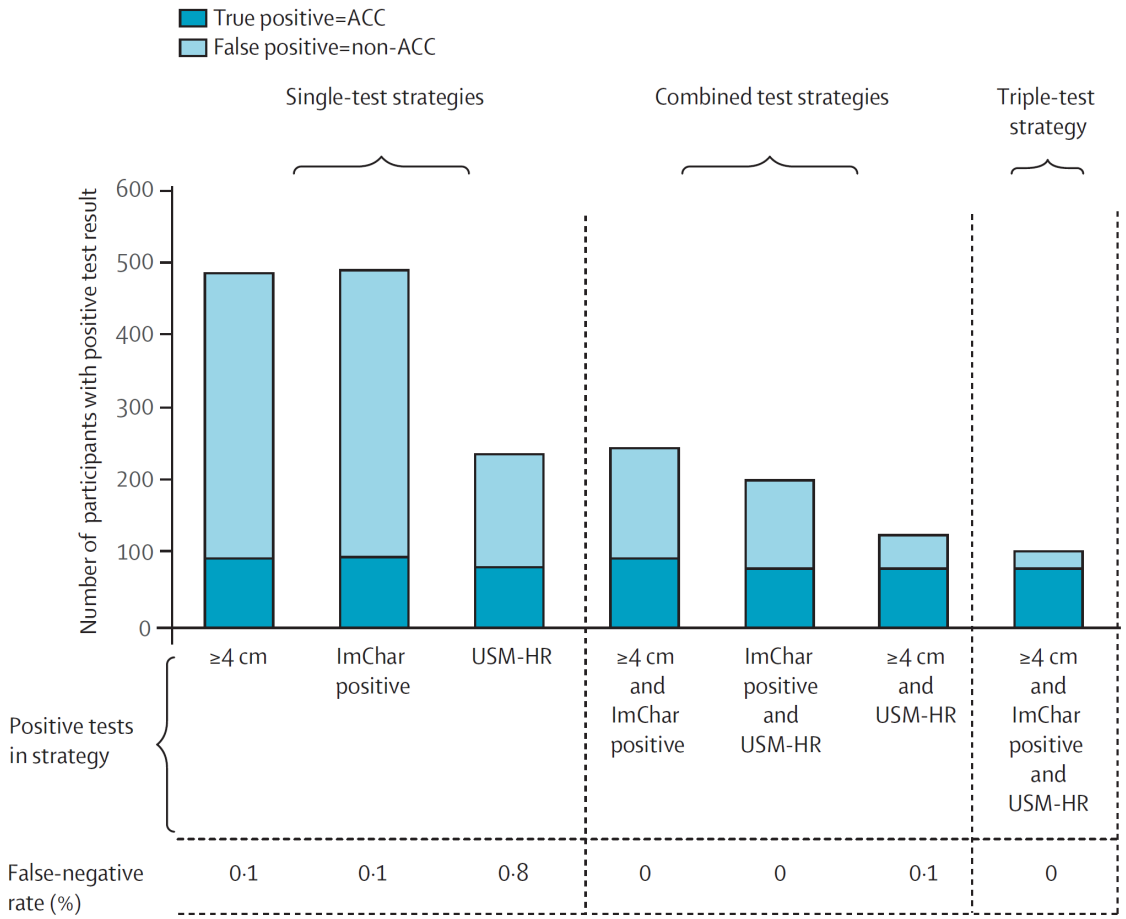
Eurine-ACT study

- Prospective ENSAT multicenter trial with 2017 patients with newly diagnosed adrenal masses > 1cm
- Primary endpoint: diagnostic accuracy of LC-MS/MS generated urinary steroid metabolomics vs. standard imaging
- Final diagnoses
  - 1767 adenomas (87.6 %)
  - 87 other benign lesions (4.3 %)
  - 98 ACC (4.9 %)
  - 65 other malignant lesions (3.2 %)

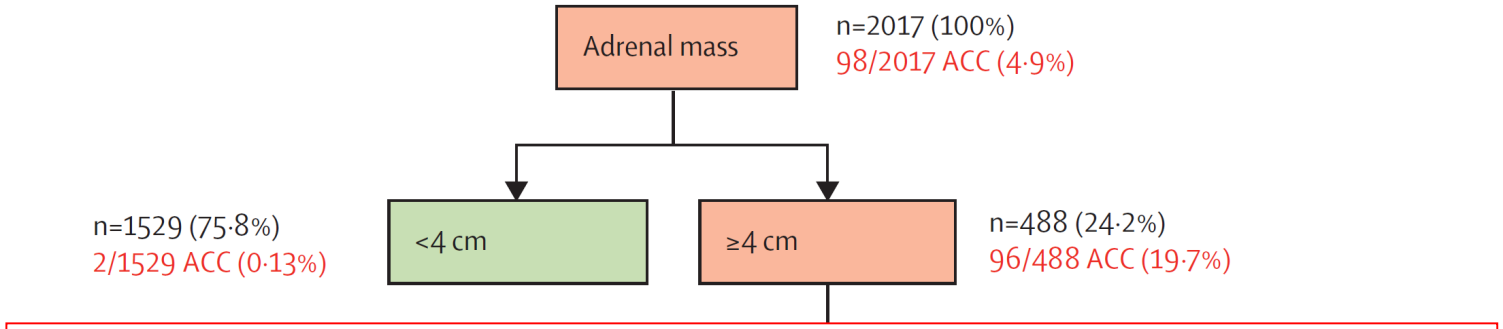
What is the most accurate diagnostic criterion?

Comparison of different diagnostic strategies:

- Tumor size ≥ 4cm
- Unenhanced CT > 20 HU (ImChar positive)
- Urinary steroidmetabolom „high risk“ (USM-HR)



Suggestion for a new diagnostic algorithm



CAVE:
this method will not help to detect metastases of extra-adrenal malignancies

- Out of 2017 adrenal tumors, 5 of 98 ACC (5.1%) would be missed
- BUT: 245 of 563 (43.5%) surgeries could have been prevented

ESE-ENSAT recommendations for pre-surgical diagnostic work-up

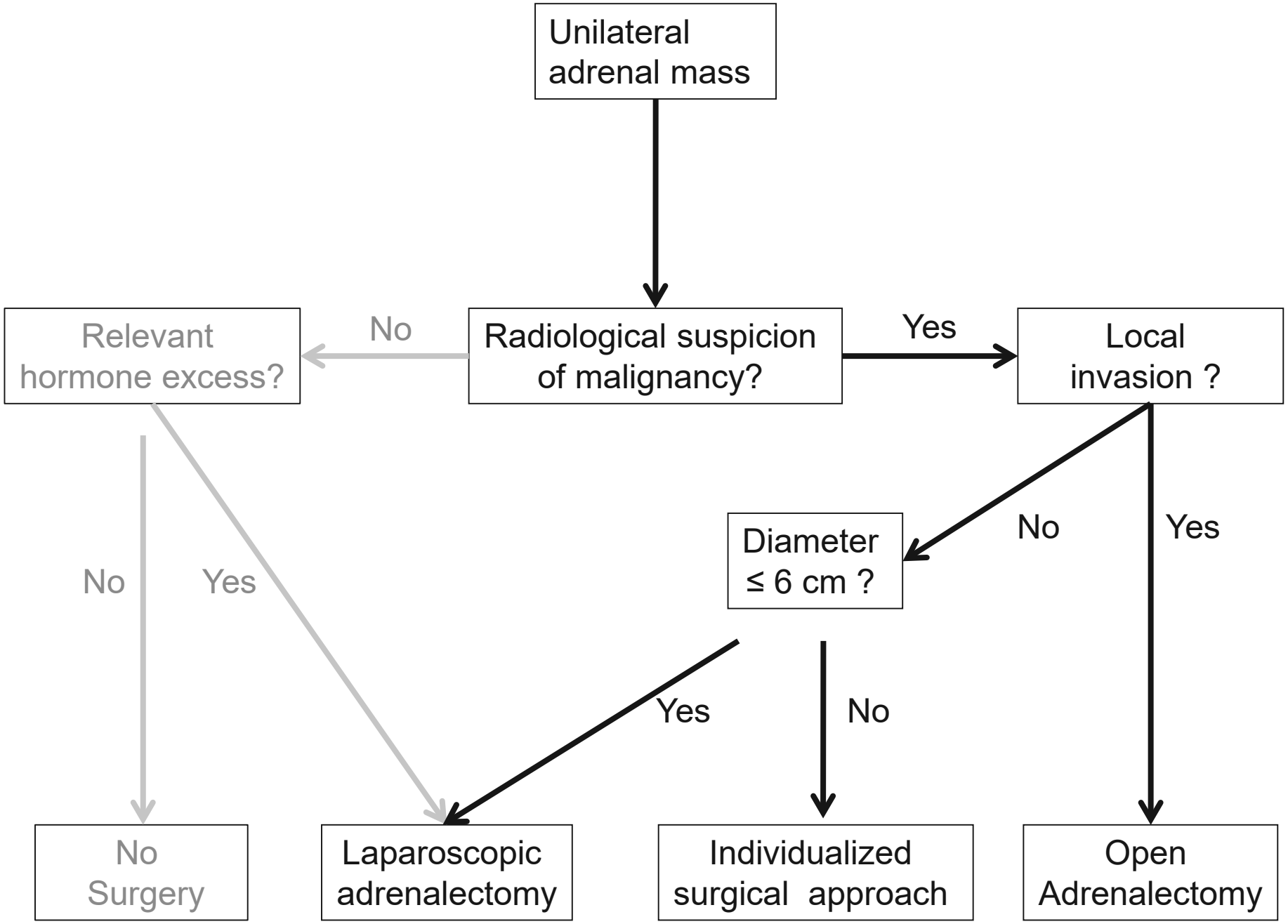
Hormonal work-up	
Glucocorticoid excess	– 1 mg dexamethasone suppression test or free cortisol in 24-h urine[a] – Basal ACTH (plasma)[b]
Sex steroids and steroid precursors[c]	– DHEA-S – 17-OH-progesterone – Androstenedione – Testosterone (only in women) – 17-beta-Estradiol (only in men and postmenopausal women) – 11-Deoxycortisol
Mineralocorticoid excess	– Potassium – Aldosterone/renin ratio (only in patients with arterial hypertension and/or hypokalemia)
Exclusion of a pheochromocytoma	– Fractionated metanephrines in 24 h urine or free plasma-metanephrines
Imaging	– CT or MRI of abdomen and pelvis – Chest CT – FDG-PET/CT[d] – Bone or brain imaging (when skeletal or cerebral metastases are suspected)

Urinary steroid profing if available

=> In 70% of ACC cases at least one of these serum hormones is clearly elevated (with urinary profiling > 95%)

=> In case of “endocrine inactive” tumor, consider a “non-ACC tumor”

Surgery



Fassnacht ESE-ENSAT guidelines et al. Eur J Endocrinol 2016

My personal key conclusion regarding surgery

- The question whether open or laparoscopic surgery is less important.
- The key question is how good is the surgeon?

⇒ It is our task to advise patients with suspected ACC and to help them to find the best surgeon (not only in their hometown)

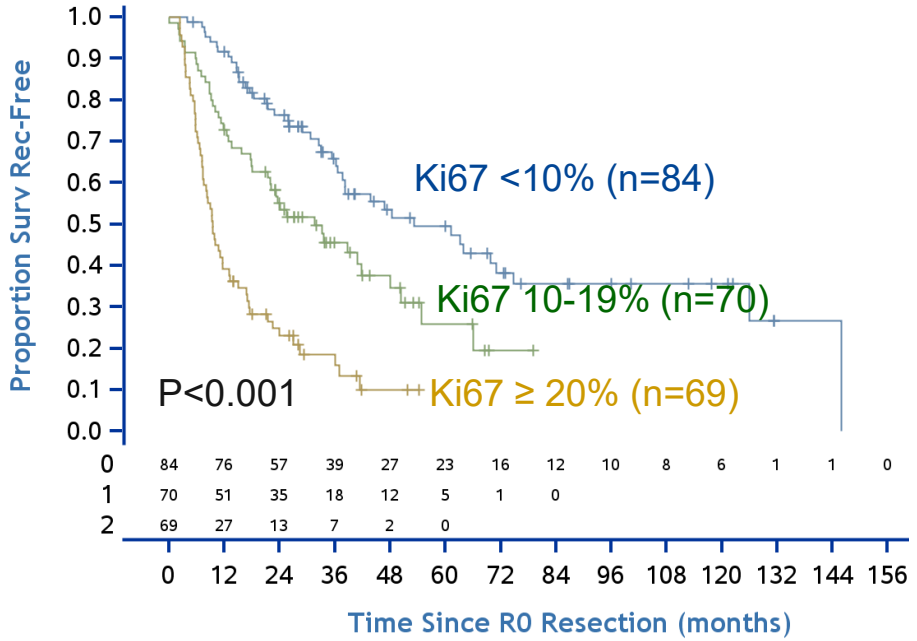
- Nevertheless, for the majority of ACC open surgery is the way to go, but some patients would benefit from laparoscopic surgery

Are there now any prognostic factors to guide us in the decision for or against adjuvant therapy?

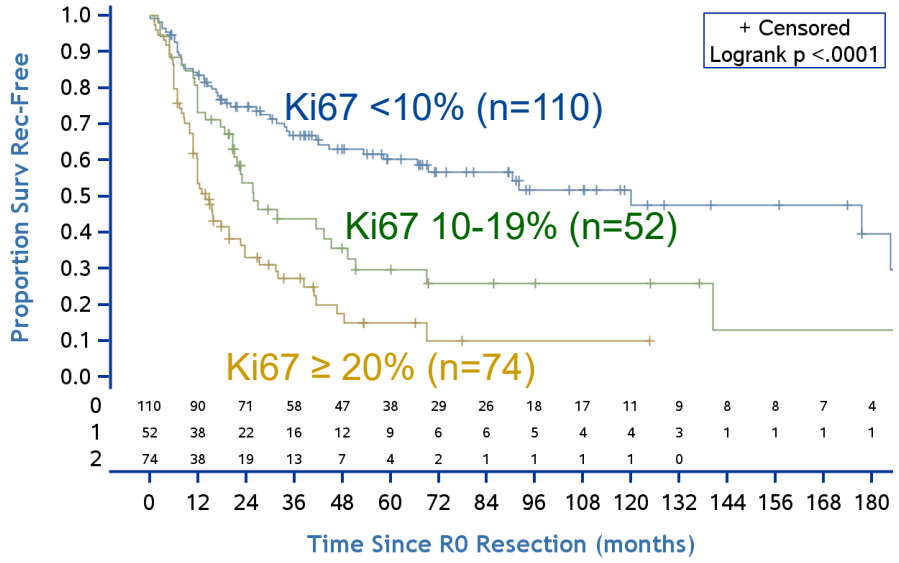
Towards reliable prognostic markers in ACC after R0 resection

- Evaluation of 15 histological markers, 3 scores, and Ki67 in a large cohort of patients

German cohort N=319



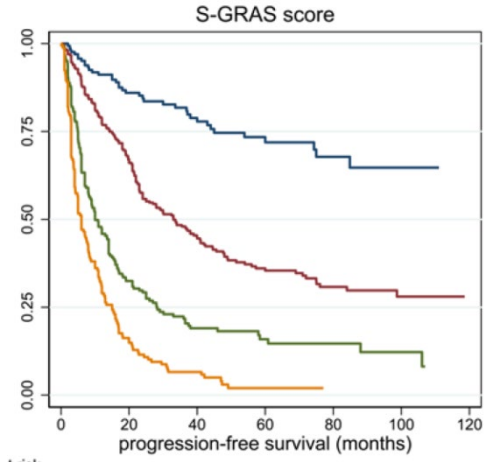
ENSAT validation cohort N=250

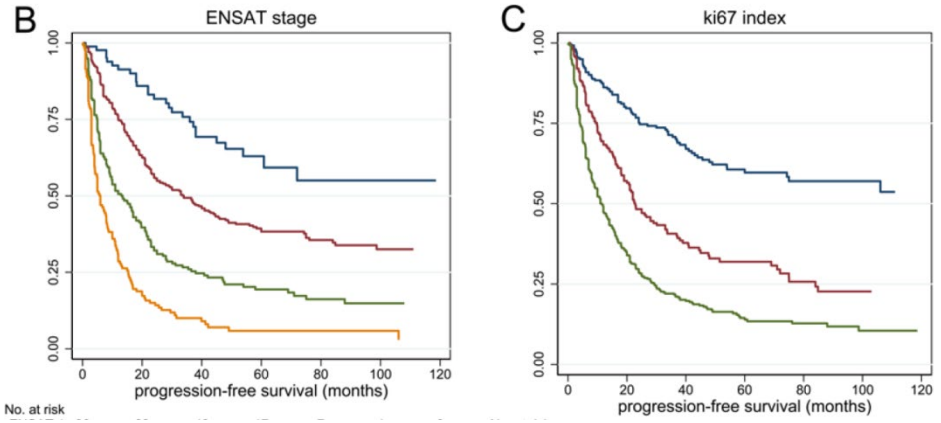


A new tool for prognostication: S-GRAS

- Based on a retrospective study with 942 well-characterized patients

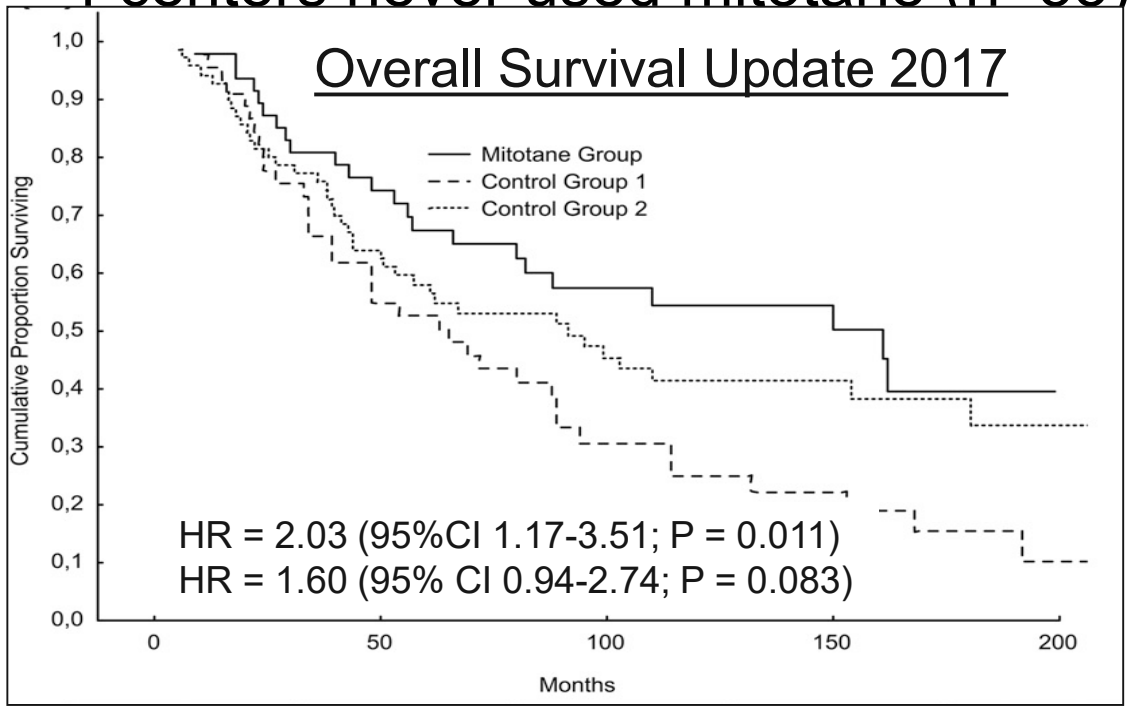
ENSAT stage	1 or 2	= 0 point
	3	= 1
	4	= 2
Grading with Ki67	<10%	= 0 point
	10-19%	= 1
	≥ 20%	= 2
Resection status	R0	= 0 points
	Rx	= 1
	R1	= 2
	R2	= 3
Age	< 50 y	= 0 point
	≥ 50 y	= 1
Symptoms	no	= 0 point
	yes	= 1
Total		**9 points**

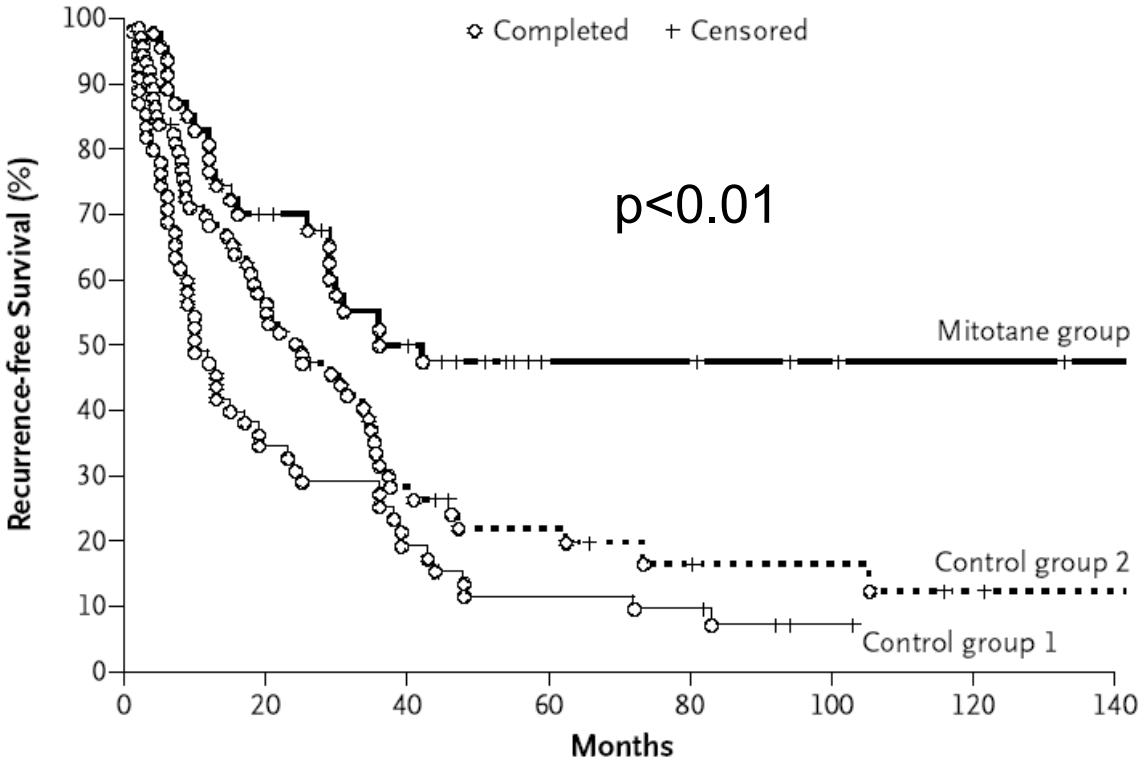




Best evidence is available for adjuvant mitotane

Large retrospective study
in 8 Italian centers
- 4 centers administered mitotane in all patients (n=42)
- 4 centers never used mitotane (n=55)





However, mitotane is disputed…

Advertisement “Times” 1946

Commentary: Adjuvant Mitotane for Adrenocortical Cancer—A Recurring Controversy

JCE&M 2008

Hui Huang and Tito Fojo

Medical Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

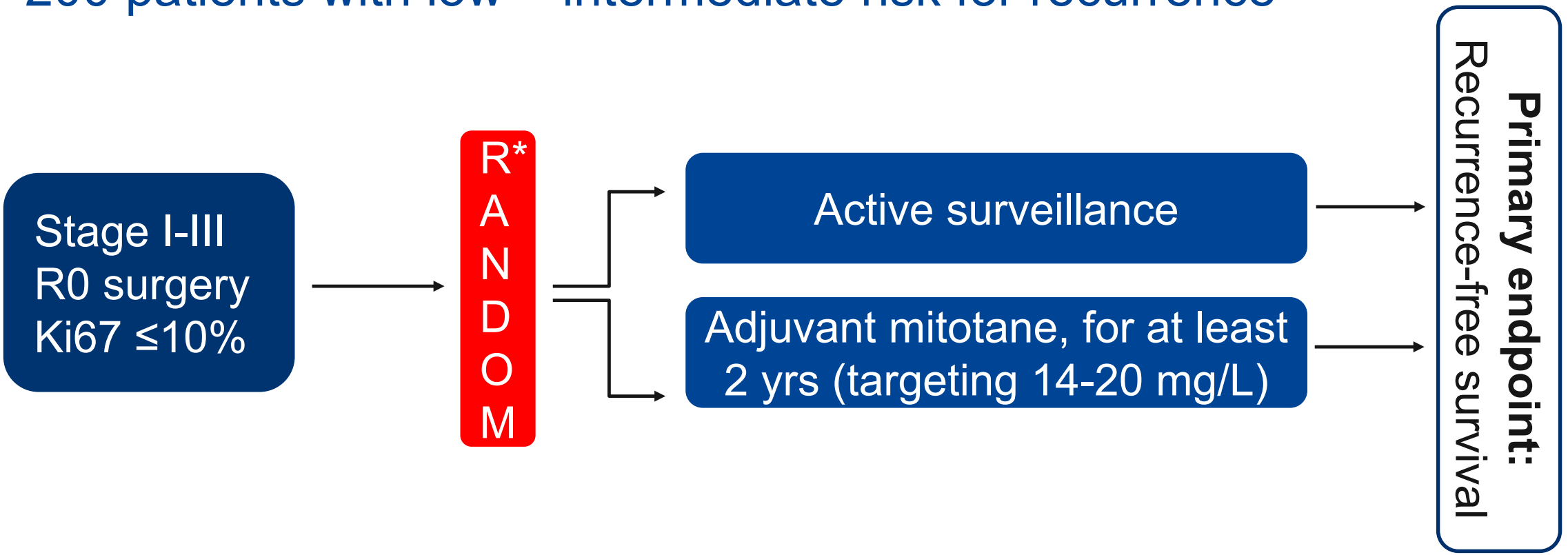
How toxic is mitotane really?

- Toxicity data are only available from 7 studies with 342 patients (with a median number of **3.8 adverse events per patient**)
- Retrospective multicenter study focusing only on adverse events
- 311 patients from 7 ENSAT centers with mitotane monotherapy
  - 69% adjuvant setting
  - 31% advanced ACC
- Median duration of mitotane treatment: 20 months (1-203)
- **Rate of AE: 9.6 per patient**

We need prospective trials…

ADIUVO-trial – an international, randomized phase III trial

► 200 patients with low + intermediate risk for recurrence



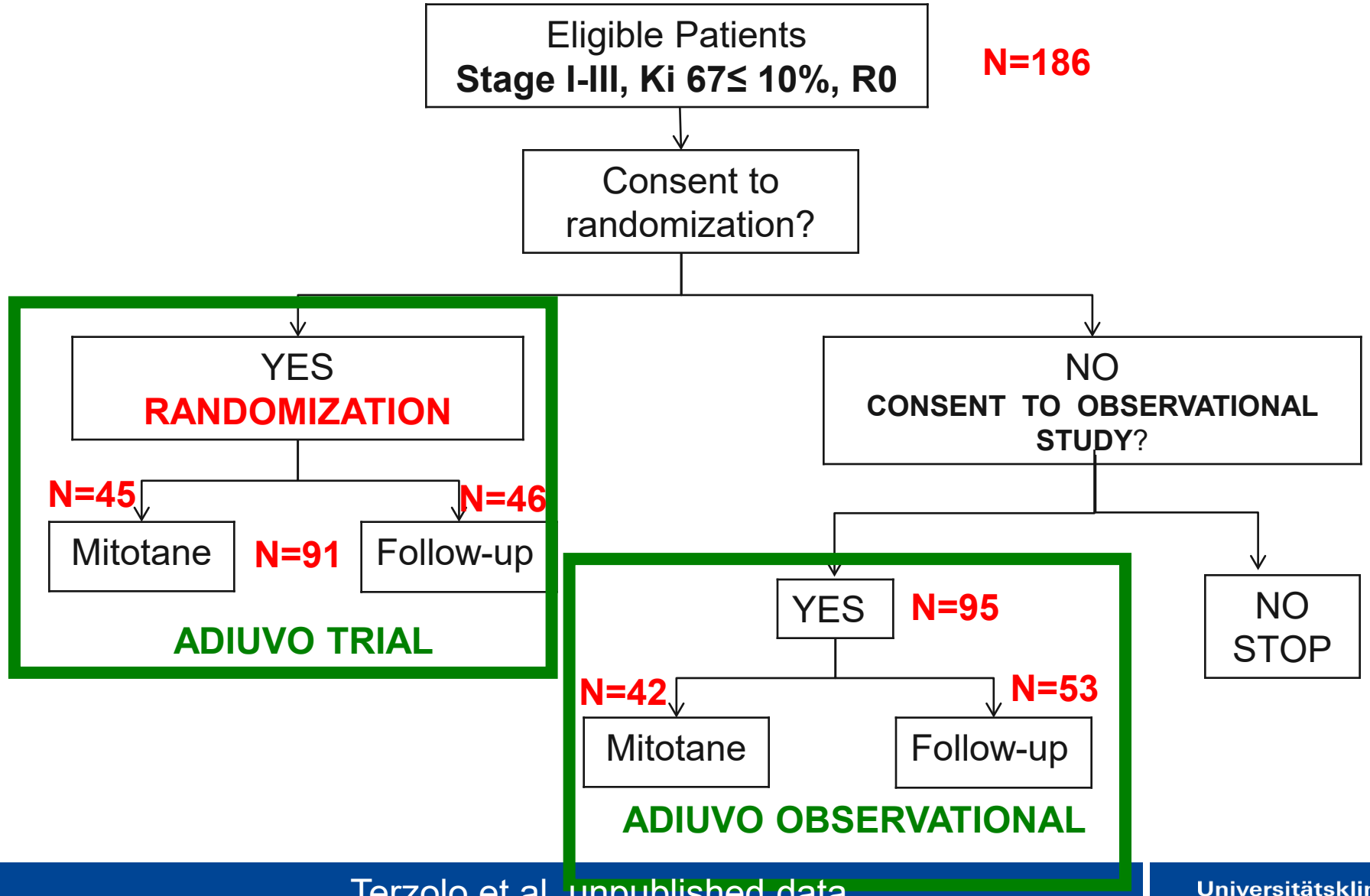
Bottlenecks and challenges…

- Only ≈ 30% of screened patients had Ki67 ≤ 10%
- Furthermore, more than half of the patients refused randomization

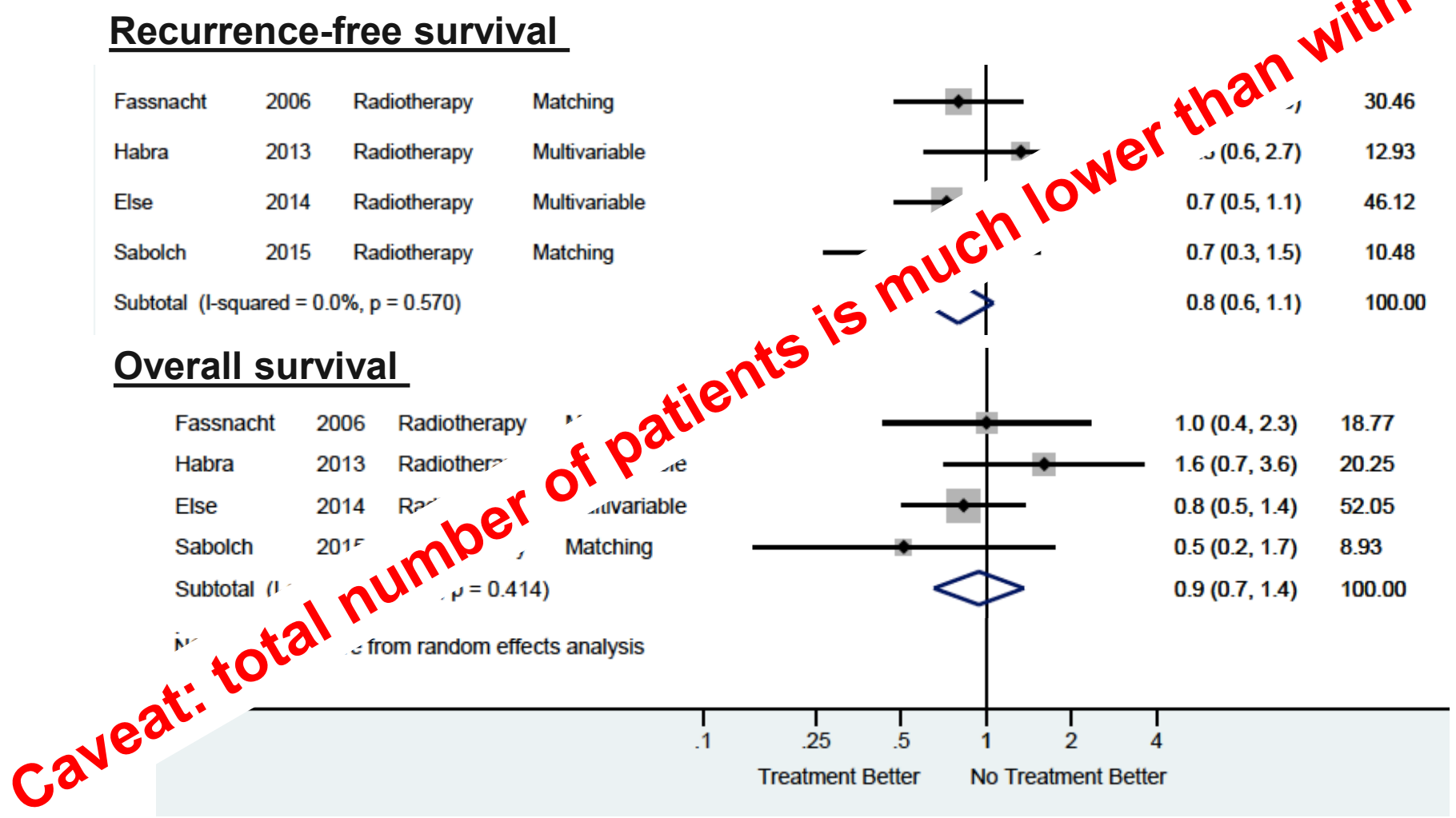
⇒Recruitment was very slooow…
⇒We started to collect also data from patients that refused randomization

- Recruitment was stopped after 10 (!) years

Study flow of ‘ADIUVO‘ and ‘ADIUVO Observational‘



Meta-analysis on adjuvant radiotherapy



Caveat: total number of patients is much lower than with mitotane

Adjuvant cytotoxic chemotherapy?

- ESE-ENSAT guidelines 2018:

> **R.7.7.** The panel did not come to a definitive consensus on adjuvant use of cytotoxic drugs. We suggest against the routine use of cytotoxic drugs in the adjuvant setting. However, the panel suggests considering adjuvant chemotherapy in selected patients with very high risk for recurrence.

⇒ Retrospective analysis (Würzburg, MD Anderson, Berlin, Brescia, Turin)

- 31 patients with adjuvant platin-based therapy
  - Cisplatin + etoposide n=16
  - Carboplat + etoposide n=8
  - Cisplatin/carboplat + etoposid + doxorubicin n=6
  - Cisplatin mono n=1

Is there a role of adjuvant platin-based therapy?

Two complementary statistical approaches:

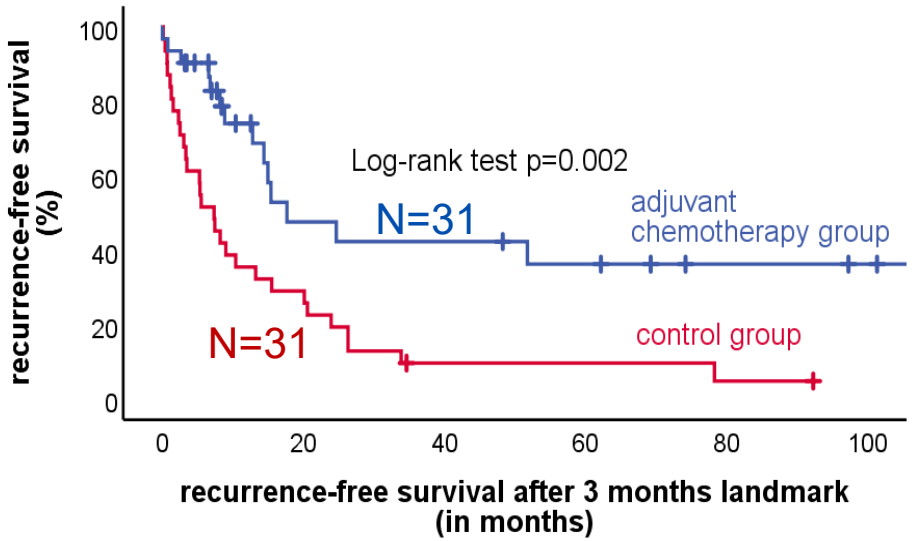
- individual matching 31 vs. 31 patients
- propensity score analysis using a cohort of 299 patients

	Adjuvant platin therapy (n=31)	Matched controls (n=31)	P value matched controls	Entire control cohort (n=268)	P value entire control group
Median tumor size mm (range)	124 (25-300)	120 (38-220)	0.79	110 (25-260)	0.45
Cortisol +/- androgens- n (%)	15 (48.4)	12 (38.7)	0.068	101 (37.7)	0.11
ENSAT tumor stage					
I - n (%)	0	0	1.0	14 (5.3)	0.026
II - n (%)	11 (35.5)	11 (35.5)		138 (52.2)	
III - n (%)	16 (51.6)	16 (51.6)		101 (38.4)	
IV - n (%)	4 (12.9)	4 (12.9)		10 (3.8)	
Venous tumor thrombus - n (%)	10 (32.3)	10 (32.3)	1.0	16 (6.3)	<0.001
Resection status					
R0 - n (%)	25 (80.6)	25 (80.6)	1.0	183 (68.3)	0.56
RX - n (%)	4 (13)	4 (13)		54 (20.1)	
R1 - n (%)	2 (6.4)	2 (6.4)		30 (11.2)	
Ki67 index - median (range)	30 (10-80)	32.1 (8-80)	0.86	20 (1-90)	0.008
<20%	7 (25)	5 (17.9)	0.55	92 (44.7)	0.014
20-39%	10 (35.7)	14 (50)		79 (38.3)	
≥40%	11 (39.3)	9 (32.1)		35 (17.0)	
Adjuvant mitotane – n (%)	28 (90.3)	28 (90.3)	1.0	120 (44.9)	<0.001

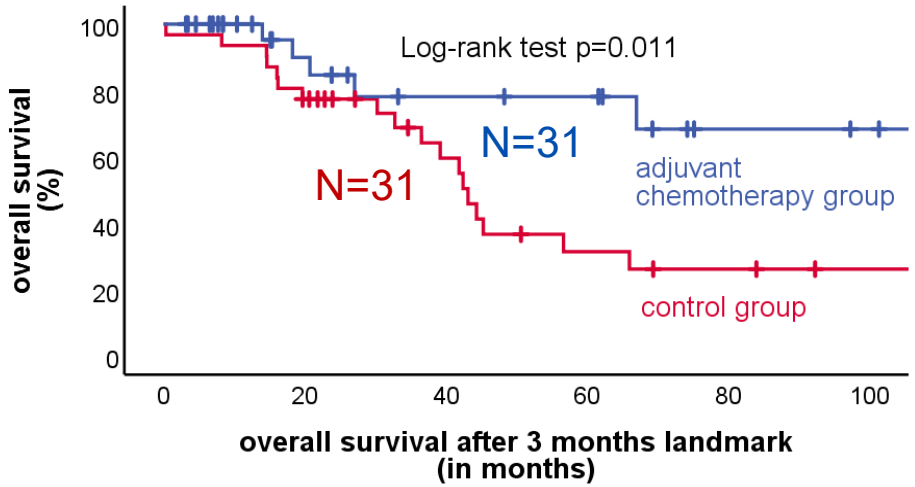
Kimpel et al. Br J Cancer 2021

Is there a role of adjuvant platin-based therapy?

Recurrence-free survival



Overall survival

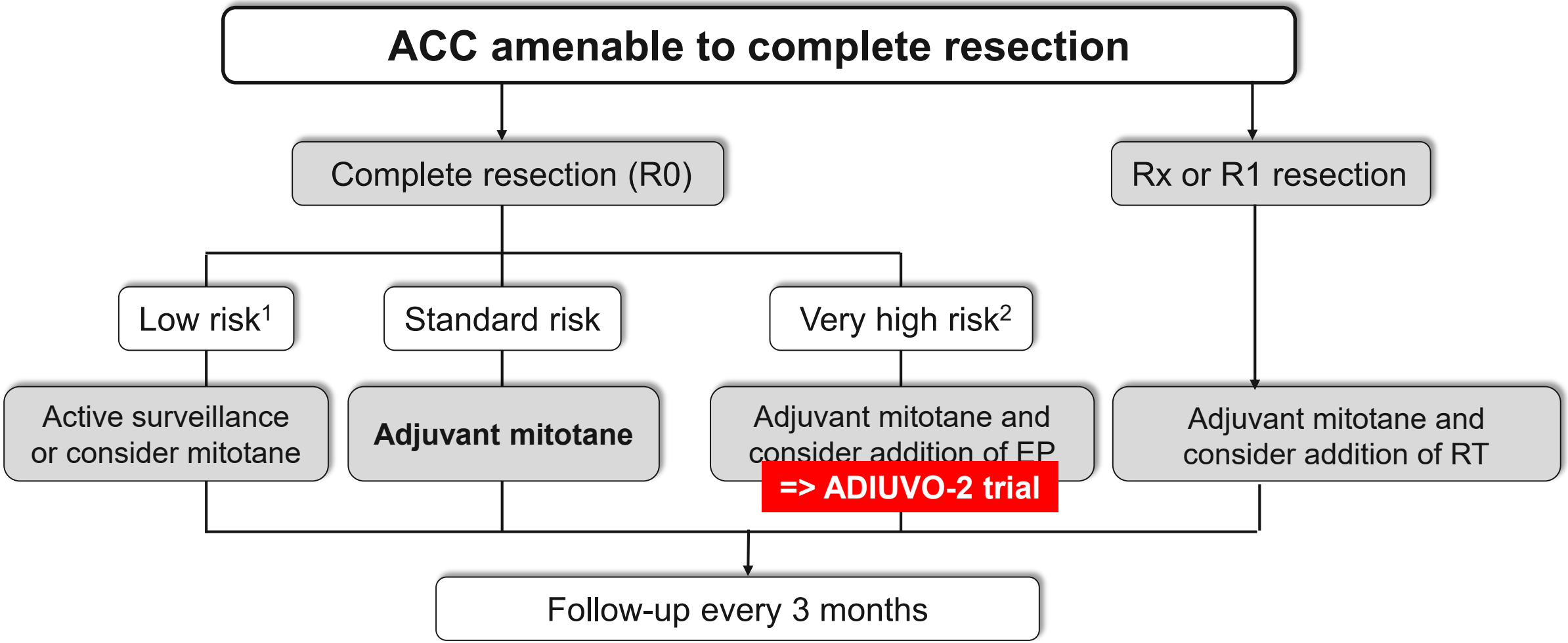


Adjusted HR of a multivariate Cox analysis:
For RFS: **0.19** (95% CI 0.09-0.42; p<0.001) for OS **0.26** (95% CI 0.09-0.72; p=0.010)

<u>Entire cohort of 299 patients</u>

After adjustment for propensity scores and accounting for immortal time bias:
HR RFS: **0.45**, 95% CI 0.29-0.89, p=0.021; OS **0.25** (95% CI 0.09-0.69; p=0.007)

Management of patients with localized ACC



[1] ENSAT I+II and Ki67 <10%

[2] Ki67 > 30%, large venous tumor thrombus, stage IV, **or** R1 resection

Treatment of advanced ACC

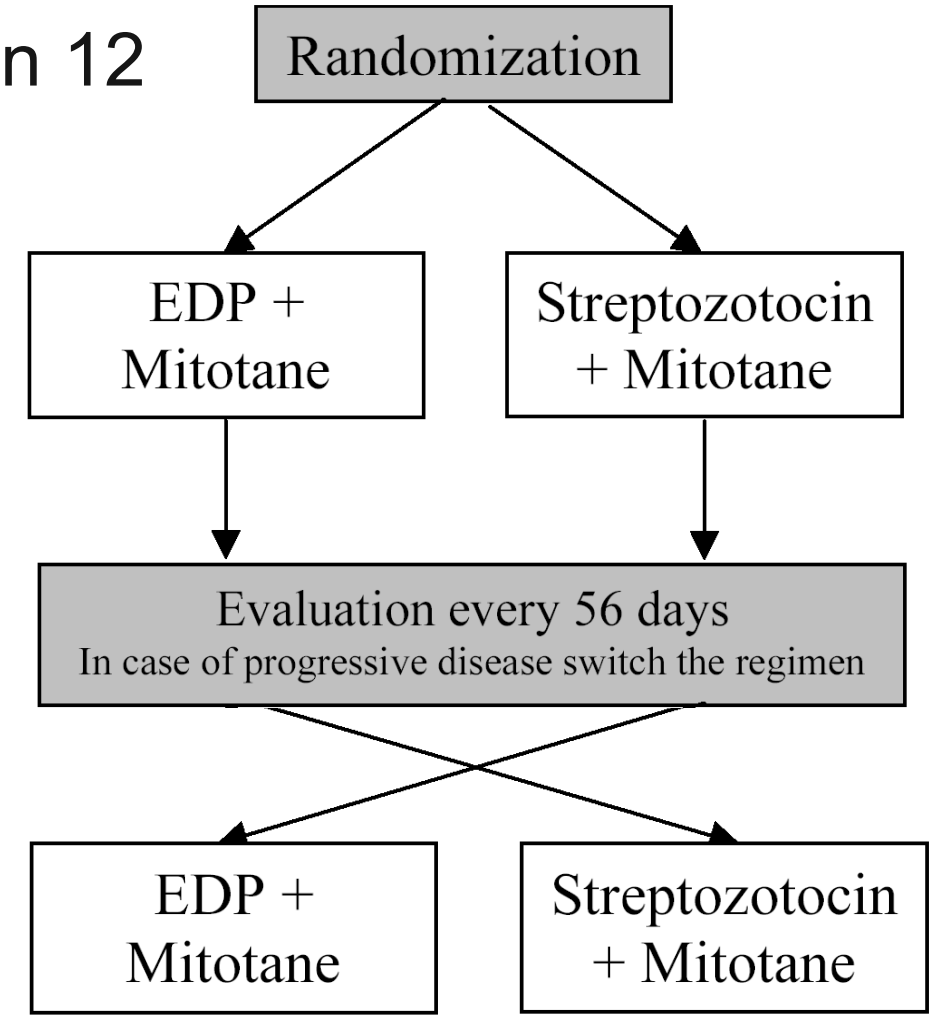
firm act study

The first randomized trial in ACC…

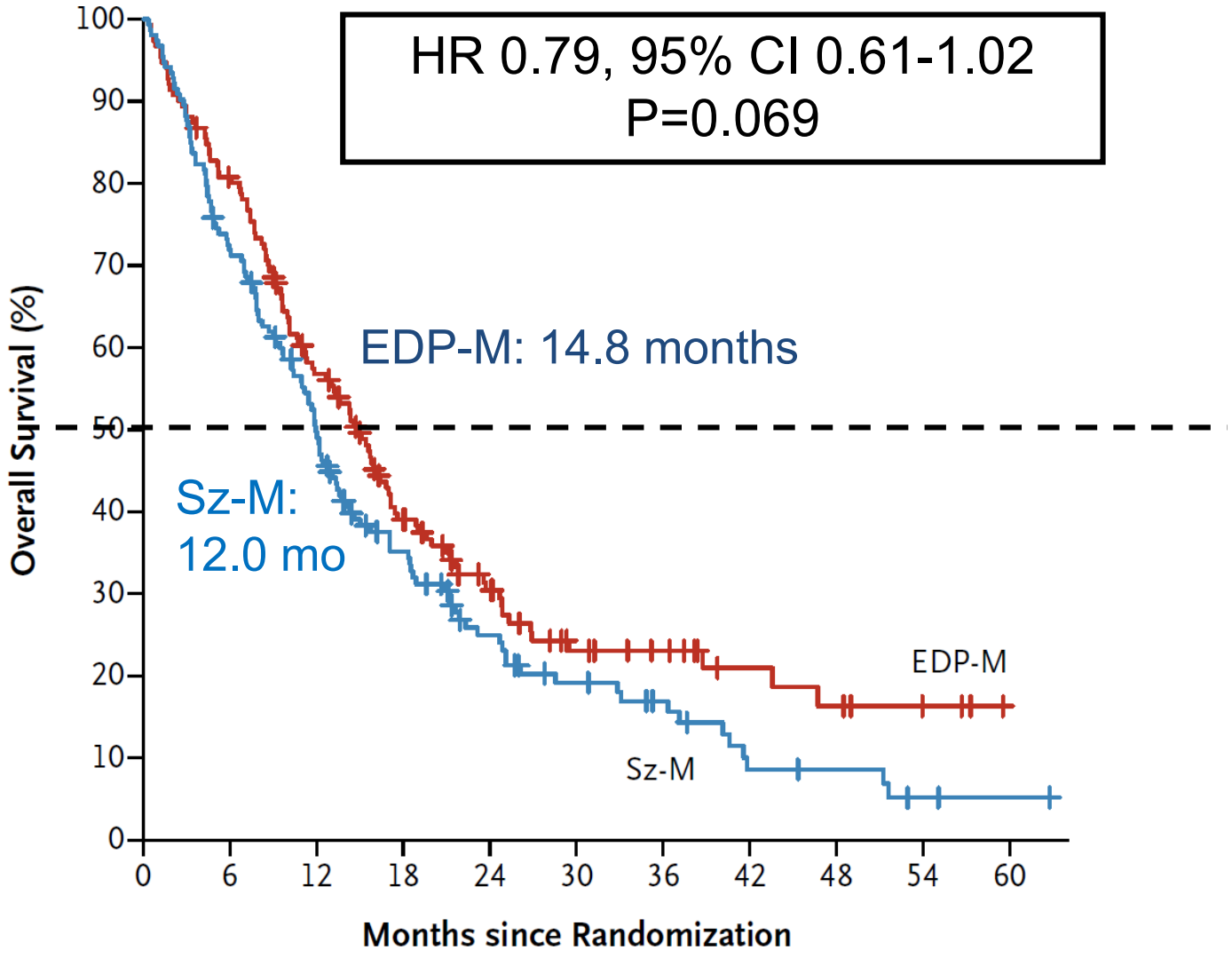
- International trial with 40 centers in 12 countries
- Enrollment June 2004 – Oct 2009
- Number of recruited patients: 304

Design:

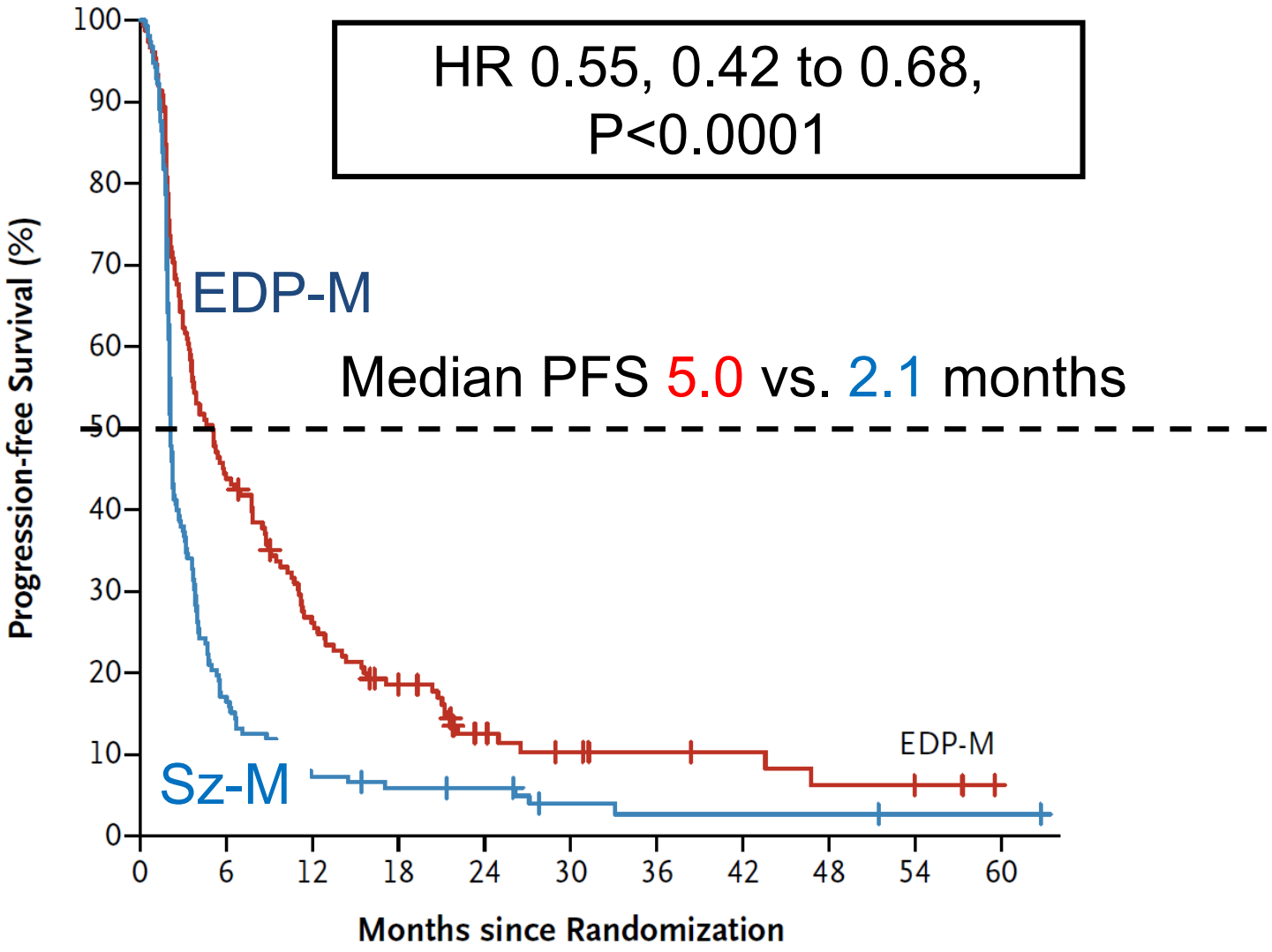
- randomized
- controlled
- international
- phase III trial



Overall survival

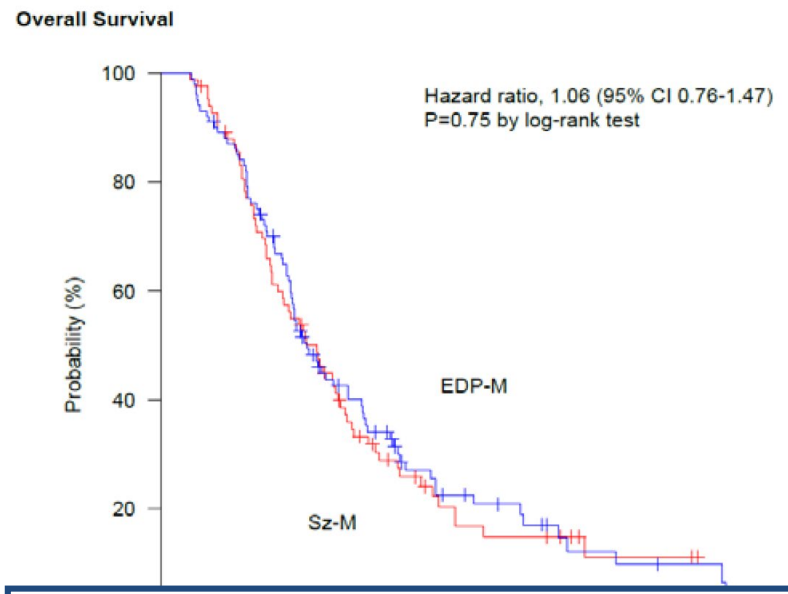


Progression-free survival

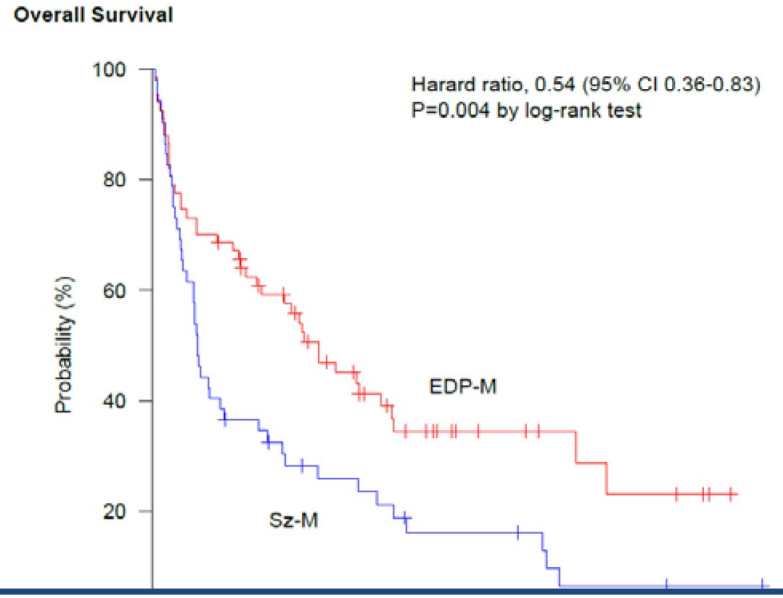


Overall survival with or without second-line therapy (FIRM-ACT trial)

Patients treated with both first- and second-line therapy



Patients treated only with first-line therapy



Adverse events and quality of life were similar in both groups,

⇒ EDP-M is now judged as standard first-line cytotoxic regimen in advanced ACC

However, the results could also justify the use of experimental drugs in first-line (?)

Is mitotane a reasonable first-line treatment?

- 127 patients with advanced ACC not amenable to curative surgery (median age 58.6 years (19.8-86 y)
- Treated with mitotane monotherapy for advanced ACC
  - 39% at the time of initial diagnosis
  - 61% at the time of recurrence
  - Median peak mitotane level: 19.6 mg/l (2.5 – 66)
- Adequate tumor evaluation (according RECIST 1.1)
- Aim:
  - Real-life data in a contemporary setting
  - Establishment of factors that could predict response to mitotane

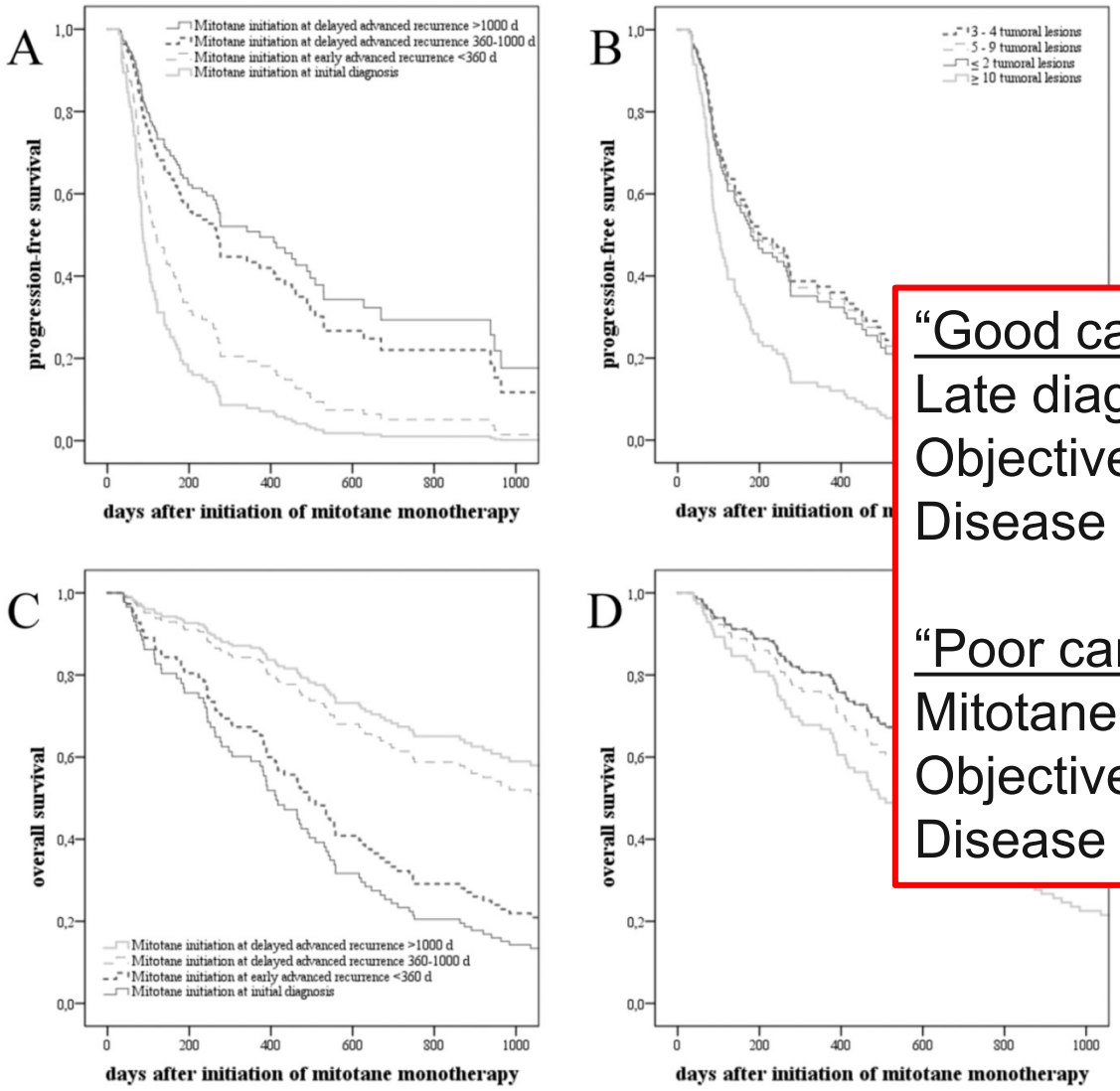
Response to mitotane in advanced ACC

Best objective response

- Complete response 3 (2.4 %)
- Partial response 23 (18.1 %)
- Stable disease 32 (25.2 %)
- Progressive disease 69 (54.3 %)

- Disease control for >6 months 50 (40.9 %)
- Disease control for >12 months 28 (22.0 %)

Predictive factors for response to mitotane



"Good candidates"

Late diagnosis of advanced recurrence + < 10 tumoral lesions

Objective response rate: 30.3 %

Disease control > 6 months 67.0 %

"Poor candidates"

Mitotane initiation < 360 days + ≥10 tumoral lesions

Objective response rate: 0 %

Disease control > 6 months: 6.3 %

What to do after failure of EDP-M?

Additional therapeutic options

- **Consider enrolment of patients in clinical trials** (www.clinicaltrial.gov)
- **Consider locoregional therapies**
- **Gemcitabine plus capecitabine** [28, 29]

  800 mg/m^2 gemcitabine on day 1 and 8 (repeated every 3 weeks)

  1500 mg capecitabine orally per day in a continuous fashion

  Mitotane can be continued (individualised decision)

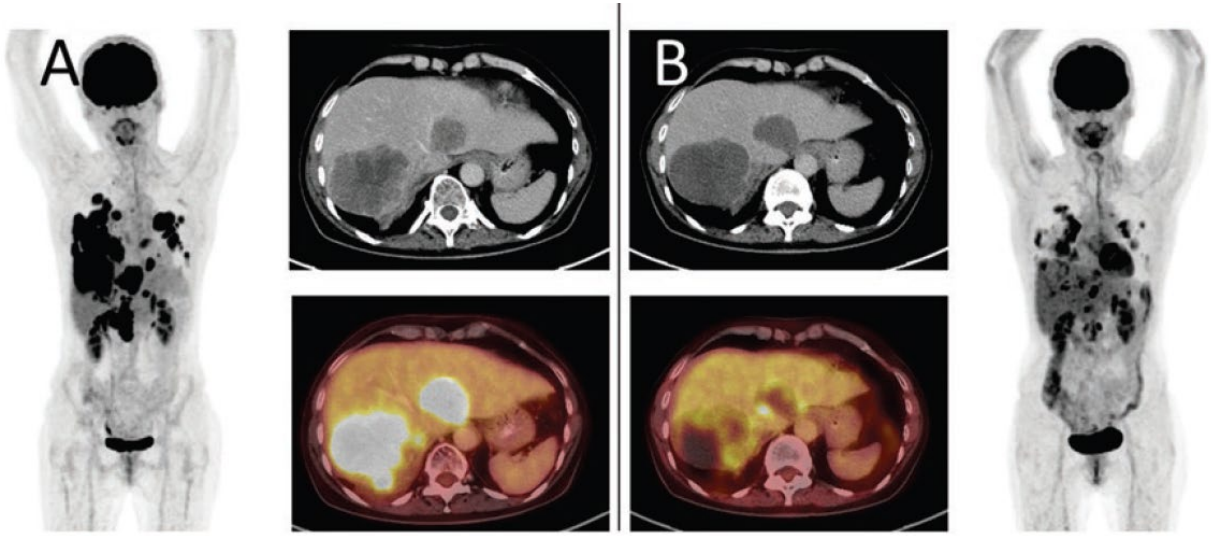
- **STZ-M** [27]

  induction: day 1–5, 1g/day STZ

  afterwards: 2g/day STZ every 21 days

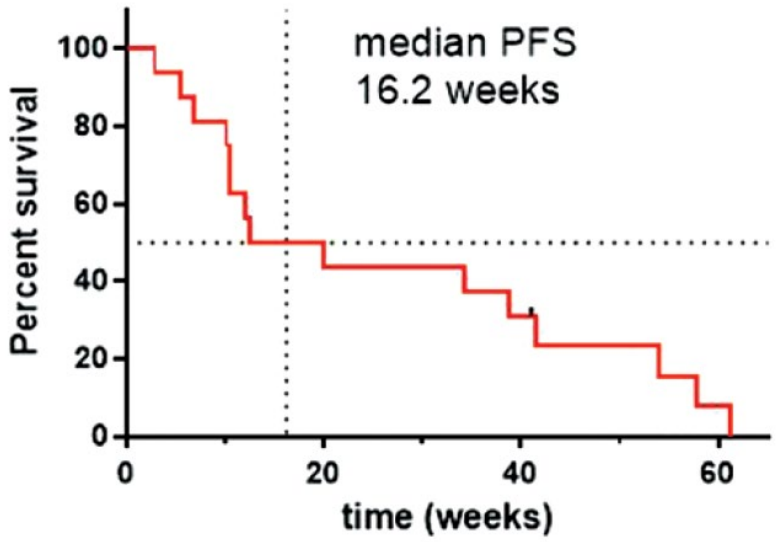
Trials with TKI failed in ACC (due to CYP3A4 induction by mitotane)
Next attempt, but now without mitotane: cabozantinib

- 16 patients with advanced ACC
- Median number of prior systemic therapy: 4 (range 0-11)



51-y-old patient

Prior cabozantinib | After 3 months cabozantinib

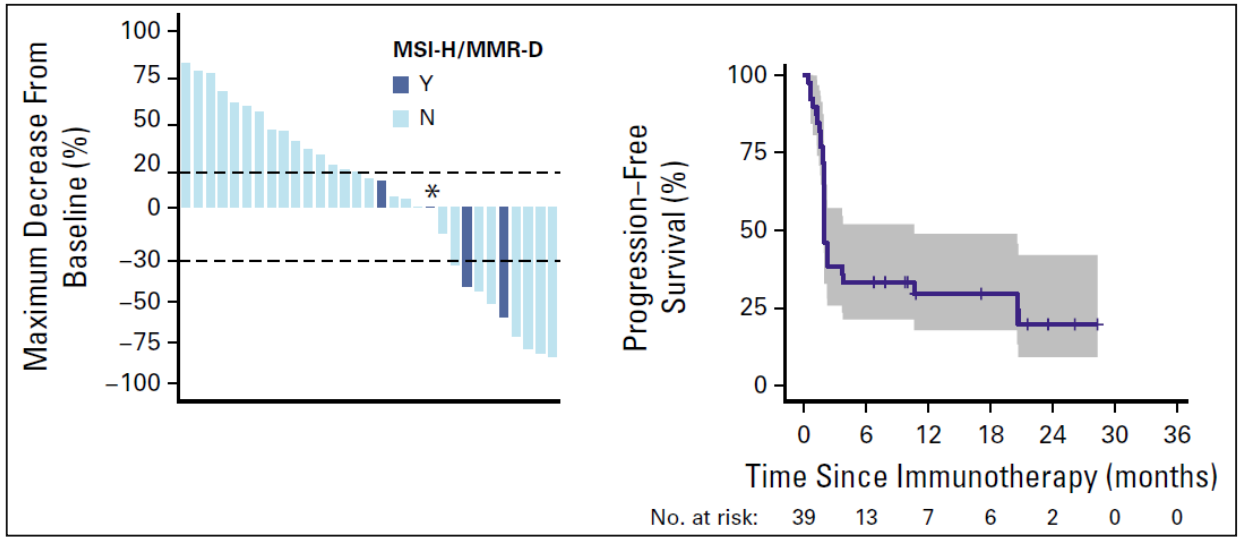


⇒ 2 parallel phase II trials are currently recruiting (organized by M. Habra and M. Kroiss)

Immunotherapy in ACC

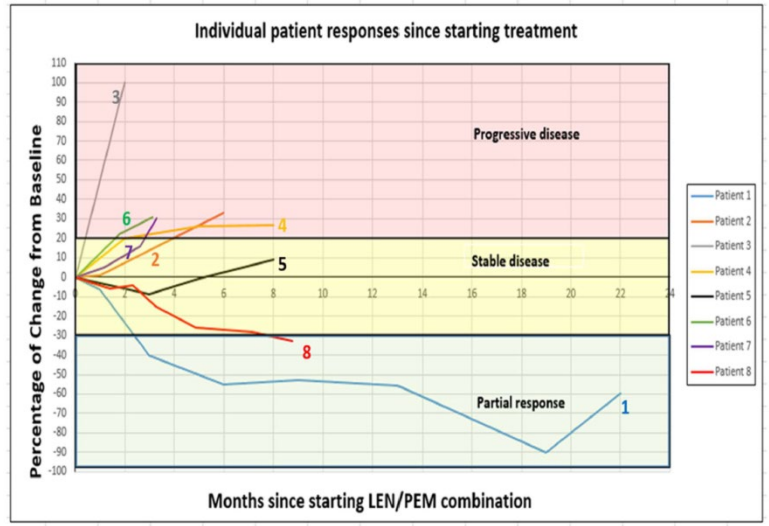
- Several (mostly small) trials have been published
- There is no doubt that a small subset of patients with ACC benefit from an immunotherapy, but the majority not

Pembrolizumab



Ray et al. J Clin Oncol 2019

Pembrolizumab + lenvatinib

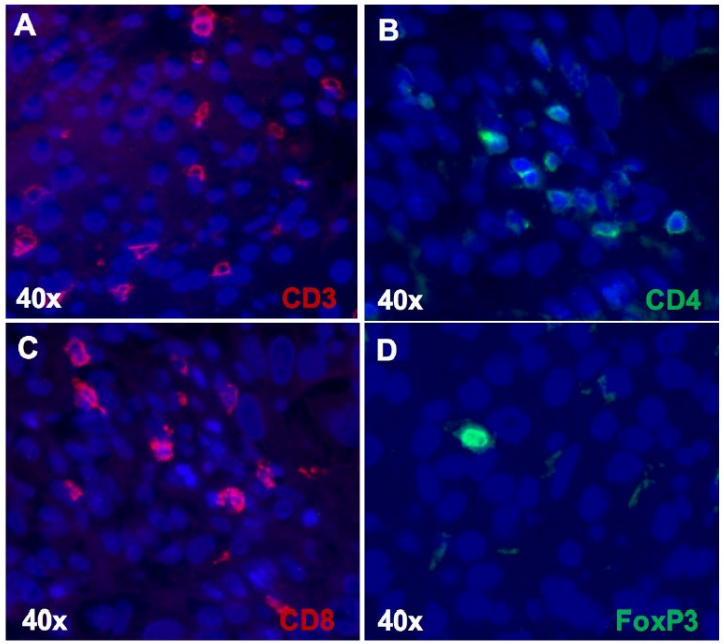


Bedrose et al. J Immunother Cancer 2020

- Key question: how can we identify the responders in advance?

Which role plays the microenvironment for an immunotherapy against ACC?

- Analysis of tumor-infiltrating lymphocytes in 146 ACC samples



Immune Cells	% of ACC	n of TILs/HPF
CD3	86.3%	7.7 (0.1-376.0)
CD4	74.0%	6.7 (0.2-109.0)
CD8	84.3%	5.7 (0.1-291.0)
FoxP3	49.3%	0.8 (0.1-18.0)

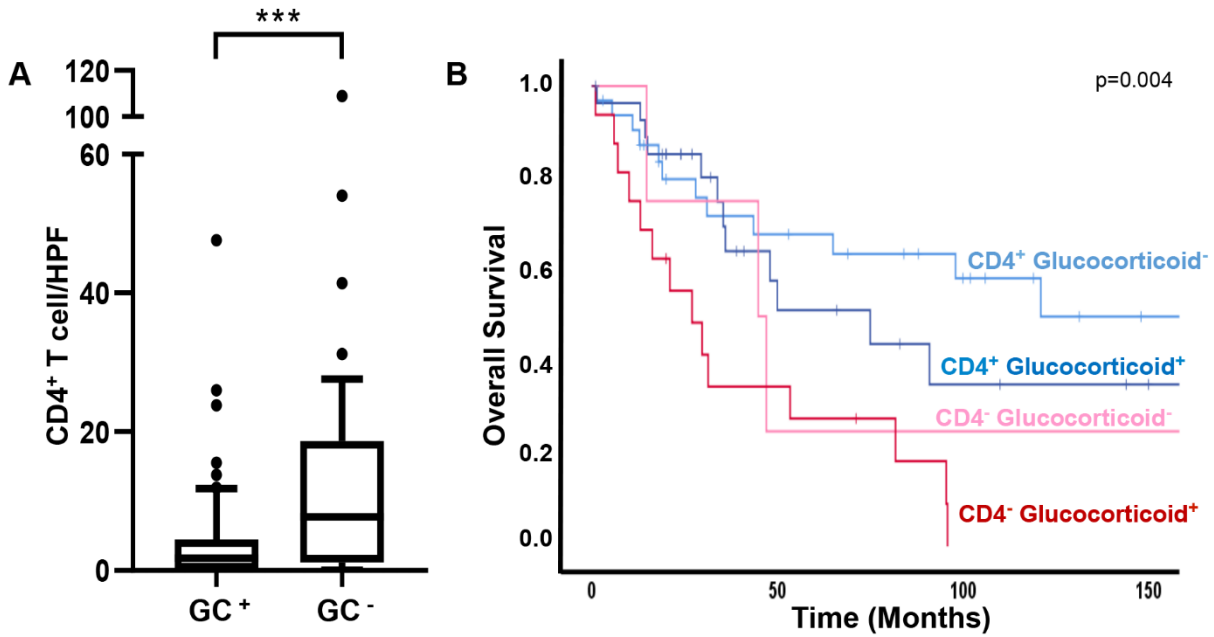


Figure 4

⇒In vivo studies are required to evaluate the therapeutical potential of this observation

Microbial Mimicry Concept for a Therapeutic Anti-Cancer Vaccine – the Spencer trial

- Hypothesis: to overcome immune resistance by
  - a therapeutic peptide vaccine composed of microbial-derived peptides mimicking cytotoxic T cell (CD8+ T cell) epitopes from the tumor associated antigens

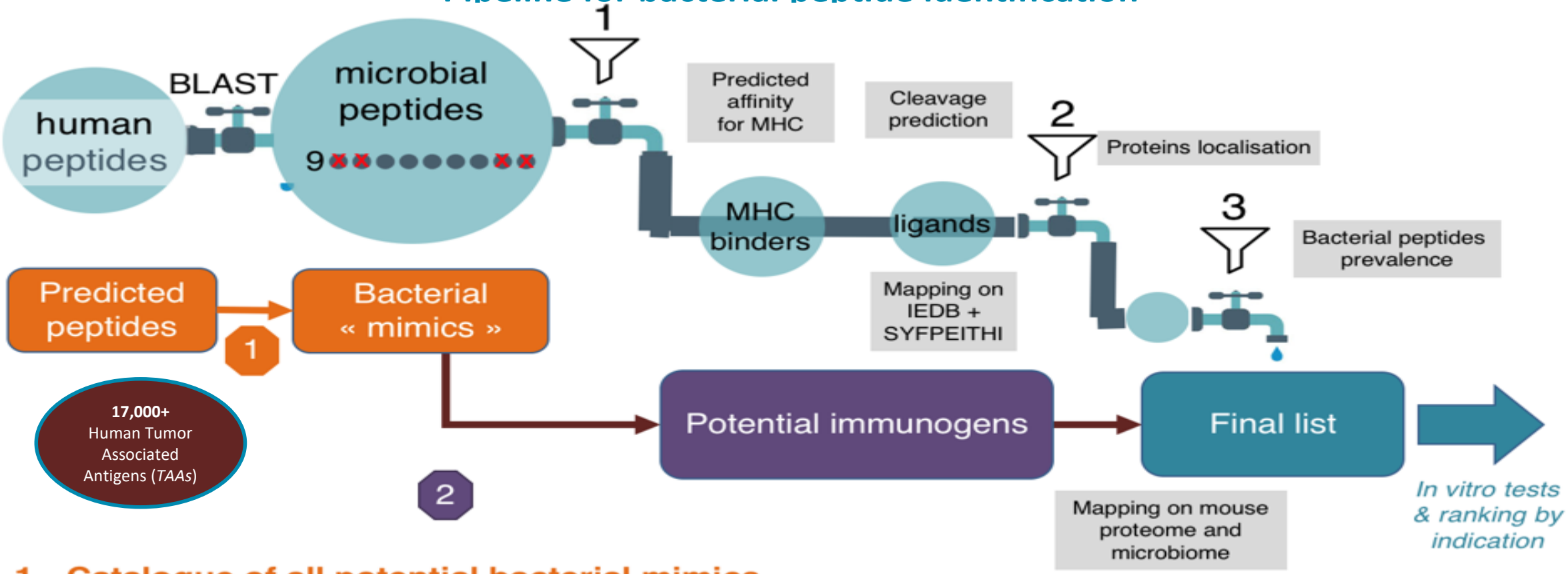
    + the helper peptide (CD4+ T cell epitope) universal cancer peptide 2 (UCP2)

    + the adjuvant Montanide ®

    + PD1 inhibitor nivolumab

EO2401 microbial-derived peptides mimicking cytotoxic T cell epitopes from TAAs

Pipeline for bacterial peptide identification



1. Catalogue of all potential bacterial mimics
2. Selection of most immunogenic peptides
3. Selection of candidates

IL13Rα2

BIRC5

FOXM1

TAAs expressed by adrenal malignancies
=>
EO2401 Therapeutic vaccine

EO2316

EO2317

EO2318

Microbial Mimicry Concept for a Therapeutic Anti-Cancer Vaccine – the Spencer trial

enterome bioscience

- Hypothesis: to overcome immune resistance by
  - a therapeutic peptide vaccine composed of microbial-derived peptides mimicking cytotoxic T cell (CD8+ T cell) epitopes from the tumor associated antigens

    + the helper peptide (CD4+ T cell epitope) universal cancer peptide 2 (UCP2)

    + the adjuvant Montanide ®

    + PD1 inhibitor nivolumab

- International phase 1/2 trial in ACC (and malignant pheo)
- Preliminary results of 33 pts. with ACC suggest some efficacy

⇒ Extension of the trial to a randomized phase II trial in planned
4:1:1 randomization EO2401+nivolumab vs EO2401 mono vs. nivolumab mono

Take home message

- Patients with suspected ACC should be treated in **specialized centers**.
- Initial diagnostic work-up (discussed in a multidisciplinary team) followed by complete surgical resection is of utmost importance.
- Most patients will likely benefit from an **adjuvant treatment with mitotane**. However, this is probably not true for “low risk patients”
- In advanced ACC, **mitotane monotherapy** or **mitotane plus EDP** are still treatments of choice.
- Monotherapy with checkpoint inhibitors is not the magic bullet
- More patients have to be treated within clinical trials.
- However, despite progress in recent years, a better understanding of the **molecular pathogenesis** is needed for real progress.

Progress is only possible as team – Thanks to all of them

Würzburg "Adrenalists"

- **Bruno Allolio**
- Matthias Kroiss
- Stefanie Hahner
- Felix Megerle
- Barbara Altieri
- Otilia Kimpel
- Michaela Haaf
- and many more

ENS@T

- Eric Baudin, France
- Jerome Bertherat, France
- Xavier Bertagna, France
- Alfredo Berruti, Italy
- Felix Beuschlein, Switzerland
- Joakim Crona, Sweden
- Harm Haak, Then Netherlands
- Darko Kastelan, Croatia
- Rossella Libe, France
- Britt Skogseid, Sweden
- Massimo Terzolo, Italy
- ……

& beyond

- Tobias Else, USA
- M.A. Habra, USA
- Gary Hammer, USA

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Funded by the European Union

Ljiljana Marina

Comorbidities in patients with adrenal incidentaloma

Comorbidities in patients with adrenal incidentaloma

Ljiljana Marina

Clinic for Endocrinology, Diabetes and Metabolic Diseases, University Clinical Centre of Serbia Faculty of Medicine, University of Belgrade

Incidentally discovered adrenal masses (AI) are reported in nearly 3% of middle aged and almost 10% of older patients undergoing abdominal imaging. After visual confirmation by CT or MRI, it is imperative to rule out the malignant nature of the adrenal mass and following that to evaluate its active hormone secretion potential. Most of these tumors are nonfunctioning (NAI), but up to 48% of patients exhibit (possible) autonomous cortisol secretion ((P)ACS) – lack of cortisol suppression after overnight 1 mg dexamethasone administration in the absence of typical signs of cortisol excess. When assessing patients with AI and (P)ACS it is important to evaluate the presence of comorbidities such as hypertension, glucose intolerance and type 2 diabetes mellitus, obesity, dyslipidemia, and osteoporosis, however, mental health issues such as depression and disturbed quality of life are emerging as important as well. Most studies highlight the overall benefit of adrenalectomy in these patients as untreated ACS carries a significant cardiometabolic burden and higher cardiovascular morbidity and mortality when compared to patients with PACS and NAI. This presentation will cover the latest updates on comorbidities in patients with NAI and (P)ACS.

Comorbidities in patients with adrenal incidentaloma

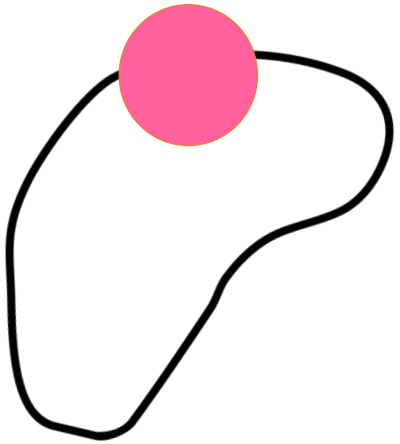
Ljiljana Marina MD PhD
Clinic for Endocrinology, Diabetes and Metabolic Diseases
University Clinical Centre of Serbia
Faculty of Medicine, University of Belgrade

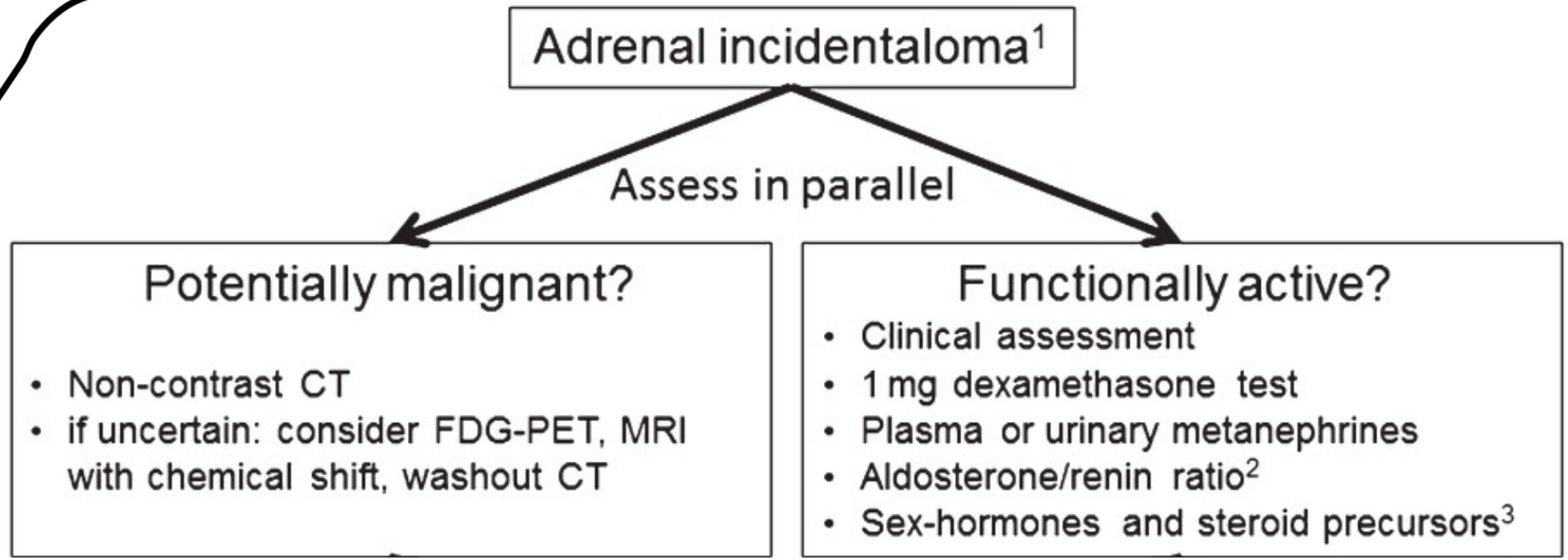
Harmonis@tion

COST ACTION CA20122

Adrenal Tumor Master Class

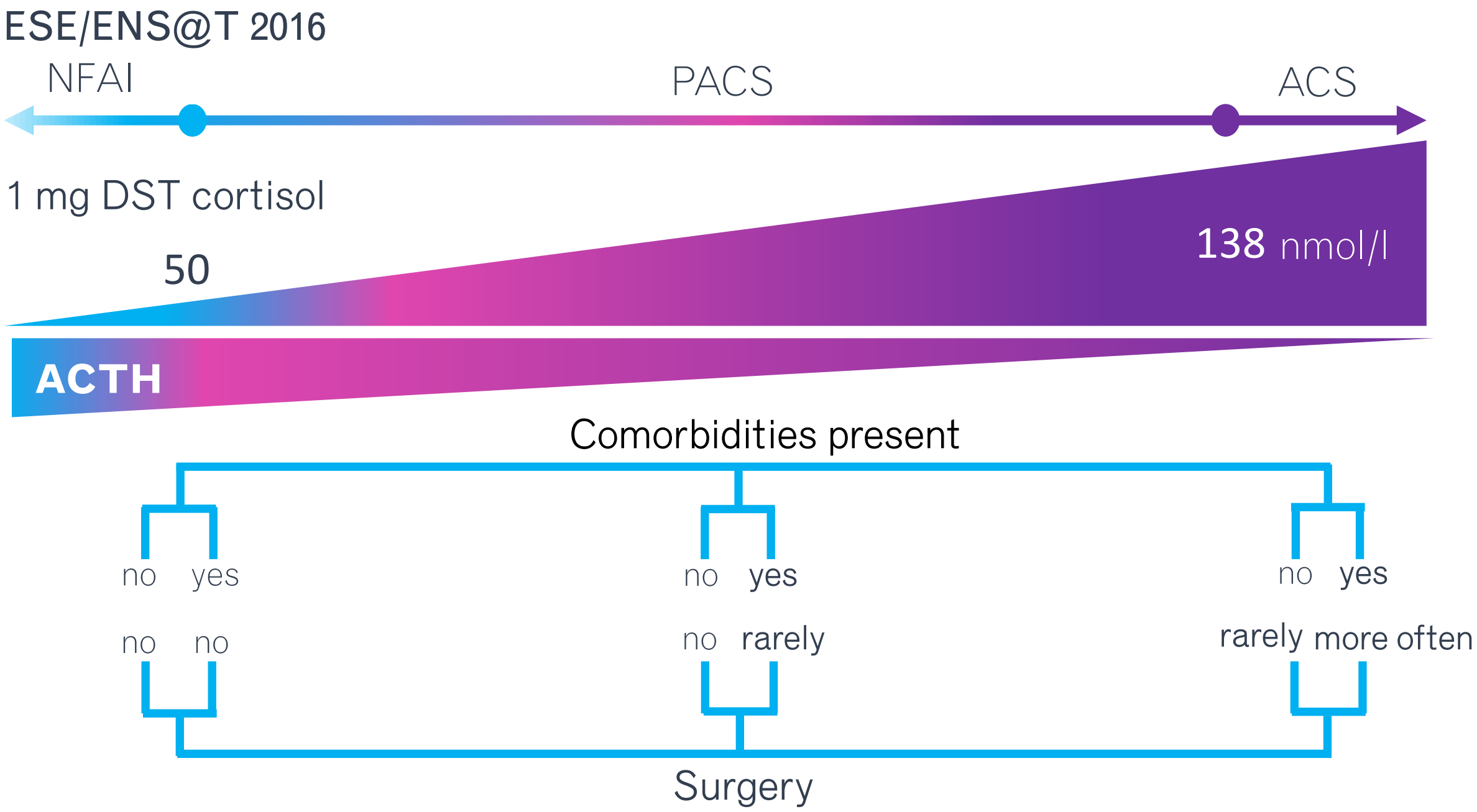
March 23, 2022



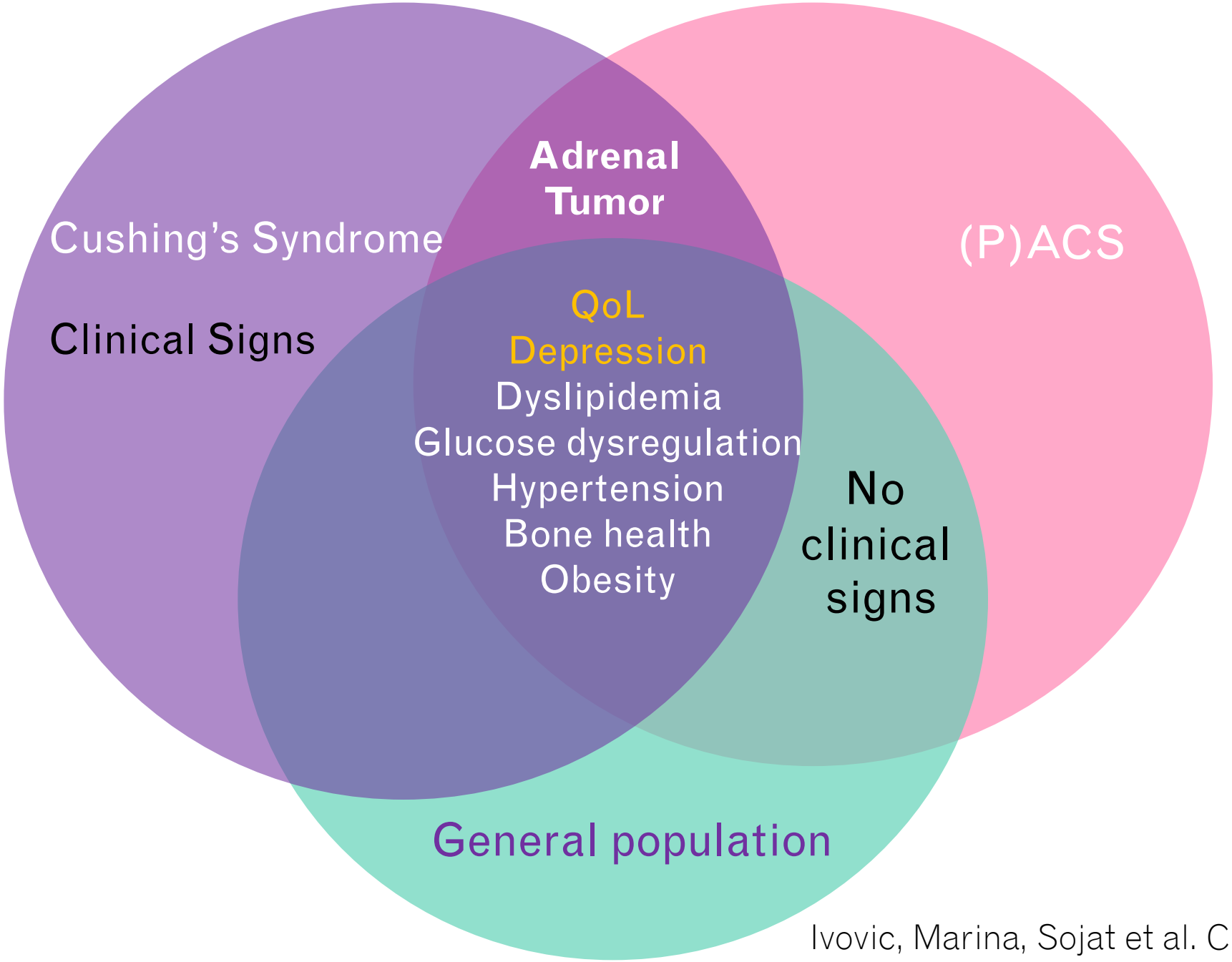


10-40% autonomous cortisol secretion
1-10% aldosterone hypersecretion

Fassnacht et al. ENSAT guideline on adrenal incidentaloma; EJE 2016



Adapted from Ivovic, Marina, Sojat et al. Curr Pharm Des 2021



Ivovic, Marina, Sojat et al. Curr Pharm Des 2021

Comorbidities in AI

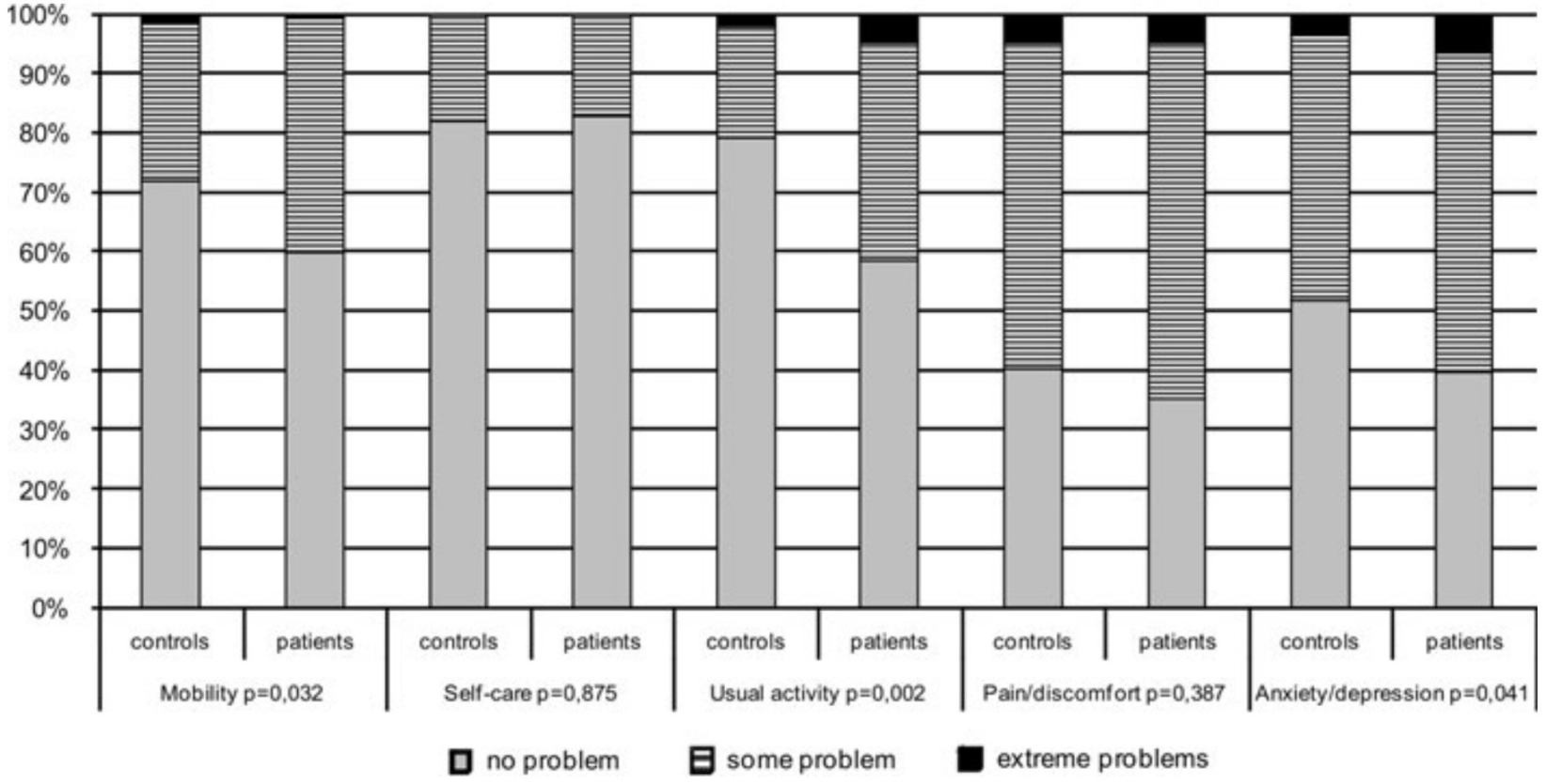
- QoL and mental health/depression
- Dyslipidemia
- Obesity
- Glucose dysregulation
- Hypertension
- Bone health

Endocrine (2011) 40:84–89
DOI 10.1007/s12020-011-9456-3

ORIGINAL ARTICLE

Health-related quality of life and fatigue in patients with adrenal incidentaloma

Darko Kastelan · Fedja Dzubur · Tina Dusek · Tamara Poljicanin · Zeljka Crncevic-Orlic · Ivana Kraljevic · Mirsala Solak · Tanja Bencevic · Izet Aganovic · Nikola Knezevic · Zeljko Kastelan · Mirko Korsic



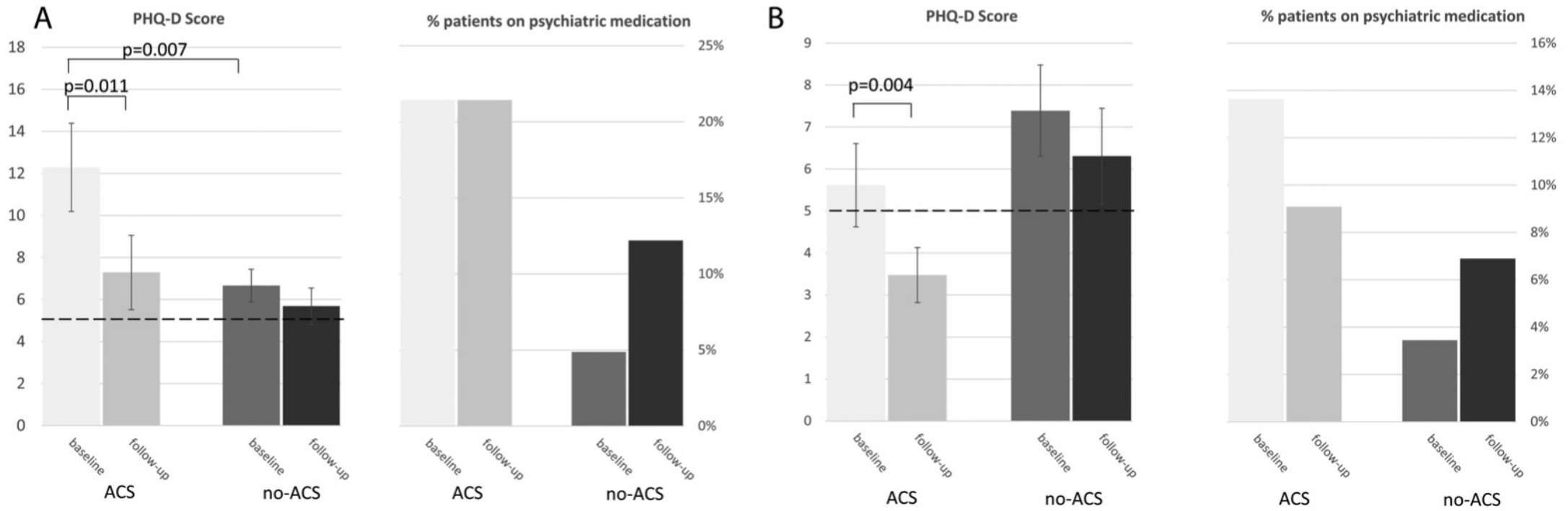
- 139 AI patients
- lower QoL
- higher anxiety/depression scores
- no difference between NAI and SCS
- cut- off value used for SCS was post 1mg DST > 83 nmol/L

Autonomous Cortisol Secretion Influences Psychopathological Symptoms in Patients With Primary Aldosteronism

Pauline Gendreitzig,[1] Heike E. Künzel,[2] Christian Adolf,[2] Laura Handgriff,[2] Lisa Müller,[2] Finn Holler,[2] Lisa Sturm,[2] Daniel A. Heinrich,[2] Martin Reincke,[2] and Marcus Quinkler[1]

The Journal of Clinical Endocrinology & Metabolism, 2021, Vol. 106, No. 6, e2423–e2433

- 298 PA patients, 46 with ACS
- Anxiety, depression and QoL



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The Journal of Clinical Endocrinology & Metabolism, 2021, Vol. 106, No. 6, e2423–e2433

- 298 PA patients, 46 with ACS

- Improvement of depression and anxiety after treatment was more pronounced in patients with PA and ACS than no-ACS

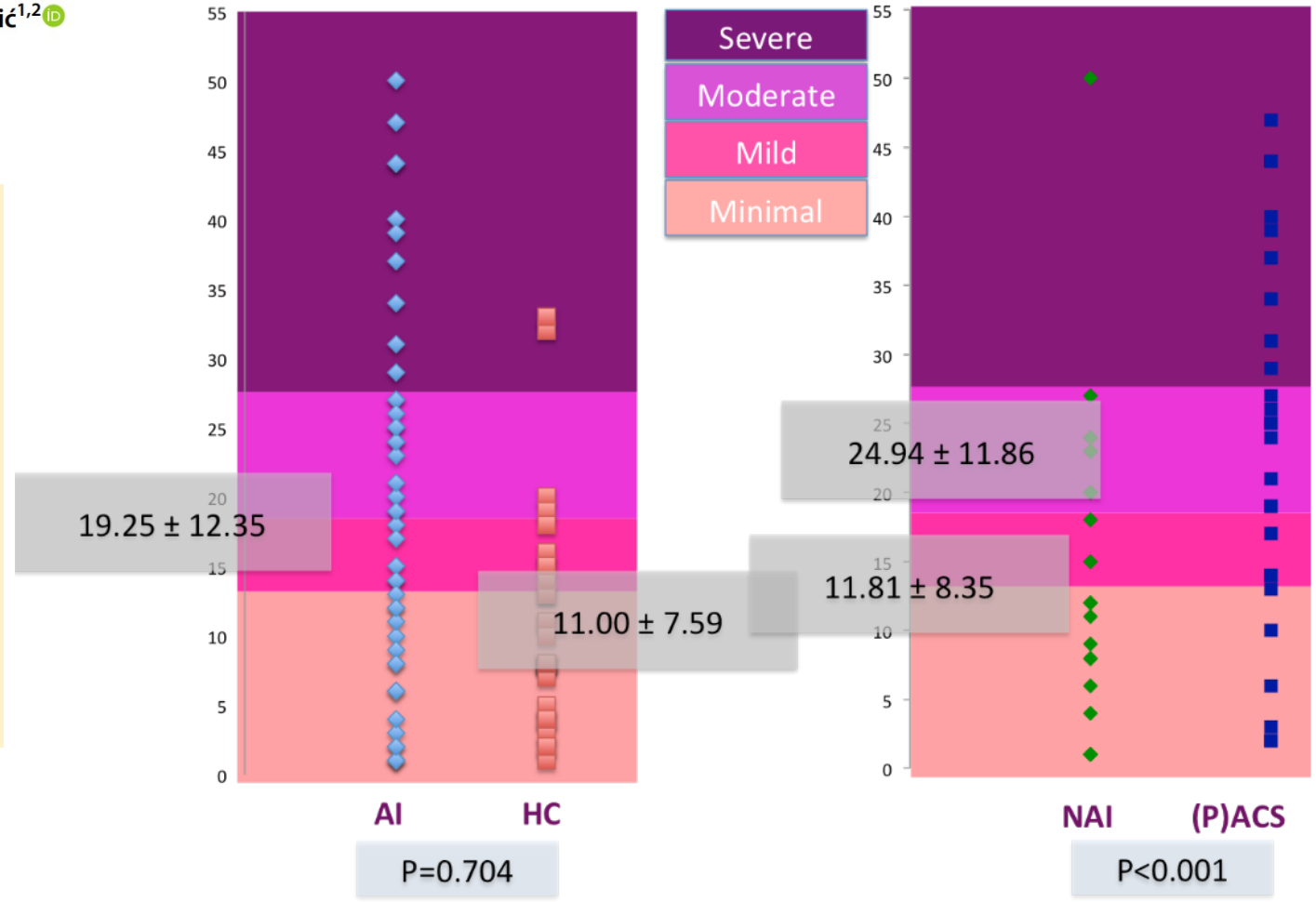
- Significant differences between sexes in depression and anxiety scores in PA patients

Depression: another cortisol-related comorbidity in patients with adrenal incidentalomas and (possible) autonomous cortisol secretion

A. S. Šojat[1] · B. Dunjić-Kostić[2,3] · L. V. Marina[1,2] · M. Ivović[1,2] · N. V. Radonjić[4] · A. Kendereški[1,2] · A. Ćirković[2,5] · M. Tančić-Gajić[1,2] · Z. Arizanović[1] · S. Mihajlović[2,6] · S. Vujović[1,2]

Journal of Endocrinological Investigation **44**, 1935–1945 (2021)

- 60 AI: 34 (P)ACS and 26 NAI
- 32 HC group
- Patients with AI and (P)ACS have significantly higher BDI-II score and significantly lower SF-36 QoL than patients with NAI and HC



European Journal of Endocrinology (2010) **162** 779–785 ISSN 0804-4643

CLINICAL STUDY

Long-term morphological, hormonal, and clinical follow-up in a single unit on 118 patients with adrenal incidentalomas

R Giordano[1], E Marinazzo[2], R Berardelli[2], A Picu[2], M Maccario[2], E Ghigo[2] and E Arvat[2]

- Significantly higher percentage of dyslipidaemia in SCS than NAI
- But no correlation between lipid profile and degree of cortisol hypersecretion

J Endocrinol Invest
DOI 10.1007/s40618-014-0232-0

ORIGINAL ARTICLE

Lipid abnormalities in patients with adrenal incidentalomas: role of subclinical hypercortisolism and impaired glucose metabolism

B. Masserini · V. Morelli · S. Palmieri · C. Eller-Vainicher · V. Zhukouskaya · E. Cairoli · E. Orsi · P. Beck-Peccoz · A. Spada · I. Chiodini

Received: 4 October 2014 / Accepted: 18 December 2014

- IGM influences lipid profile regardless of the presence of SH
- In the absence of glucose alterations, SCS has no effect on lipid pattern

Annals of Internal Medicine

ORIGINAL RESEARCH

Cardiometabolic Disease Burden and Steroid Excretion in Benign Adrenal Tumors

A Cross-Sectional Multicenter Study

Alessandro Prete, MD; Anuradhaa Subramanian, MSc; Irina Bancos, MD; Vasileios Chortis, MD, PhD; Stylianos Tsagarakis, MD, PhD; Katharina Lang, MD; Magdalena Macech, MD; Danae A. Delivanis, MD; Ivana D. Pupovac, MD; Giuseppe Reimondo, MD; Ljiljana V. Marina, MD, PhD; Timo Deutschbein, MD; Maria Balomenaki, MD; Michael W. O'Reilly, MD, PhD; Lorna C. Gilligan, MD, PhD; Carl Jenkinson, PhD; Tomasz Bednarczuk, MD, PhD; Catherine D. Zhang, MD; Tina Dusek, MD, PhD; Aristidis Diamantopoulos, MD; Miriam Asia, MSc; Agnieszka Kondracka, MD, PhD; Dingfeng Li, MD; Jimmy R. Masjkur, MD; Marcus Quinkler, MD; Grethe Å. Ueland, MD, PhD; M. Conall Dennedy, MD, PhD; Felix Beuschlein, MD; Antoine Tabarin, MD, PhD; Martin Fassnacht, MD; Miomira Ivović, MD, PhD; Massimo Terzolo, MD; Darko Kastelan, MD, PhD; William F. Young Jr., MD; Konstantinos N. Manolopoulos, MD, PhD; Urszula Ambroziak, MD, PhD; Dimitra A. Vassiliadi, MD; Angela E. Taylor, PhD; Alice J. Sitch, PhD; Krishnarajah Nirantharakumar, MD; and Wiebke Arlt, MD, DSc; for the ENSAT EURINE-ACT Investigators*

- the prevalence of dyslipidemia did not differ between NFAT (28.8%) and MACS-2 (35.9%)

THERAPY OF ENDOCRINE DISEASE

Improvement of cardiovascular risk factors after adrenalectomy in patients with adrenal tumors and subclinical Cushing's syndrome: a systematic review and meta-analysis

Irina Bancos[1], Fares Alahdab[2], Rachel K Crowley[3], Vasileios Chortis[4,5], Danae A Delivanis[1], Dana Erickson[1], Neena Natt[1], Massimo Terzolo[6], Wiebke Arlt[4,5], William F Young Jr[1] and M Hassan Murad[2]

European Journal of Endocrinology (2016) **175**, R283–R295

- no improvement in dyslipidemia after surgery in NAI and SCS

European Journal of Endocrinology
(2016) **175**, R283–R295

THERAPY OF ENDOCRINE DISEASE

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Ann Intern Med. 2016 October 18; 165(8): 533–542. doi:10.7326/M16-0547.

"Non-Functional" Adrenal Tumors and the Risk of Incident Diabetes and Cardiovascular Outcomes: A Cohort Study

Diana Lopez, MD[1,2], Miguel Angel Luque-Fernandez, PhD, MPH, MSc[3,4], Amy Steele, BA[1,5], Gail K. Adler, MD, PhD[1,2], Alexander Turchin, MD, MS[1,2,6], and **Anand Vaidya, MD, MMSc[1,2]**

- 34.6% with SCS are obese and 18.8 % with NAI
- SCS are twice as likely to gain weight during follow-up than NAI
- significant improvement in obesity after surgery in SCS but not in NAI

Comorbidities in AI

- QoL and mental health/depression
- Dyslipidemia
- Obesity
- Glucose dysregulation
- Hypertension
- Bone health

Clinical Endocrinology (2011) **74**, 300–305 doi: 10.1111/j.1365-2265.2010.03928.x

ORIGINAL ARTICLE

The size of adrenal incidentalomas correlates with insulin resistance. Is there a cause-effect relationship?

Giovanna Muscogiuri*, Gian Pio Sorice*, Annamaria Prioletta*, Teresa Mezza*, Clelia Cipolla*, Enrica Salomone*, Andrea Giaccari*,†, Alfredo Pontecorvi* and Silvia Della Casa*

- 36 patients (19 with AI and 17 HC), hyperinsulinaemic euglycaemic clamp
- 82.5% NAI, 12.5% SCS, 5% CS
- AI patients were more insulin resistant than HC
- ATS was the most powerful predictor of IR
- High prevalence of IR in NAI suggests involvement in AI growth
- Metformin should be considered in AI patients

METABOLISM CLINICAL AND EXPERIMENTAL 62 (2013) 786–792

Available online at www.sciencedirect.com

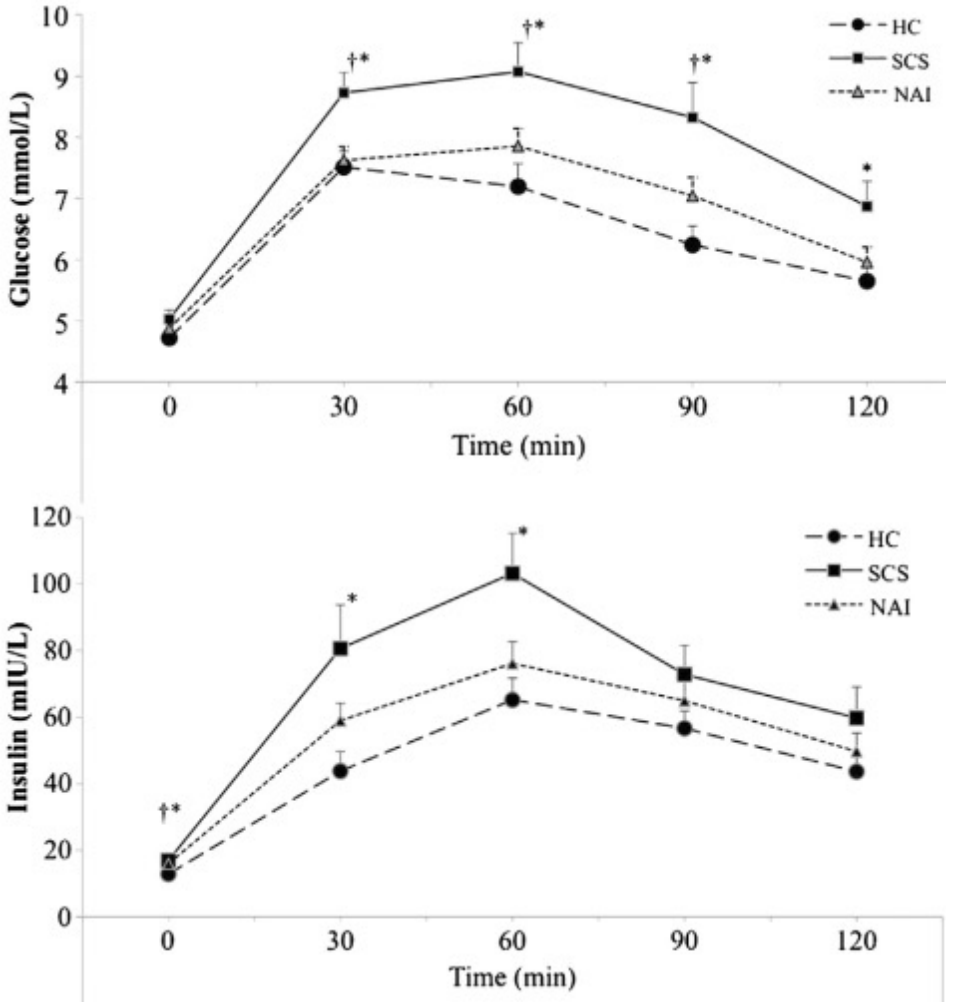
Metabolism

www.metabolismjournal.com

Nondiabetic patients with either subclinical Cushing's or nonfunctional adrenal incidentalomas have lower insulin sensitivity than healthy controls: Clinical implications

Miomira Ivović[a], Ljiljana V. Marina[a,], Svetlana Vujović[a], Milina Tančić-Gajić[a], Miloš Stojanović[a], Nevena V. Radonjić[b], Milan Gajić[c], Ivan Soldatović[c], Dragan Micić[a]*

- 142 patients: 70 NAI, 37 SCS and 35 HC
- HOMA, MATSUDA, TyG, ISI composite, G/I
- no difference in IS indexes between SCS and NAI
- SCS higher prevalence of IGT and higher AUC for glucose than NAI
- NAI: an „intermediate" form between SCS and HC



Annals of Internal Medicine

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Cardiometabolic Disease Burden and Steroid Excretion in Benign Adrenal Tumors

A Cross-Sectional Multicenter Study

Alessandro Prete, MD; Anuradhaa Subramanian, MSc; Irina Bancos, MD; Vasileios Chortis, MD, PhD; Stylianos Tsagarakis, MD, PhD; Katharina Lang, MD; Magdalena Macech, MD; Danae A. Delivanis, MD; Ivana D. Pupovac, MD; Giuseppe Reimondo, MD; Ljiljana V. Marina, MD, PhD; Timo Deutschbein, MD; Maria Balomenaki, MD; Michael W. O'Reilly, MD, PhD; Lorna C. Gilligan, MD, PhD; Carl Jenkinson, PhD; Tomasz Bednarczuk, MD, PhD; Catherine D. Zhang, MD; Tina Dusek, MD, PhD; Aristidis Diamantopoulos, MD; Miriam Asia, MSc; Agnieszka Kondracka, MD, PhD; Dingfeng Li, MD; Jimmy R. Masjkur, MD; Marcus Quinkler, MD; Grethe Å. Ueland, MD, PhD; M. Conall Dennedy, MD, PhD; Felix Beuschlein, MD; Antoine Tabarin, MD, PhD; Martin Fassnacht, MD; Miomira Ivović, MD, PhD; Massimo Terzolo, MD; Darko Kastelan, MD, PhD; William F. Young Jr., MD; Konstantinos N. Manolopoulos, MD, PhD; Urszula Ambroziak, MD, PhD; Dimitra A. Vassiliadi, MD; Angela E. Taylor, PhD; Alice J. Sitch, PhD; Krishnarajah Nirantharakumar, MD; and Wiebke Arlt, MD, DSc; for the ENSAT EURINE-ACT Investigators*

- the prevalence of T2DM did not differ between NFAT (32.1%) and MACS-2 (32.6%), but MACS-2 had increased T2DM severity than NFAT patients

THERAPY OF ENDOCRINE DISEASE

Improvement of cardiovascular risk factors after adrenalectomy in patients with adrenal tumors and subclinical Cushing's syndrome: a systematic review and meta-analysis

Irina Bancos[1], Fares Alahdab[2], Rachel K Crowley[3], Vasileios Chortis[4,5], Danae A Delivanis[1], Dana Erickson[1], Neena Natt[1], Massimo Terzolo[6], Wiebke Arlt[4,5], William F Young Jr[1] and M Hassan Murad[2]

European Journal of Endocrinology (2016) **175**, R283–R295

- there was a significant improvement in T2DM in SCS after adrenalectomy
- insufficient data and small cohorts for NAI patients

Annals of Internal Medicine

ORIGINAL RESEARCH

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Annals of Internal Medicine

REVIEW

Natural History of Adrenal Incidentalomas With and Without Mild Autonomous Cortisol Excess

A Systematic Review and Meta-analysis

Yasir S. Elhassan, MBBS; Fares Alahdab, MD; Alessandro Prete, MD; Danae A. Delivanis, MD, PhD; Aakanksha Khanna, MD; Larry Prokop, MLS; Mohammad H. Murad, MD, MPH; Michael W. O'Reilly, PhD; Wiebke Arlt, MD, DSc; and Irina Bancos, MD

- Hypertension was the most reported comorbid condition
- More frequently found in MACE than NFAT: 64.0% vs. 58.2%
- During follow-up: a new diagnosis of hypertension, 8.4% vs. 5.2% and worsening of preexisting hypertension,13.4% vs. 4.8%.
- Prevalence and severity of hypertension were higher in MACS-2 and CS than NFAT

European Journal of Endocrinology
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- significant improvement in hypertension in both SCS and NAI

MECHANISMS IN ENDOCRINOLOGY

Endogenous subclinical hypercortisolism and bone: a clinical review

I Chiodini[1], C Eller Vainicher[1], V Morelli[1,2], S Palmieri[1,2], E Cairoli[1,2], A S Salcuni[3], M Copetti[4] and A Scillitani[5]

European Journal of Endocrinology
(2016) **175**, R265–R82

- SH may increase risk of vertebral fractures
- Glucocorticoids impair osteoblastic function and increase bone resorption
- Most studies found a reduction in trabecular BMD measured at spine by DXA
- SH probably afects trebecular bone at sipne and possibly cortical bone at femur
- AI patients with SH, who underwent the surgical removal of the adrenal mass, had a strong reduction in the probability of a new vertebral fracture

Cardiovascular events and mortality in patients with adrenal incidentalomas that are either non-secreting or associated with intermediate phenotype or subclinical Cushing's syndrome: a 15-year retrospective study

Lancet Diabetes Endocrinol
2014; 2: 396–405

Guido Di Dalmazi, Valentina Vicennati, Silvia Garelli, Elena Casadio, Eleonora Rinaldi, Emanuela Giampalma, Cristina Mosconi, Rita Golfieri, Alexandro Paccapelo, Uberto Pagotto, Renato Pasquali

- Higher incidence of CV events in stable SCS and worsening SCS than NAI
- Higher all cause mortality in stable SCS and worsening SCS than NAI

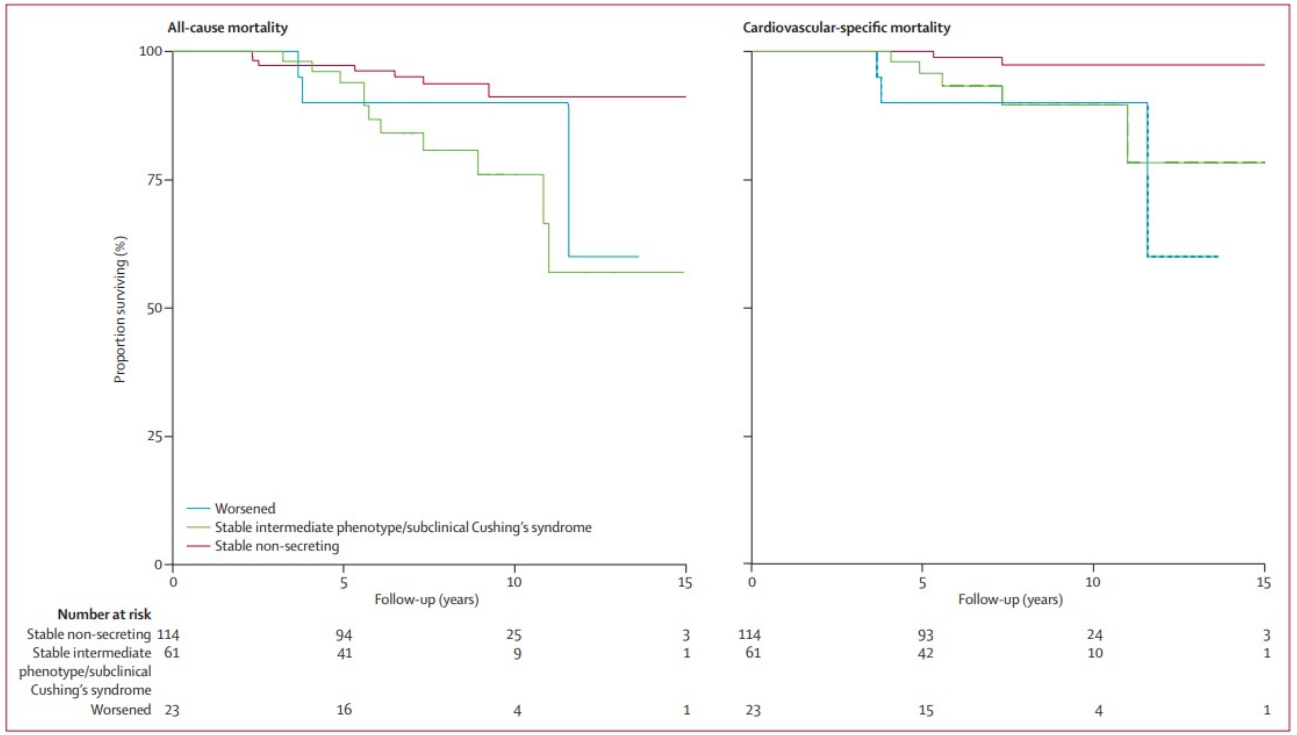

Figure 2: Kaplan-Meier curves showing all-cause and cardiovascular-specific mortality

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ORIGINAL RESEARCH

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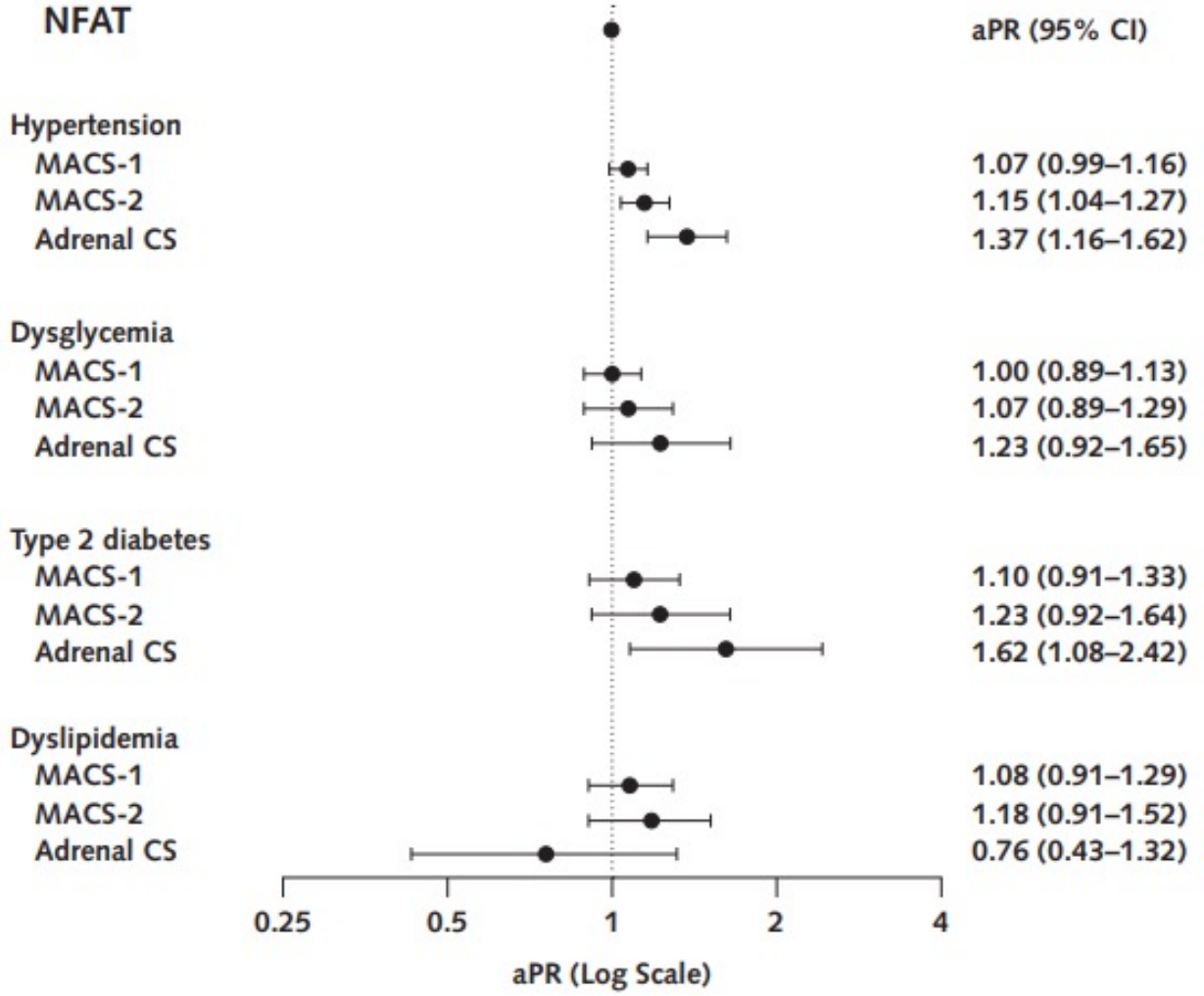
- MACS-2 vs NFAT
  - affects women more than men
  - increased prevalence and severity of hypertension
  - increased severity of T2DM
  - the prevalence of dyslipidemia did not differ
- MACS-2 carries an increased cardiometabolic burden



Why does it affect women more than men?

CLINICAL ENDOCRINOLOGY

ORIGINAL ARTICLE

Luteinizing hormone and insulin resistance in menopausal patients with adrenal incidentalomas: The cause-effect relationship?

Ljiljana V. Marina, Miomira Ivović, Milina Tančić-Gajić, Zorana Arizanović, Dragana Raković, Jelena Milin-Lazović, Aleksandra Kendereški, Dragan Micić, Svetlana Vujović

First published: 30 December 2017 | https://doi.org/10.1111/cen.

Review

Cell PRESS

Is the adrenal cortex a target for gonadotropins?

Sophie Bernichtein[1,3], Maria Alevizaki[2] and Ilpo Huhtaniemi[1]

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[2] Endocrinology, Metabolism and Diabetes Unit, Evgenidion Hospital and Department of Medical Therapeutics, ALEXANDRA Hospital, Athens University School of Medicine, Athens, Greece
[3] Current address: INSERM U845, Centre de Recherche Croissance et Signalisation, Faculté de Médecine Necker, 156 rue de Vaugirard, 75730 Paris Cedex 15, France

0013-7227/01/$03.00/0
Printed in U.S.A.

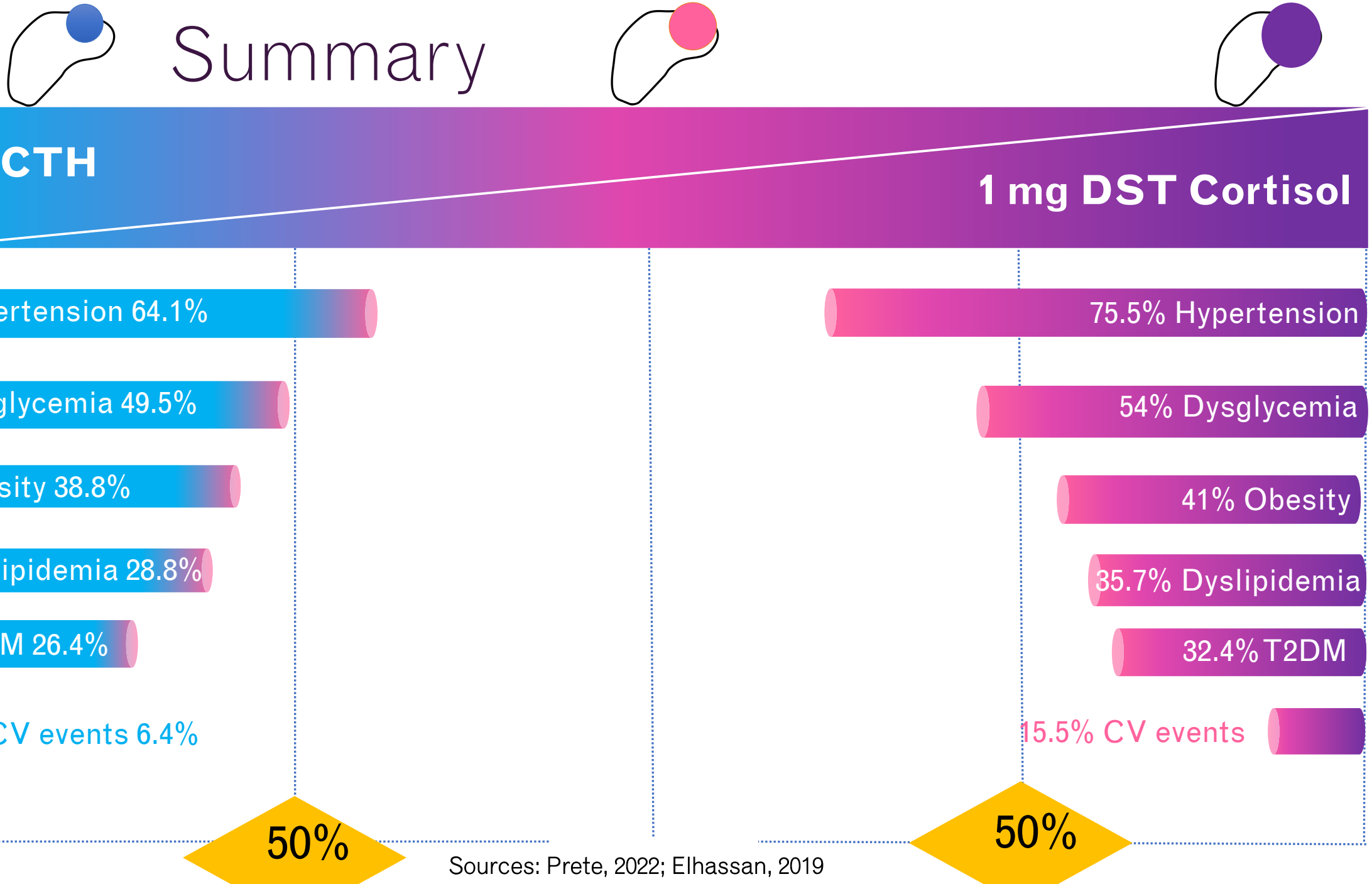
The Journal of Clinical Endocrinology & Metabolism 86(11):5534–5540

Aberrant Membrane Hormone Receptors in Incidentally Discovered Bilateral Macronodular Adrenal Hyperplasia with Subclinical Cushing's Syndrome

ISABELLE BOURDEAU, PIERRE D'AMOUR, PAVEL HAMET, JEAN-MARIE BOUTIN, AND ANDRÉ LACROIX

Division of Endocrinology, Department of Medicine, Research Center, Hôtel-Dieu and Hôpital Saint-Luc du Centre Hospitalier de l'Université de Montréal, Montréal, Canada H2W 1T8

Summary

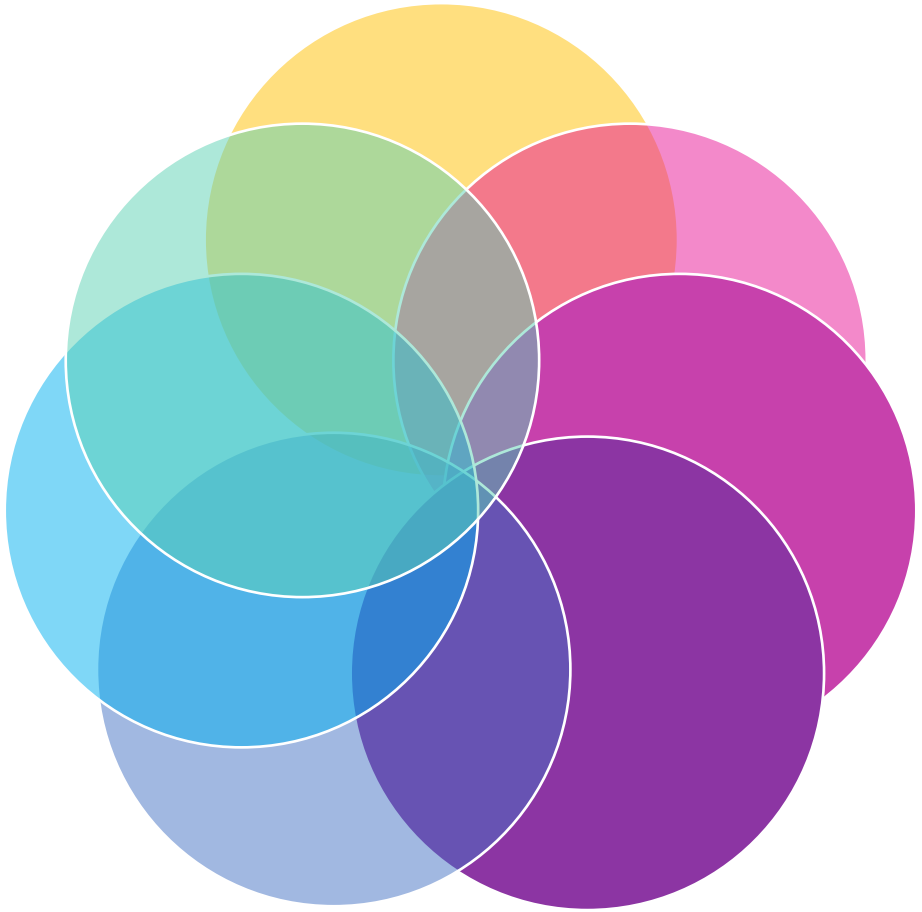


Sources: Prete, 2022; Elhassan, 2019

Summary

Sex

Medical therapy if surgery delayed or not possible



Age

Time / duration / progression

Comorbidities

Surgery

Personal preference

Funded by the European Union

Felix Beuschlein

Diagnosis and subtype differentiation of primary aldosteronism

Diagnosis and subtype differentiation of primary aldosteronism

Felix Beuschlein

University Hospital Zurich, Switzerland

Primary aldosteronism (PA) has emerged as the most frequent cause of curable arterial hypertension. Around 7% of hypertensive patients in population based studies and up to 20% of patients with resistant hypertension attending specialized centers are affected by primary aldosteronism. Patients are mainly suffering from severe hypertension and varying degrees of hypokalemia and metabolic alkalosis. Early detection and treatment of affected patients reduces cardiovascular, renal and metabolic co-morbidities and mortality. The majority of patients can be classified as either unilateral aldosterone producing adenoma or bilateral adrenal hyperplasia. Differential diagnostic work-up incorporates adrenal imaging and adrenal vein sampling. Treatment options diverge between the two subtypes, with unilateral adrenalectomy being the treatment of choice in aldosterone producing adenoma and administration of mineralocorticoid-antagonists the therapy for patients with bilateral adrenal hyperplasia. Current guidelines offer recommendations towards case finding and diagnostic approaches for affected patients. Epidemiological evidence and data from international patient registries provide the basis for algorithms that aid in risk stratification and assessment of likely therapeutic response and clinical outcome. Finally, new technological approaches including steroid and metabolic signatures might streamline diagnostic pathways. The presentation will provide an update on the current state of the art in personalized approaches and the yet achieved spectrum of precision medicine for patients with primary aldosteronism.

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Diagnosis and subtype differentiation of primary aldosteronism

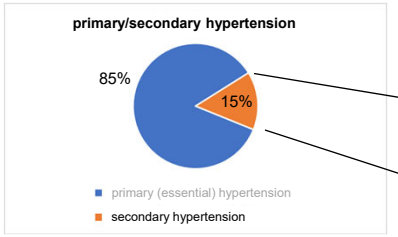
Felix Beuschlein

23 – 25 March 2022

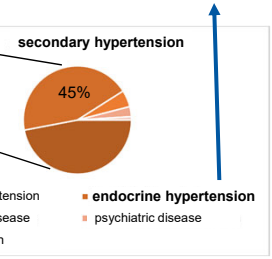
COST Harmonis@tion Adrenal Tumor Master Class

Arterial Hypertension

In an European population >60 years,
hypertension is the **most prevalent clinical diagnosis**.
About 20% of the adult population have elevated blood pressure



Primary aldosteronism



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Commentary 251

Primary Aldosteronism: Are We Missing the Wood for the Trees?

Author J. W. Funder
Affiliation Prince Henry's Institute, Clayton, Victoria, Australia

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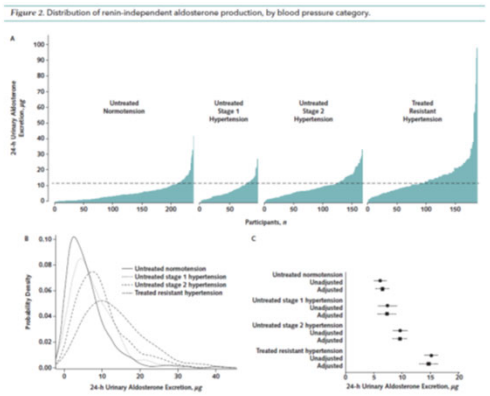
Funder JW. Horm Metab Res. 2012 Mar;44(3):251-3

The Unrecognized Prevalence of Primary Aldosteronism



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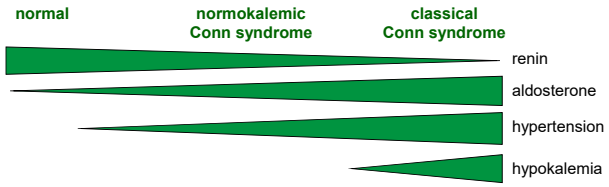
Brown JA, et al. Ann Intern Med. 2020;173:10-20. doi:10.7326/M20-0065

Spectrum of primary aldosteronism



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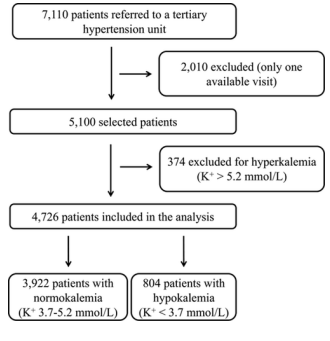
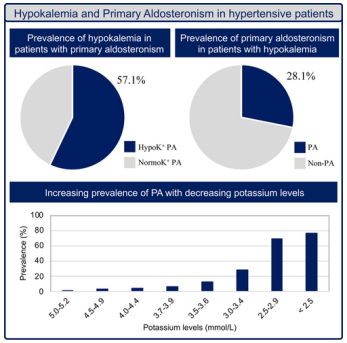
Prevalence of hypokalemia and PA in hypertensive patients





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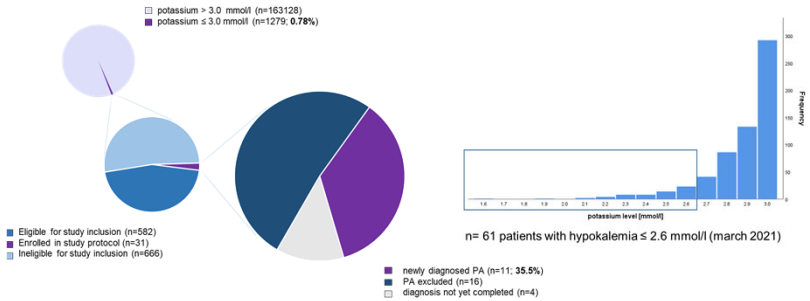
Burello J et al., Hypertension. 2020;75:1025–1033

Incidence of primary aldosteronism in patients with hypokalemia (IPAHK+)

- a total of 163,128 outpatient potassium measurements have been performed at USZ outpatient clinics. 1279 measurements, corresponding to 0.78%, revealed potassium levels ≤ 3.0 mmol/l



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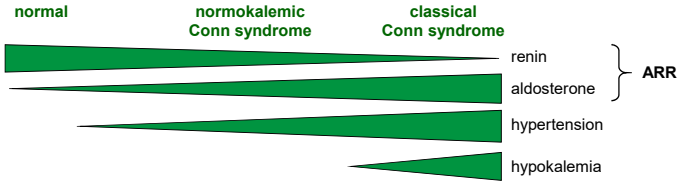
Spectrum of primary aldosteronism



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Influence of medication on ARR

- ß-blocker
- central alpha-agonist

→ aldo ↑ renin → false positive result

- ACE inhibitors
- diuretics
- Ang II receptor
- MRA

→ negative results

one week before determination of ARR:
- verapamil: max 2x240mg
- doxazosin (4-8mg/d) **or** urapidil (max 2x90mg)
- if required: dihydralazin (max 2x50mg)

- calcium channe
- alpha blockers
- vasodilator

→ aldo ↔ renin → little influence on diagnostics

pause 4 weeks prior testing – pause 1 week prior testing – maintain and/or adapt dosage

Consensus German Conn registry

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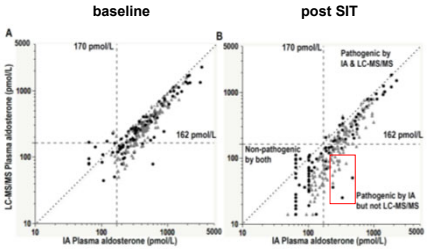
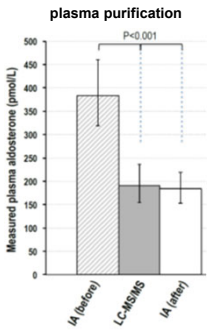
Influence of the assay on ARR





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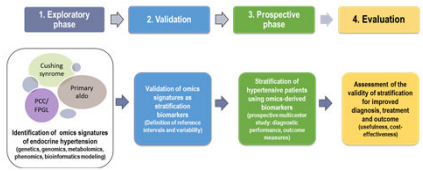
Eisenhofer G et al. J Clin Endocrinol Metab. 2021 Dec 28

Differential diagnosis of hypertension (ENSAT-HT)

- EU funded Horizon 2020 research and innovation project that investigates specific types of hypertension and whether it is possible to define profiles based on systems biology "-omics" approaches
- plasma and urine steroidobolomics, plasma metanephrines, miRNA profiles, urine and plasma NMR profiles, small metabolites
- retrospective and prospective phase



ens@t-ht

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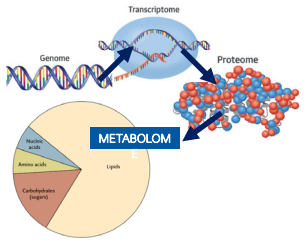
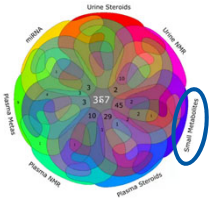
Differential diagnosis of hypertension (ENSAT-HT)

- retrospective phase: complete sample set from 282 patients with different forms of hypertension
- plasma targeted metabolomics:

  high-throughput parallel identification and quantification of pre-selected metabolites with known chemical and biochemical annotation





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Differential diagnosis of hypertension (ENSAT-HT)

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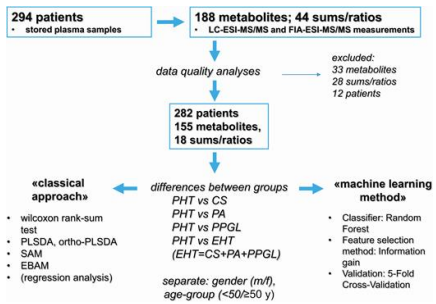

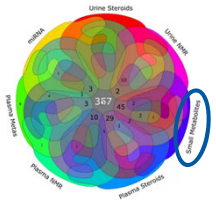

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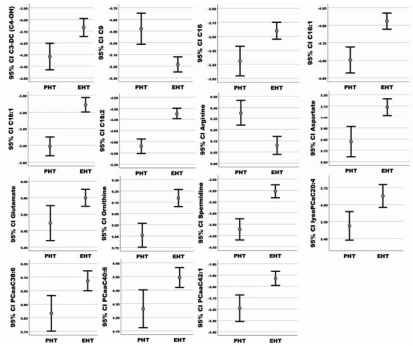
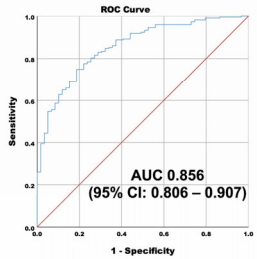
Erlic Z et al. JCEM, 2021, Vol. 106, No. 4, 1111–1128

Differential diagnosis of hypertension (ENSAT-HT)

Asp
Arg
C16
C16:1
C18:1
C18:2
C3-DC C4-OH
C9
Glu
lysoPC a C20:4
Orn
PC aa C38:6
PC aa C40:6
PC aa C42:1
Spermidine





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Erlic Z et al. JCEM, 2021, Vol. 106, No. 4, 1111–1128

1. screening — aldosterone/renin ratio

2. proof of diagnosis — sodium infusion test
oral sodium loading
urinary aldost.-18-glucuronide
captopril testing

1. Screening — aldosterone/renin ratio

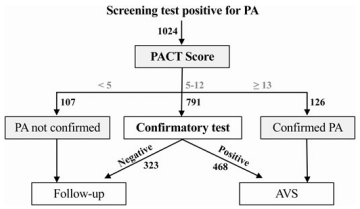
no confirmatory testing if hypokalemia and high aldosterone (>20 ng/dL)

spironolactone therapy for patients, in whom no further testing is possible or desired

Funder et al. J Clin Endocrinol Metab. 2016 May;101(5):1889-916

A

Variable	Label	Points
Sex	Female	0
	Male	2
Antihypertensive medication (DDD)	< 1	0
	≥ 1	1
PRA at screening (ng/mL/h)	< 0.20	3
	0.20-0.99	1
	≥ 1.00	0
Aldosterone at screening (ng/dL)	< 16.0	0
	16.0-27.9	2
	≥ 28.0	4
Lowest potassium (mEq/L)	< 3.7	4
	3.7-3.9	2
	≥ 4.0	0
Organ damage	Yes	2
	No	0



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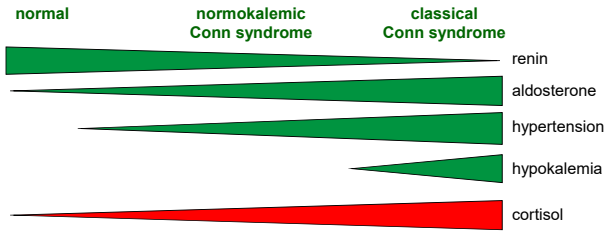
Burello J al. J Clin Endocrinol Metab. 2021 Mar 25;106(4):e1708-e1716

Further hormonal spectrum of primary aldosteronism



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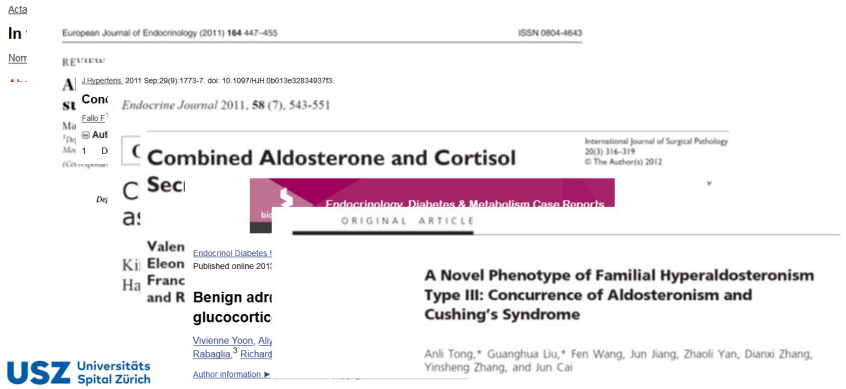
Indication of co-secretion of aldosterone and cortisol from the literature



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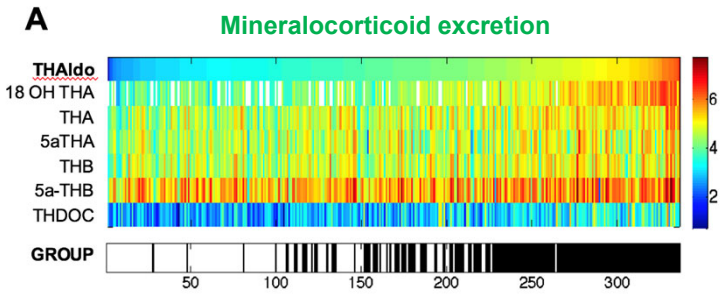
Steroid metabolomics in primary aldosteronism



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Arlt et al. JCI Insight. 2017 Apr 20;2(8).

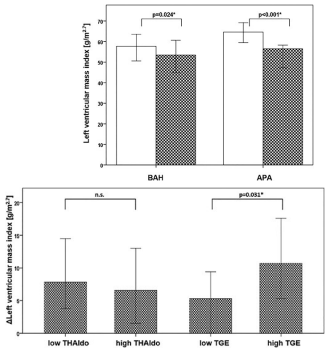
Glucocorticoid Excretion and Metabolic Risk in primary aldosteronism

Approximate percentage change (95% CI; p) per unit change in the metabolic risk parameter

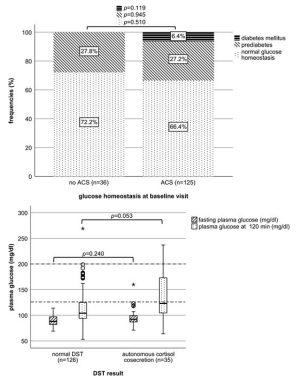
	Total Glucocorticoids	TetraHydroAldo
BMI	2.5 (0.8 4.2), **p=0.004**	1.1(-1.2, 3.4), p=0.35
Waist circumference	1.3 (0.6-2.0), **p<0.001**	0.3 (-0.6, 1.2), p=0.56
Fasting insulin	0.8 (0.4-1.3), **p=0.001**	0.1 (-0.5, 0.7), p=0.82
120 min insulin (oGTT)	0.2 (0.1, 0.3), **p=0.003**	0.1 (-0.1, 0.2), p=0.32
HOMA-IR	3.4 (1.3-5.4), **p=0.001**	0.0 (-2.5, 2.6), p=0.98
HbA1c	18.1 (1.8, 38.0), p=0.07	6.7 (-15.8, 29.2), p=0.56
LDL cholesterol	0.2 (0.0, 0.5), p=0.05	0.1 (-0.2, 0.4), p=0.38
HDL cholesterol	-0.9 (-1.4, -0.4), **p<0.001**	-0.4 (-1.1, 0.2), p=0.21
Systolic blood pressure	0.4 (0.0, 0.8), p=0.07	0.6 (0.1, 1.1), p=0.02
Diastolic blood pressure	0.9 (0.3, 1.5), **p=0.007**	0.3 (-0.5, 1.2), p=0.48

Cortisol co-secretion in primary aldosteronism

Left ventricular hypertrophy



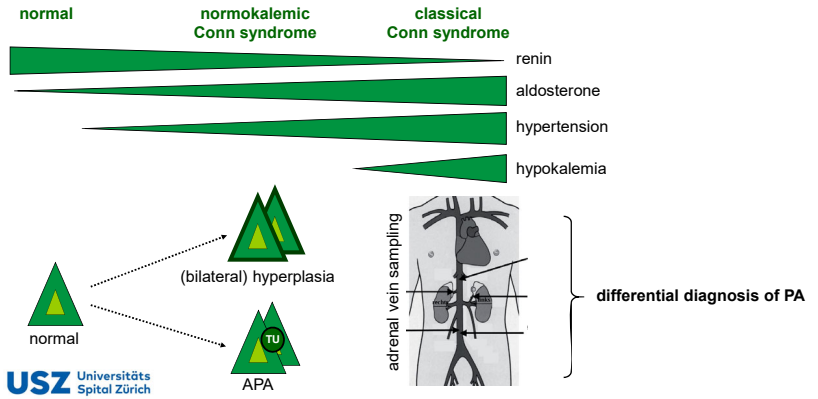
Glucose metabolism



Adolf C et al. JCEM, 2018 Dec 1;103(12):4543-4552

Gerards J et al. JCEM, 2019 Aug 1;104(8):3192-3202

Spectrum of primary aldosteronism



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1. screening	aldosterone/renin ratio
2. proof of diagnosis	sodium infusion test orale sodium loading urinary aldoster.-18-glucuronide captopril testing
3. differential diagnosis	adrenal CT- or MRI

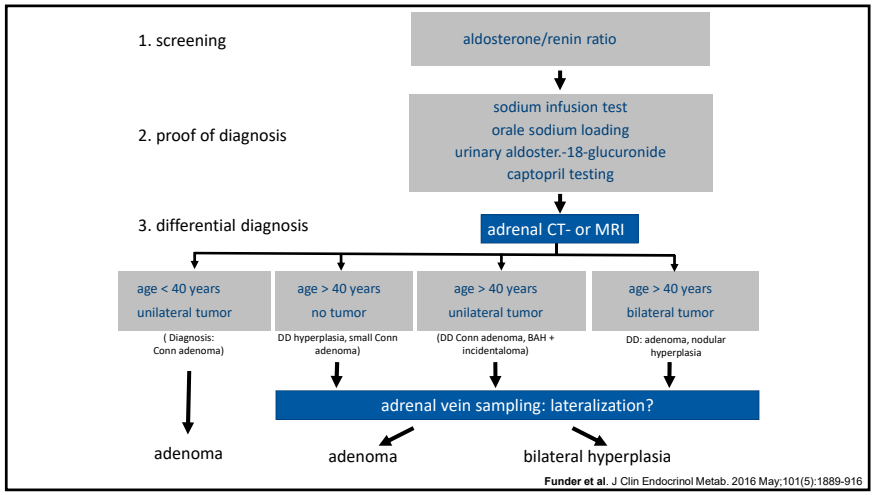
Questions to answer:
- exclusion of a (very rare) aldosterone producing ACC
- presence/absence of an adrenal adenoma
- information on general adrenal morphology
- information on vascular situation (in case of AVS)

Funder et al. J Clin Endocrinol Metab. 2016 May;101(5):1889-916

1. screening	aldosterone/renin ratio
2. proof of diagnosis	sodium infusion test orale sodium loading urinary aldoster.-18-glucuronide captopril testing
3. differential diagnosis	adrenal CT- or MRI

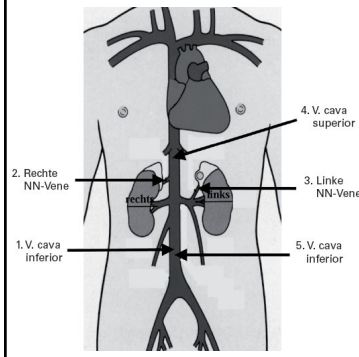
spironolactone therapy for patients, in whom no further testing is possible or desired

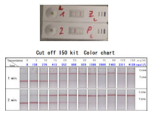
Funder et al. J Clin Endocrinol Metab. 2016 May;101(5):1889-916



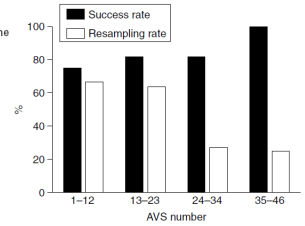
Funder et al. J Clin Endocrinol Metab. 2016 May;101(5):1889-916

Performance of adrenal vein sampling





rapid cortisol measurement



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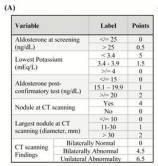
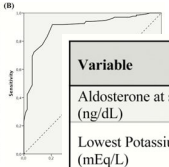
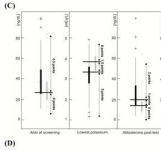
Diederich et al. Med. Klinik 2007

Betz M et al., Eur J Endocrinol. 2011 Aug;165(2):301-6

Clinical prediction scores for lateralization – the Torino example

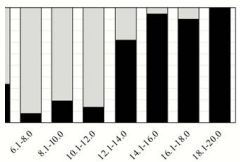






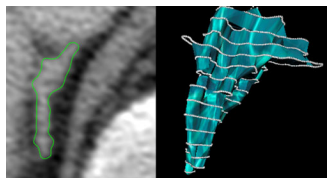
Variable	Label	Points
Aldosterone at screening (ng/dL)	</= 25	0
	> 25	0.5
Lowest Potassium (mEq/L)	< 3.4	5
	3.4 - 3.9	1.5
	>/= 4	0
Aldosterone post-confirmatory test (ng/dL)	</= 15	0
	15.1 – 19.9	1
	>/= 20	2
Nodule at CT scanning	Yes	4
	No	0
Largest nodule at CT scanning (diameter, mm)	</= 10	0
	11-30	1
	> 30	2
CT scanning Findings	Bilaterally Normal	0
	Bilaterally Abnormal	4.5
	Unilateral Abnormality	6.5

ertiary hypertension unit. Diagnostic
nd tested in a training (N = 150)
ation cohort (N = 65), respectively.
ated in an external independent

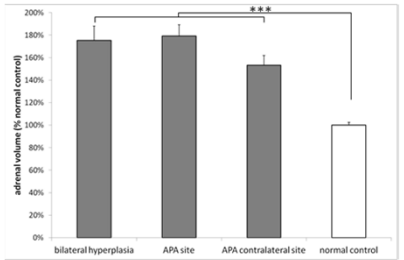


Burello J et al., JCEM, October 2020, Pages e3706–e3717

Adrenal volumetry in primary aldosteronism



Schneller J, et al. Acad Radiol. 2014;21(11):1465-74



p-values	bilateral hyperplasia	APA site	APA contralateral site	normal control
bilateral hyperplasia	-	0.81	0.15	4.64×10^{-19}
APA site	0.81	-	0.06	5.01×10^{-25}
APA contralateral site	0.15	0.06	-	4.49×10^{-15}
normal control	4.64×10^{-19}	5.01×10^{-25}	4.49×10^{-15}	-

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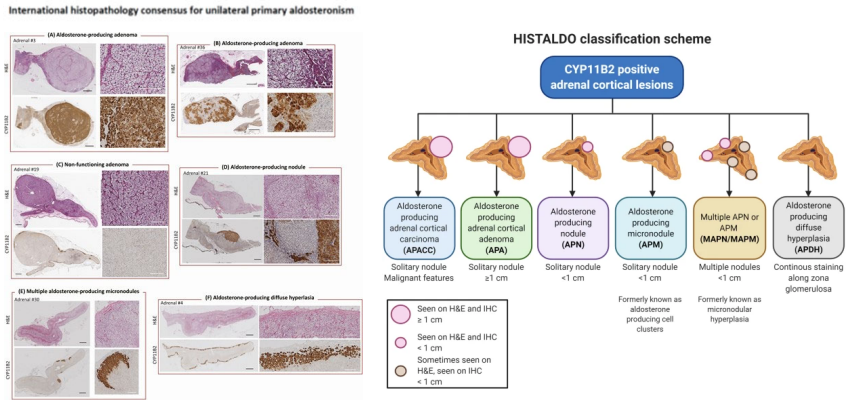
Degenhart C, et al. Clin Endocrinol (Oxf). 2017 Mar;86(3):325-331

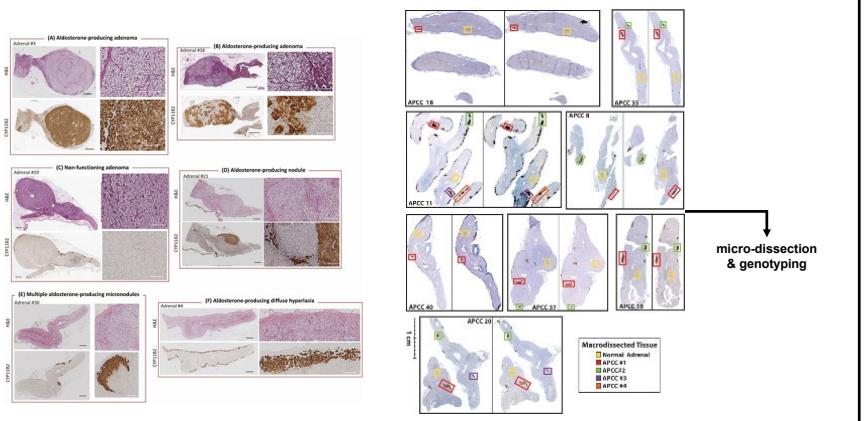
Morphological spectrum of primary aldosteronism



Williams TA et al. J Clin Endocrinol Metab. 2021 Jan 1;106(1):42-54

Juhlin CC et al. Endocr Pathol. 2021 Mar;32(1):102-133

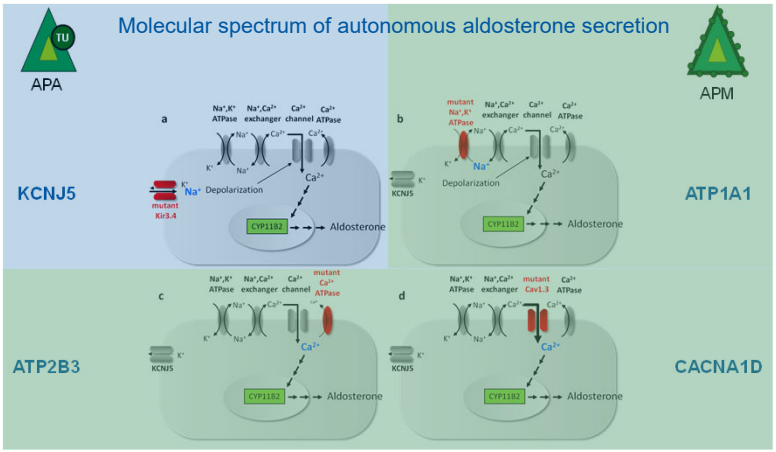
Morphological spectrum of primary aldosteronism – and underlying molecular marker



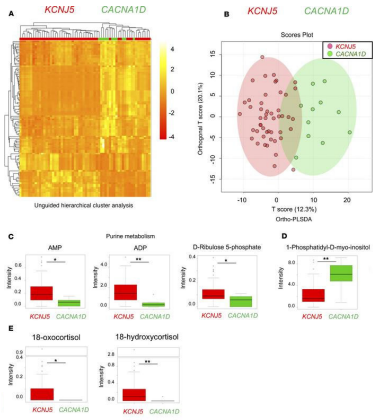
Williams TA et al. J Clin Endocrinol Metab. 2021 Jan 1;106(1):42-54

Nishimoto et al. Proc Natl Acad Sci U S A. 2015 Aug 18

Molecular spectrum of autonomous aldosterone secretion



Tissue Metabolomics: Specific molecular signatures

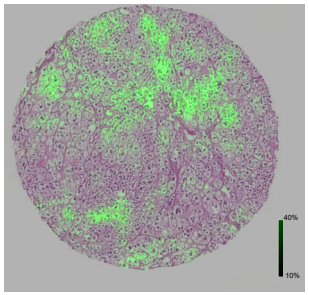


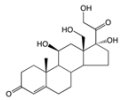
Murakami M, et al. JCI Insight. 2019 Sep 5;4(17)

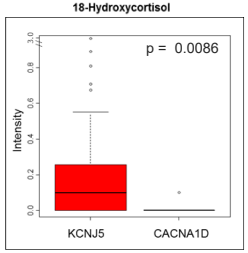
Tissue Metabolomics: Specific molecular signatures

18-Hydroxycortisol is significantly increased in KCNJ5 patients

KCNJ5 patient tissue:







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Murakami M, et al. JCI Insight. 2019 Sep 5;4(17)

Genotype specific steroid profile

Steroid	Wild-Type (n=36)	*KCNJ5* (n=27)	ATPase (n=9)	*CACNA1D* (n=7)	*P* Value
Aldosterone	0.109 (0.067–0.261)	0.148 (0.097–0.500)	0.453*† (0.179–2.278)	0.062 (0.046–1.56)	0.007
18-Oxocortisol	0.015 (0.010–0.146)	0.315*† (0.079–0.768)	0.047 (0.030–0.251)	0.012 (0.010–0.185)	0.000
18-Hydroxycortisol	0.772 (0.489–1.937)	2.243*† (1.259–7.243)	1.675 (0.790–3.490)	0.608 (0.371–1.650)	0.001
11-Deoxycorticosterone	0.078 (0.039–0.187)	0.117 (0.068–0.266)	0.144† (0.109–1.338)	0.050 (0.034–0.072)	0.011
Corticosterone	1.800 (0.691–3.916)	2.388† (1.148–7.825)	4.366† (1.874–16.617)	0.778 (0.691–1.008)	0.023
Pregnenolone	0.281 (0.120–0.383)	0.303 (0.161–0.478)	0.547 (0.349–5.568)	0.256 (0.165–0.413)	0.065
Progesterone	0.134 (0.049–0.309)	0.123 (0.086–0.385)	0.158 (0.090–1.209)	0.109 (0.051–0.155)	0.467
17-Hydroxyprogesterone	0.914 (0.502–0.914)	0.541 (0.323–1.270)	0.953 (0.716–9.868)	0.644 (0.468–0.968)	0.100
11-Deoxycortisol	0.243 (0.125–0.553)	0.318 (0.218–0.759)	0.346 (0.186–2.493)	0.185 (0.124–0.255)	0.221
Cortisol	112.8 (54.4–162.0)	111.1 (54.4–164.0)	169.8 (108.2–280.0)	56.3 (50.9–108.4)	0.046
21-Deoxycortisol	0.080 (0.014–0.103)	0.082 (0.010–0.104)	0.103 (0.016–1.625)	0.078 (0.009–0.092)	0.481
Cortisone	16.2 (12.0–20.5)	16.9 (13.4–19.3)	18.1 (14.5–20.9)	13.5 (11.7–19.0)	0.659
DHEA	0.488 (0.290–0.813)	0.624 (0.387–1.225)	0.517 (0.254–1.020)	0.518 (0.227–0.807)	0.719
DHEA-SO_4	942 (591–1297)	988 (424–1322)	723 (364–1305)	651 (529–1725)	0.765
Androstenedione	0.745 (0.452–1.213)	0.623 (0.494–1.305)	0.650 (0.483–2.160)	0.643 (0.524–0.850)	0.999

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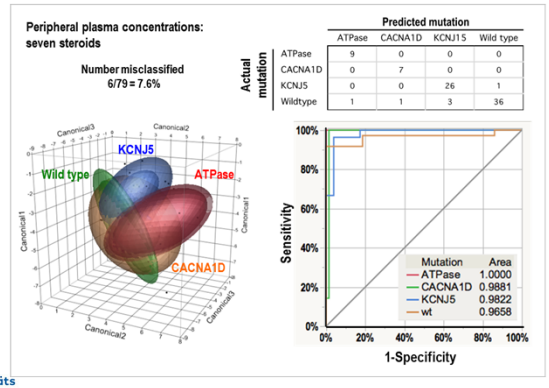
Williams TA, et al. Hypertension. 2016 Jan;67(1):139-45.

Genotype specific steroid profile



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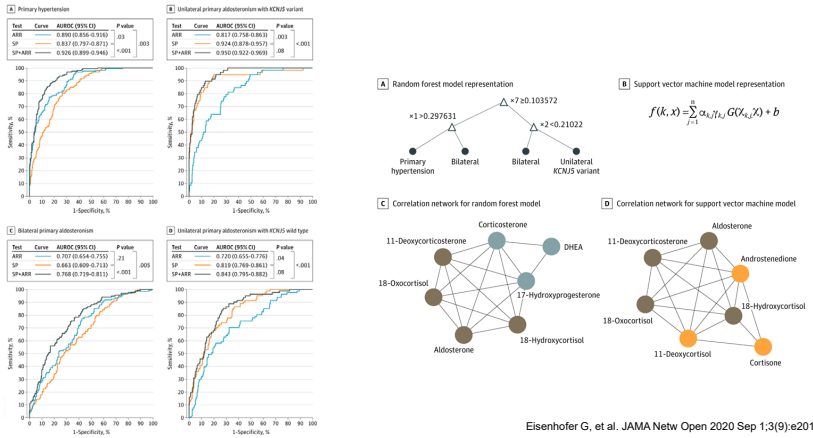
Williams TA, et al. Hypertension. 2016 Jan;67(1):139-45.

Steroid profiles in the diagnostic and differential diagnostic of PA

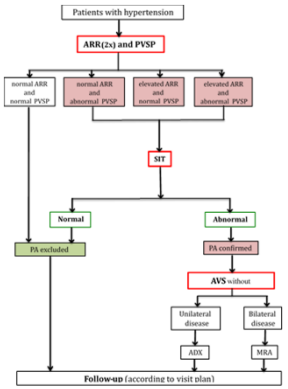


Eisenhofer G, et al. JAMA Netw Open 2020 Sep 1;3(9):e2016209

A multicentre PROspective study

on the diagnostic value of Steroid profiling in primary ALDOsteronism

ARR: aldosterone/renin ratio;
PVSP: peripheral venous steroid profiling;
AVS: adrenal venous sampling;
ADX: adrenalectomy;
MRA: mineralocorticoid receptor antagonist.



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Outcomes after adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures and analysis of remission rates in an international cohort

Tracy A Williams, Jacques W M Lenders, Paolo Mulatero, Jacopo Burrello, Marietta Rottenkolber, Christian Adolf, Fumitoshi Satoh, Laurence Amar, Marcus Quinkler, Jaap Deinum, Felix Beuschlein, Kanako K Kitamoto, Uyen Pham, Ryo Morimoto, Hironobu Umakoshi, Aleksander Prejbisz, Tomaz Kocjan, Mitsuhide Naruse, Michael Stowasser, Tetsuo Nishikawa, William F Young Jr, Celso E Gomez-Sanchez, John W Funder, Martin Reincke, for the Primary Aldosteronism Surgery Outcome (PASO) investigators*

Retrospective analysis (28 center - **14** countries, **5** continents)

Phase 1 - Build consensus for standardised criteria for outcome of ADX

Phase 2 - Apply consensus to assess outcome in referral centres

Phase 3 - Identify pre-surgical correlates of outcome

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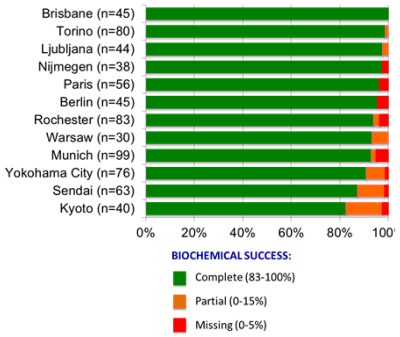
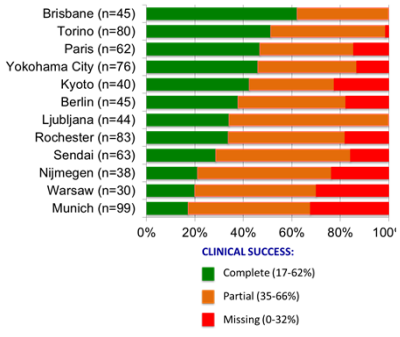
Williams et al. Lancet Diabetes Endocrinol. 2017 Sep;5(9):689-699.

Biochemical outcome rates
(n= 699)



Clinical outcome rates
(n=705)



Williams et al. Lancet Diabetes Endocrinol. 2017 Sep;5(9):689-699.

BASELINE INDEPENDENT CORRELATES OF CLINICAL REMISSION

CLINICAL REMISSION = COMPLETE SUCCESS

Variable	Clinical outcome	
	Odds Ratio (95% CI)	P value
Age (years)	**0.95 (0.93-0.98)**	<0.01
Lowest serum K^+ (mmol/L)	1.43 (0.95-2.15)	0.09
Anti-HT medication (DDD)	0.80 (0.70-0.90)	<0.01
eGFR (mL/min/1.73m²)	1.01 (1.00-1.02)	0.08
SBP (mmHg)	0.99 (0.98-1.01)	0.30
BMI (kg/m²)	0.98 (0.94-1.03)	0.38
Sex (ref. female)	**2.25 (1.40-3.62)**	<0.01
LVH (ref. absent)	1.61 (1.01-2.59)	0.05

HT, hypertension; DDD, defined daily dose; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; LVH, left ventricular hypertrophy (assessed by echocardiography).

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Summary

- There are substantial hurdles in the diagnosis and differential diagnosis of PA
- Clinical characteristics of a cohort contribute to wide variation in differential diagnosis and clinical outcome rates
  - Younger patients and females particularly benefit from a favorable surgical outcome
  - Useful to inform and counsel patients with unilateral PA prior to adrenalectomy concerning the expected post-operative clinical benefit
- Omics including «Steroid metabolomics», tissue and plasma metabolomics provide a rich source of phenotypic annotations
- Whether these features will lead to improvements in (differential) diagnostics of PA patients is currently under exploration

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Thank you very much for your attention!

Universität Zürich[UZH]

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

Funded by the European Union

Henri Timmers

Management of pheochromocytoma and paraganglioma

Management of pheochromocytoma and paraganglioma

Henri Timmers

Radboud University Medical Center, Netherlands

Pheochromocytoma/paraganglioma rare tumors originating from the neural crest with an incidence of eight cases per million per year. The likelihood of the diagnosis can be estimated based on risk factors and specific symptoms. The highest diagnostic performance is achieved by using LCMS for measuring catecholamine metabolites in plasma or 24 hour urine collections. Pre-analytical factors are important to consider when taking plasma samples and drug interference has to be taken into account. Regarding the localisation of these tumors after a biochemical diagnosis has been established, different anatomical and functional imaging techniques are available with an eminent role for PET scanning. Approximately 40% of cases are related to a germ line mutation and in 30% of the sporadic cases a somatic mutation can be identified. Regarding the pre-operative management alpha adrenergic blockade is still considered gold standard, although this issue is now a matter of debate. Surgery itself is usually performed in a minimally invasive approach either retroperitoneoscopically or trans peritoneal. After surgery patients needs to be followed at least 10 years depending on their risk factors of recurrence. Usually by yearly measurements of metanephrines. Regarding the management of metastatic pheochromocytomas and paragangliomas the focus is on two major issues. The first is the management of catecholamine induced symptoms and complications. The second involves anti-tumour treatments including radionuclides treatment, chemotherapy, targeted treatment and local palliative treatments. Recently the first prospective randomised placebo controlled trial on sunitinib has been presented. Regarding the tumour screening in patients at risk, a guideline on the management of a symptomatic SDHX mutation carriers has been published. Special considerations apply to the management of pheochromocytoma/paraganglioma during pregnancy.

Management of pheochromocytoma/paraganglioma

Henri Timmers, MD, PhD
Radboud University Medical Center, Nijmegen, the Netherlands
Dept. of Internal medicine, section of Endocrinology

Radboudumc

Consensus Document

Genetics, diagnosis, management and future directions of research of phaeochromocytoma and paraganglioma: a position statement and consensus of the Working Group on Endocrine Hypertension of the European Society of Hypertension

Jacques W.M. Lenders[a,b], Michiel N. Kerstens[c], Laurence Amar[d], Aleksander Prejbisz[e], Mercedes Robledo[f], David Taieb[g], Karel Pacak[h], Joakim Crona[i], Tomáš Zelinka[j], Massimo Mannelli[k], Timo Deutschbein[l], Henri J.L.M. Timmers[a], Frederic Castinetti[m], Henning Dralle[n], Jřri Widimský[j], Anne-Paule Gimenez-Roqueplo[o], and Graeme Eisenhofer[b,p]

Lenders et al., J Hypertension 2020

Epidemiology

Incidence 8 cases per million per year; doubled from 1995 to 2015

Prevalence

- in autopsy studies 0.05%
- in adult outclinic patients with hypertension 0.2-0.6%
- in children with hypertension 1.7%
- in patients with adrenal incidentaloma 7%

Berends et al. Eur J Intern Med. 2018

Indications for screening and clinical presentation

TABLE 1. Signs and symptoms in patients with and without PPGL enrolled in the full study cohort (*N* = 2017) and three different patient subpopulations according to their clinical presentation[a]

	All patients		Signs and symptoms		Incidentaloma		Surveillance	
	PPGL	No PPGL	PPGL	No PPGL	PPGL	No PPGL	PPGL	No PPGL
N	243	1774	89 **7%**	1170	88 **19%**	387	66 **23%**	217
BMI (kg/m^2)	25[b]	28	25[b]	28	25[b]	29	25[b]	27
Heart rate (b/min)	79[b]	72	81[b]	72	78[b]	73	77[b]	70
Pallor (%)	26[b]	13	37[b]	15	21[b]	10	18[b]	7
Sweating (%)	46[b]	28	55[b]	30	47[b]	25	32[b]	21
Palpitations (%)	46[b]	36	65[b]	44	46[b]	22	21	19
Tremor (%)	25[b]	15	33[b]	18	21[b]	8	18[b]	6
Nausea /vomiting (%)	21[b]	11	26[b]	12	18	11	20[b]	10
Symptom score ≥3[c]	42	18	56	21	40	13	28	1
Hypertension (%)	83	85	95	93	80	78	71	58
Headaches (%)	38	39	46	45	39	29	27	25
Weakness (%)	42[b]	35	51[b]	36	34	32	42	32
Panic/anxiety (%)	25	25	37	32	19	14	15[b]	7
Flushing (%)	20	23	21	27	23	17	14	14
Constipation (%)	14[b]	9	17[b]	9	10	9	14	7

Geroula et al., Eur J Endocrinol. 2019

Clinical presentation and indications for screening

- Likelihood of a PPGL can be assessed by combining the indication for screening and the presence of specific clinical features
- Sustained hypertension is aspecific for PPGL

Lenders et al., J Hypertension 2020

Diagnostic performance LC-MS

Prospective PMT study: 2056 patients screened, 236 confirmed PPGL

Table 2. Diagnostic sensitivities and specificities of plasma and urinary panels of *O*-methylated metabolites.

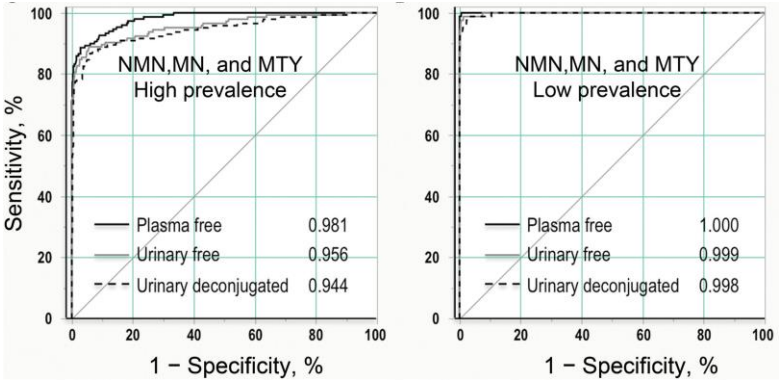
Group[a]	Sensitivity, %	Specificity, %
All patients (NMN and MN)		
Plasma free	96.6 (228/236)[b]	94.9 (1727/1820)[c]
Urinary free	92.9 (210/226)	94.5 (1660/1756)[c]
Urinary deconjugated	92.9 (210/226)	92.8 (1630/1757)
All patients (NMN, MN, and MTY)		
Plasma free	97.9 (231/236)[b]	94.2 (1714/1820)[c]
Urinary free	93.4 (211/226)	94.2 (1655/1756)[c]
Urinary deconjugated	92.9 (210/226)	92.1 (1619/1757)

b $P < 0.05$, higher sensitivity of plasma than both urinary panels

c $P < 0.02$, higher specificity of panels for plasma and urinary free metabolites than urinary deconjugated metabolites

Eisenhofer et al. Clin Chemistry 2018

Diagnostic performance LC-MS



- High risk patients: plasma free better than urinary free / deconjugated
- Low risk patients: no difference between plasma and urine

Eisenhofer et al. Clin Chemistry 2018

Biochemical diagnosis

- To avoid false-positive results, testing for plasma-free metanephrines requires blood sampling after at least 20 min of supine rest
- In non-emergency situations, biochemical testing should precede imaging
- Plasma or urinary metanephrines in the normal range reliably exclude a PPGL in symptomatic patients
- Plasma metanephrines >2x the URL indicate a high likelihood of a PPGL and the patient can proceed to imaging studies

Lenders et al., J Hypertension 2020

Drug interference

TABLE 2. Medications that may cause falsely elevated results for plasma and urinary metanephrines due to pharmacodynamic interference

	Plasma and urine		
	Normetanephrine	Metanephrine	3-Methoxytyramine
Antidepressants	++	–	–
Phenoxybenzamine	++	–	–
MAO-inhibitors	++	++	–
Sympathomimetics	+	+	–
Levodopa	–	–	+++

++ clear increase; + mild increase; –no increase.
MAO, monoamine oxidase.

Lenders et al., J Hypertension 2020

Imaging

CT Characteristics of Pheochromocytoma: Relevance for the Evaluation of Adrenal Incidentaloma

Letizia Canu,[1,2] Janna A. W. Van Hemert,[1] Michiel N. Kerstens,[3] Robert P. Hartman,[4] Aakanksha Khanna,[5] Ivana Kraljevic,[6] Darko Kastelan,[6] Corin Badiu,[7] Urszula Ambroziak,[8] Antoine Tabarin,[9] Magalie Haissaguerre,[9] Edward Buitenwerf,[3] Anneke Visser,[10] Massimo Mannelli,[2] Wiebke Arlt,[11] Vasileios Chortis,[11] Isabelle Bourdeau,[12] Nadia Gagnon,[12] Marie Buchy,[13] Francoise Borson-Chazot,[13] Timo Deutschbein,[14] Martin Fassnacht,[14,15] Alicja Hubalewska-Dydejczyk,[16] Marcin Motyka,[16] Ewelina Rzepka,[16] Ruth T. Casey,[17] Benjamin G. Challis,[17] Marcus Quinkler,[18] Laurent Vroonen,[19] Ariadni Spyroglou,[20,21] Felix Beuschlein,[20,21] Cristina Lamas,[22] William F. Young,[5] Irina Bancos,[5] and Henri J. L. M. Timmers[1]

Retospective evaluation of 1011 patients with PPGL

- Unenhanced attenuation ≤10 HU in 0.5%
- High washout (APW≥60% and/or RPW≥40%) similar to adenoma in 34%

Canu et al. J Clin Endocrinol Metab 2019

Imaging

- Functional imaging is essential in patients with a high risk of recurrence or multifocal disease
- The choice of the radiopharmaceutical for functional imaging depends on genotype, biochemical phenotype, size and location

Table 3 Proposed clinical algorithm for nuclear imaging investigations in cases of phaeochromocytomas and paragangliomas

	First choice	Second choice	Third choice (if [^{18}F]FDOPA or [^{68}Ga]Ga-SSA is not available)
PHEO (sporadic)	[^{18}F]FDOPA or [^{123}I]MIBG	[^{68}Ga]SSA	[^{18}F]FDG
Inherited PHEO (except *SDHx*): *NF1*/*RET*/*VHL*/*MAX*	[^{18}F]FDOPA	[^{123}I]MIBG or [^{68}Ga]SSA	[^{18}F]FDG
HNPGL (sporadic)	[^{68}Ga]SSA	[^{18}F]FDOPA	[^{111}In]SSA/[^{99m}Tc]SSA
Extra-adrenal sympathetic and/or multifocal and/or metastatic and/or *SDHx* mutation	[^{68}Ga]SSA	[^{18}F]FDG and [^{18}F]FDOPA	[^{18}F]FDG and [^{123}I]MIBG or [^{18}F]FDG and [^{111}In]SSA/[^{99m}Tc]SSA

Taïeb et al. Eur J Nucl Med Mol Imaging 2019

Genetics

Gene	Prevalence of PVs in PCC/PGL	Inheritance
EGLN1/PHD2	0.6%	AD
FH	1%	AD
EPAS1	1%	AD; somatic mosaicism
MAX	0.8–1%	
NF1	3%	AD
RET	6%	AD
SDHA	<1–3%	AD
SDHAF2	<0.1–0.1%	AD; paternal inheritance
SDHB	9–10%	AD
SDHC	1%	AD
SDHD	2–9%	AD; paternal inheritance
TMEM127	0.6–2.1%	AD
VHL	4–7%	AD

Wachtel and Fishbein, Curr Opin Endocrinol Diabetes Obes 2021

Genetics

- 80% of all PPGLs can be explained by genetic alterations (40% germline); all patients with a PPGL should be considered for genetic testing
- Lack of family history for PPGL does not preclude the presence of a germline mutation
- NGS is the preferred technique to analyse all relevant genes in a single test
- Suspicion of a variant of unknown significance requires complementary testing to establish pathogenicity

Lenders et al., J Hypertension 2020

Approach to the Patient: Perioperative Management of the Patient with Pheochromocytoma or Sympathetic Paraganglioma

Annika M.A. Berends,[1] Michiel N. Kerstens,[1] Jacques W.M. Lenders,[2,3] and Henri J.L.M. Timmers[2]

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Berends et al. JCEM 2020

Complications of PPGL surgery

- Dramatic fall in mortality (from 40% to 1-3%) and morbidity (current cardiovascular complication rate 9%)
- Due to advances in
  - medical management: α-adrenergic blockade introduced in 1949 *
  - anaesthesiology
  - surgery (minimally invasive)
  - early diagnosis (adrenal incidentaloma and carrier screening)

** Grimson et al J Am Med Assoc 1949*

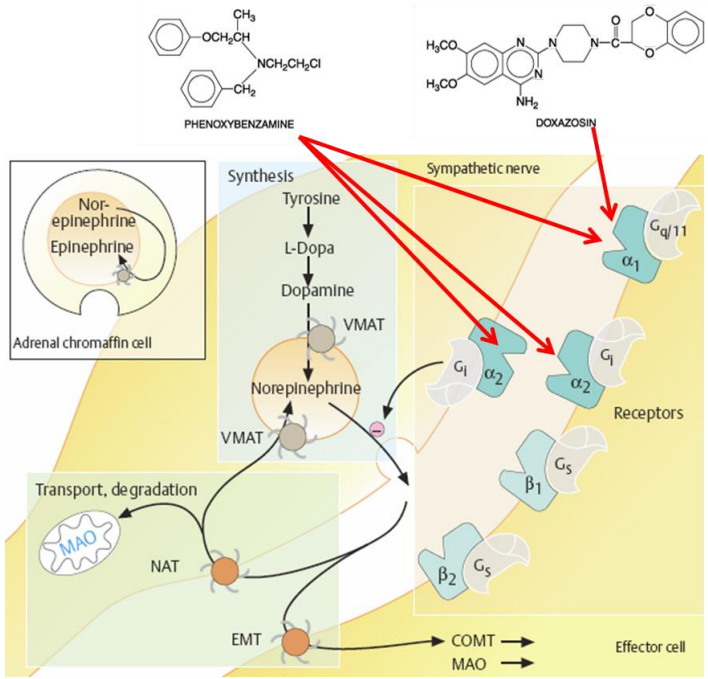
Goals of pre-surgical management

- Prevent anesthesia and surgery induced catecholamine storm and its consequences on the cardiovascular system

BUT ALSO

- Prevent PRE-operative complications
- Relieve of symptoms
- Control blood pressure, heart rate, volume status, glucose metabolism, bowel motility

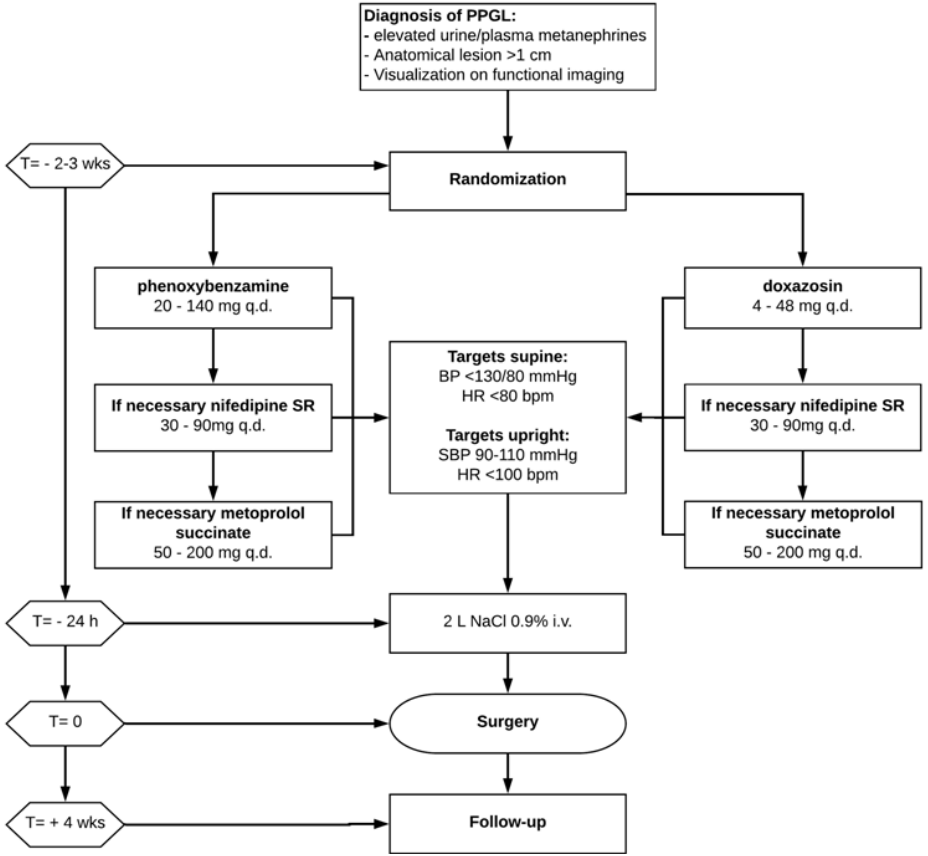
α-adrenergic receptor blockers



PRESCRIPT trial

First prospective study on pre-treatment in PPGL

To compare the efficacy of phenoxybenzamine and doxazosin



Buitenwerf et al. J Clin Endocrinol Metab. 2020

PRESCRIPT trial

144 patients, 30 day post-operative follow-up, no mortality

Phenoxy vs doxa

- No differences in cummulative time outside BP range (SBP>160, MAP <60 mmHg) 11.1% vs 12.2%, NS
- No differences in post-op hypotension & complications
- More intraoperative hemodynamic instablility with doxa: higher need of vasodilator drugs
- No differences in side effects: 85% grade I-II, transient

Buitenwerf et al. J Clin Endocrinol Metab. 2020

Debate on the necessity of blockade

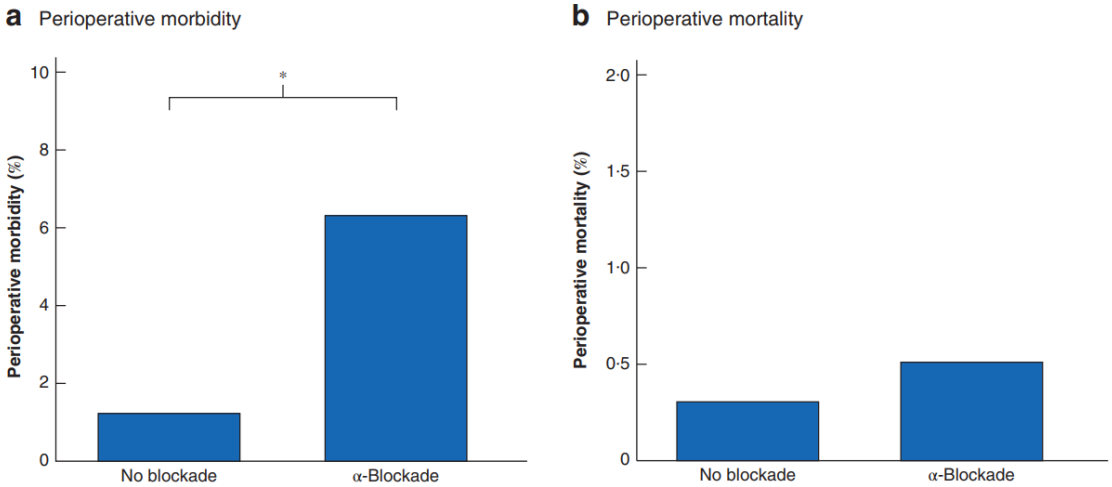
International multicentre review of perioperative management and outcome for catecholamine-producing tumours

H. Groeben[1], M. K. Walz[2], B. J. Nottebaum[1], P. F. Alesina[2], A. Greenwald[7], R. Schumann[8], M. W. Hollmann[17], L. Schwarte[18], M. Behrends[10], T. Rössel[3,4], C. Groeben[3,4], M. Schäfer[5], A. Lowery[20], N. Hirata[21], M. Yamakage[21], J. A. Miller[22], T. J. Cherry[22], A. Nelson[11], C. C. Solorzano[12], B. Gigliotti[9], T. S. Wang[13], J. K. G. Wietasch[19], P. Friederich[6], B. Sheppard[14], P. H. Graham[15], T. N. Weingarten[16] and J. Sprung[16]

Groeben et al. British J of Surgery 2020

Debate on the necessity of blockade

- 1860 patients in 21 centers
- 343 without intense alphablokkade
- Retrospective, non-randomized
- No data on vasoactive drugs and fluids required (determinants of outcome)
- Results from high volume center is not generally applicable



Groeben et al. British J of Surgery 2020

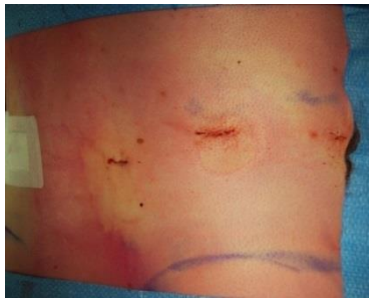
Presurgical and surgical management

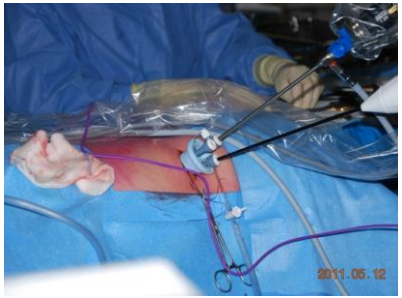
- Presurgical a-adrenergic receptor blocker remains the mainstay for preventing life-threatening perioperative cardiovascular complications
- Normotensive patients should also receive presurgical medical treatment
- In patients with head and neck paraganglioma, presurgical adrenergic blockade is only indicated in patients with positive biochemistry

Lenders et al., J Hypertension 2020

Surgical management

- Scopic adrenalectomy
  - posterior retroperitoneoscopic
  - transperitoneal laparoscopic (BMI >45 and tumor >7cm)
- Open adrenalectomy (‘large and invasive’)

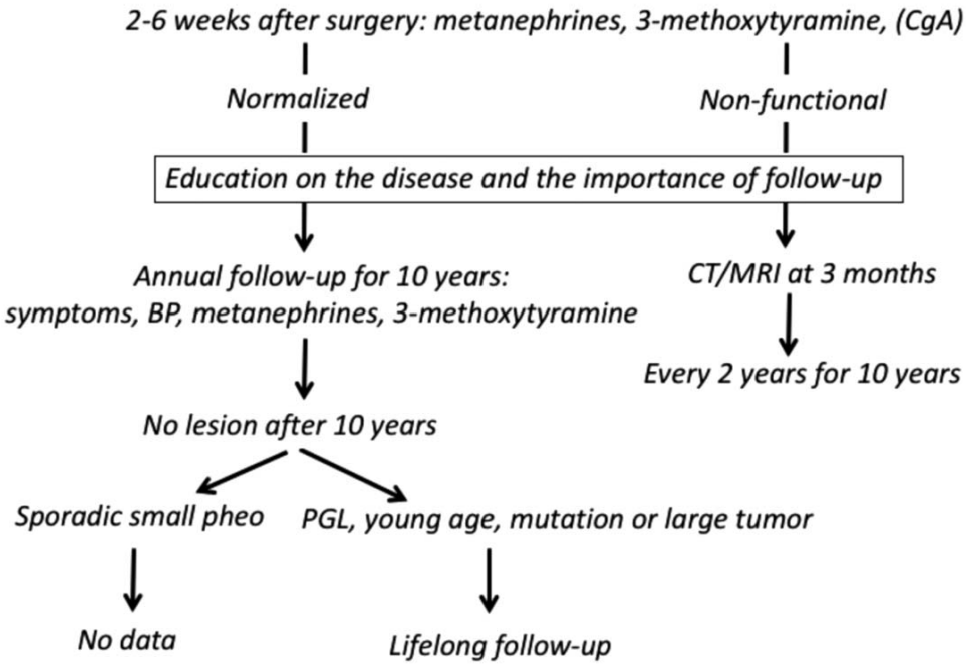




Langenhuijsen et al. J Endourol 2013
Walz et al. World J Surg. 2010

European Reference Network | eUROGEN *Urogenital Diseases*

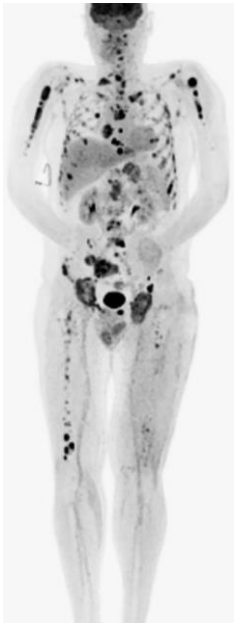
Post-surgical follow-up



Lenders et al., J Hypertension 2020; Plouin et al. European Journal of Endocrinology 2016

Metastatic PPGL

- All PPGLs are potentially malignant; no reliable histological / molecular markers
- Metastatic defined by lesions in tissues where chromaffin cells are normally absent: lymph nodes, bone, liver, lung
- *SDHB* mutations, tumour size >5 cm, multifocality and dopaminergic biochemical phenotype are risk factors for developing metastases
- 40-50% of patients with metastatic PPGL harbour *SDHB* mutations

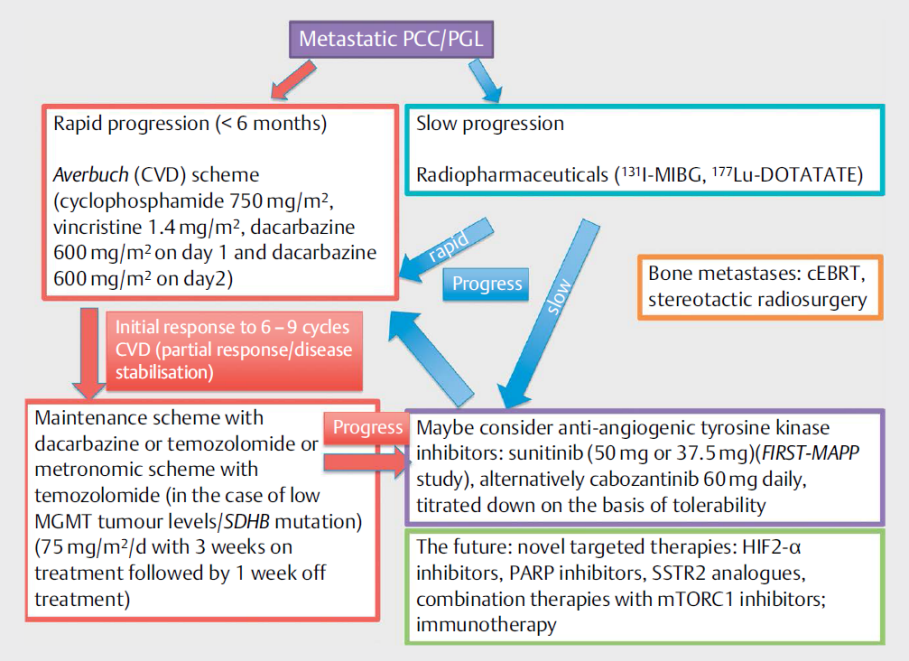


Lam et al. Endocrine pathology 2017; Chrisoulidou et al. Endocr Relat Cancer 2007

Management of metastatic PPGL

Management of catecholamine induced symptoms / complications

- adrenergic blockade
- laxatives
- (metyrosine)



Nölting, Grossman, Pacak. Exp Clin Endocrinol Diabetes 2019

2021 ESMO congress

First International Randomized STudy in MAlignant Progressive Pheochromocytoma and Paraganliomas (FIRSTMAPPP): an academic double blind phase II trial assessing Sunitinib antitumor efficacy

Baudin E, Goichot B, Berutti A, Hadoux J, Moalla S, Laboureau S, Svenja Nölting, de la Fouchardiere C, Strasburger C, Timo Deutschbein , Zovato S, Amar L, Tabarin A, Niccoli P, Timmers H, Faggiano AJ, Beuschlein F, Attard M, Texier M, Fassnacht M; for the ENDOCAN-COMETE and ENSAT networks.

Endocrine Oncology, Gustave Roussy - Cancer Campus, Villejuif, France, Internal medicine and Endocrinology, Hopital de Hautepierre - Hopitaux Universitaires de Strasbourg, Strasbourg, France, Department of Medical and Surgical Specialties, Radiological Sciences, and Publi, Azienda Ospedaliera Spedali Civili di Brescia, Brescia, Italy, Endocrine Oncology, Institut Gustave Roussy, Villejuif, France, Imaging departement, Institut Gustave Roussy, Villejuif, France, 6Endocrinology, Hopitaux Universitaire d'Angers, Angers, France, Medizinische Klinik und Poliklinik IV, MediziKlinikum der Universität München, Munchen, Germany, Department of Medical Oncology, Léon Bérard Center, Lyon, Lyon, France, Department of Endocrinology and Metabolism, Charité Universitätsmedizin Berlin, Berlin, Germany, Department Internal Medicine I, Division of Endocrinology and Diabetes, University Hospital Würzburg, Würzburg, Germany, Oncology, IOV - Istituto Oncologico Veneto IRCCS, Padova, Italy, PARIS, HEGP - Hopital Europeen Georges-Pompidou - AP-HP, Paris, France, Department of Endocrinology, University of Bordeaux, Bordeaux, France, Internal Medicine, Radboud University Medical Center, Nijmegen, Nijmegen, Netherlands, Oncology, Institut Paoli Calmette, Marseille, France, Department of Clinical and Molecular Medicine, Sapienza University of Rome, Rome, Italy, Endocrine Research, LMU Klinikum der Universität München, Munich, Germany, Ile de France, Gustave Roussy - Cancer Campus, Villejuif, France, Biostatistics, Institut Gustave Roussy, Villejuif, France, Department of Internal Medicine I - Division of Endocrinology, University Hospital Würzburg, Würzburg, Germany

International consensus on initial screening and follow-up of asymptomatic *SDHx* mutation carriers

Laurence Amar[1,2]✉, Karel Pacak[3], Olivier Steichen[4], Scott A. Akker[5], Simon J. B. Aylwin[6], Eric Baudin[7], Alexandre Buffet[2,8], Nelly Burnichon[2,8], Roderick J. Clifton-Bligh[9,10], Patricia L. M. Dahia[11], Martin Fassnacht[12], Ashley B. Grossman[13,14,15], Philippe Herman[16], Rodney J. Hicks[17], Andrzej Januszewicz[18], Camilo Jimenez[19], Henricus P. M. Kunst[20,21], Dylan Lewis[22], Mitsuhide Naruse[23], Mercedes Robledo[24,25], David Taïeb[26], David R. Taylor[6], Henri J. L. M. Timmers[27], Giorgio Treglia[28,29,30], Nicola Tufton[5], William F. Young[31], Jacques W. M. Lenders[27,32], Anne-Paule Gimenez-Roqueplo[2,8] and Charlotte Lussey-Lepoutre[2,33]✉

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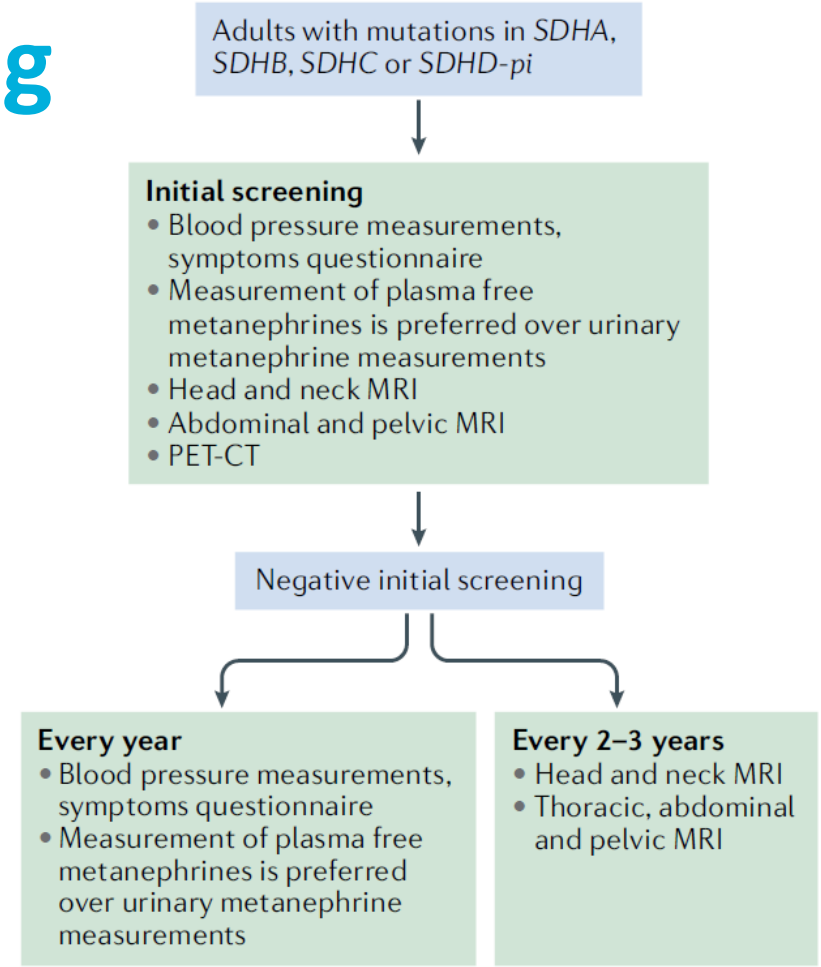
"OK, all those in favour of delegating decision-making, shrug your shoulders"

Amar et al. Nat Rev Endocrinol. 2021

SDHx tumorscreening

- *SDHB* from 6-10 yo
- *SDHA/C/D*-pi from 10-15 yo
- From 70 yo 1x/5 y
- From 80 yo and no tumor: follow-up staken
- Metanephrines + MRI head/neck/chest/abdomen/pelvis
- PET-CT at 1st screening adults



Amar et al. Nat Rev Endocrinol. 2021

Positive Impact of Genetic Test on the Management and Outcome of Patients With Paraganglioma and/or Pheochromocytoma

Alexandre Buffet,[1,2] Laurène Ben Aim,[3] Sophie Leboulleux,[4] Delphine Drui,[5] Delphine Vezzosi,[2] Rossella Libé,[6] Christiane Ajzenberg,[7] Daniele Bernardeschi,[8] Bertrand Cariou,[5] Frédéric Chabolle,[9] Olivier Chabre,[10] Vincent Darrouzet,[11] Brigitte Delemer,[12] Rachel Desailloud,[13] Bernard Goichot,[14] Annabelle Esvant,[15] Lucile Offredo,[1] Philippe Herman,[16] Sandrine Laboureau,[17] Hervé Lefebvre,[18] Peggy Pierre,[19] Isabelle Raingeard,[20] Yves Reznik,[21] Jean-Louis Sadoul,[22] Julien Hadoux,[4] Antoine Tabarin,[23] Igor Tauveron,[24] Delphine Zenaty,[25] Judith Favier,[1] Jérôme Bertherat,[6,26] Eric Baudin,[4] Laurence Amar,[26,27] and Anne-Paule Gimenez-Roqueplo,[1,3,26] for the French Group of Endocrine Tumors (GTE) and COMETE Network

Buffet et al. JCEM 2019

Pheochromocytoma/paraganglioma and pregnancy

- Estimated incidence: 0.007% (30.246 pregnancies in 20 years)
- Untreated: high maternal and fetal morbidity and mortality
- ‘The great mimick’: pre-eclampsia, hyperemesis, gestational diabetes, thyreotoxicosis

Harrington et al. World J Surgery 1999; Lenders. Eur J of Endocrinology 2012
Biggar et al. Brittish J Surgery 2013

Pregnancy and phaeochromocytoma/ paraganglioma: clinical clues affecting diagnosis and outcome – a systematic review

K Langton,[a,b] N Tufton,[c] S Akker,[c] J Deinum,[b,d,e] G Eisenhofer,[a,b,d] HJLM Timmers,[e] MEA Spaanderman,[f,g] JWM Lenders[b,d,e]

- Systematic review
- 157 papers on 204 pregnancies in 200 patients

PPGL diagnosis	
During pregnancy	144/204 (70.6%)
After pregnancy	45/204 (22.1%)
Postmortem[c]	15/204 (7.4%)

Langton et al. BJOG 2021

Clinical clues

	OR (95% CI)	*P*-value
Factors associated with a higher probability of an antepartum diagnosis of PPGL		
Admission for history of PPGL, mutation, adrenal mass	5.23 (1.19–22.99)	0.029
Sweating	3.33 (1.40–7.91)	0.0064
Palpitations	2.66 (1.25–5.69)	0.0117
Triad[a]	3.43 (0.98–11.92)	0.053
Hypertension at admission	1.94 (1.02–3.69)	0.048

Langton et al. BJOG 2021

Maternal and fetal outcomes in phaeochromocytoma and pregnancy: a multicentre retrospective cohort study and systematic review of literature

*Irina Bancos, Elizabeth Atkinson, Charis Eng, William F Young Jr, Hartmut P H Neumann, on behalf of the International Pheochromocytoma and Pregnancy Study Group**

- Multi-centre retrospective study, 1980-2019
- Combination of
  - Registry of 197 pregnancies in 186 patients
  - Systematic review of 7 reports (>5 cases) on 63 pregnancies in 55 patients

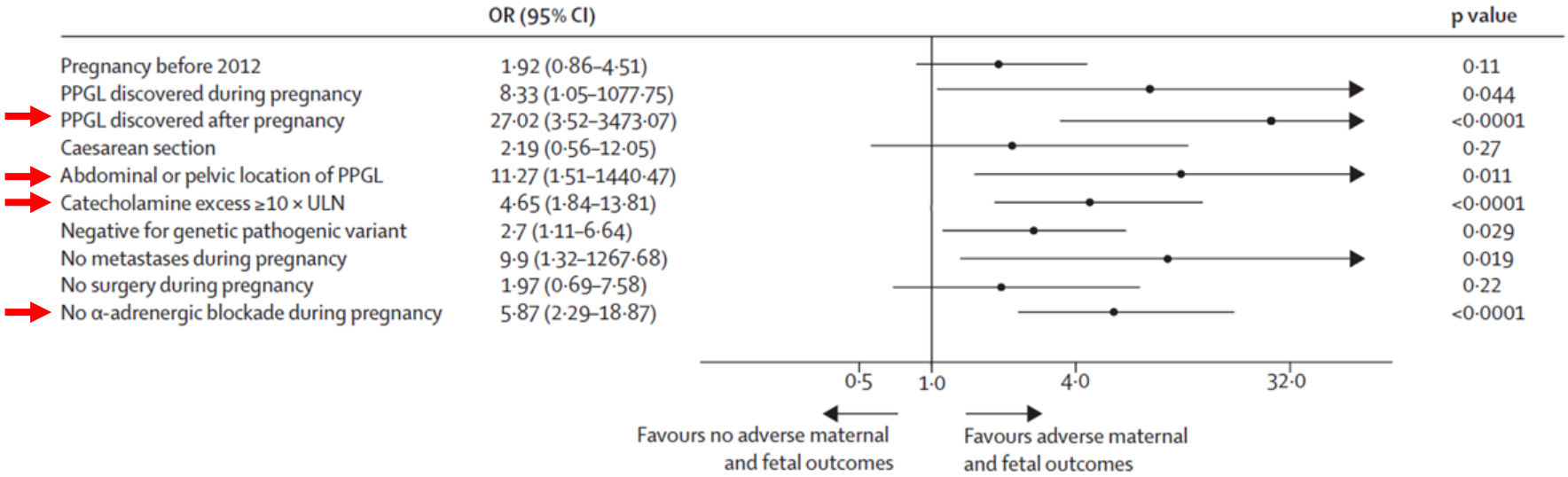
Bancos et al. Lancet Diabetes Endocrinol 2021

Outcome

- Maternal and fetal outcome evaluable in 230 (92%) pregnancies
- 14% complications of catecholamine excess or death
  - 9% fetal death
  - 6% severe maternal complication or death (1%)
  - 2% maternal plus fetal complications

Bancos et al. Lancet Diabetes Endocrinol 2021

Determinants of outcome



Bancos et al. Lancet Diabetes Endocrinol 2021

Medical treatment

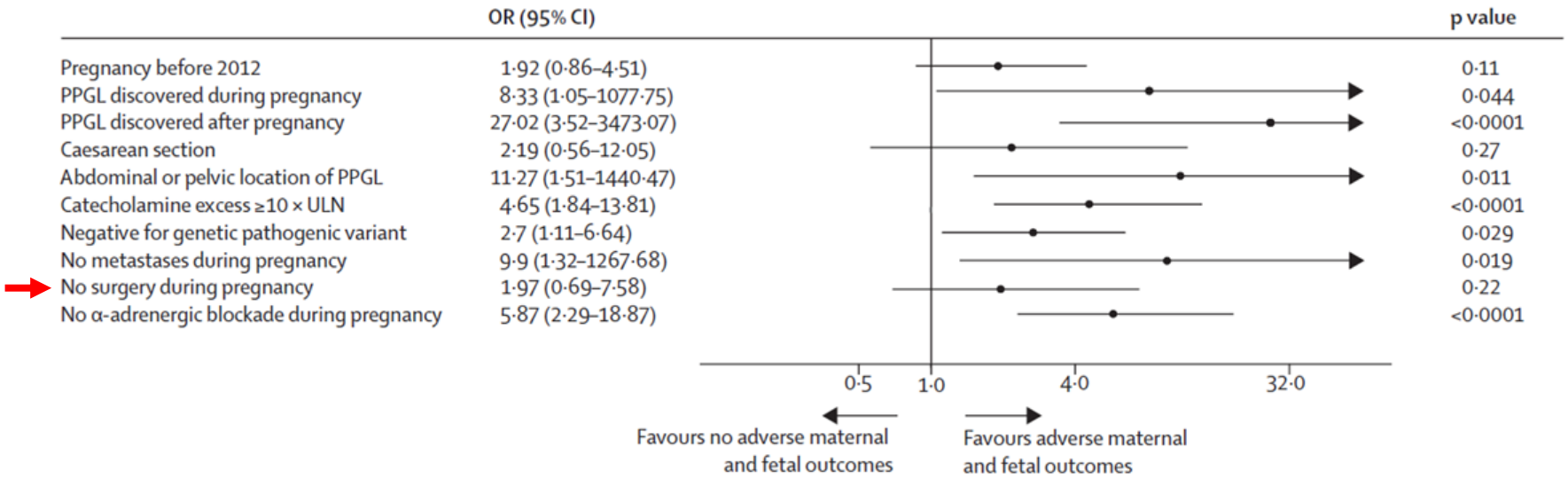
Drug	Starting dose	Incremental dose steps[a]	Dose range	Comments
phenoxybenzamine	10mg q.d.	20mg	10-140mg	Preferably started at least 7-14 days prior to surgery, also in case of normotension. Doses higher than starting dose are administered b.i.d.
or				
doxazosin ER	4mg q.d.	4mg	4-56mg	
nifedipine ER	30mg q.d.	30mg	30-90mg	Add-on to α-adrenergic receptor blockade in case of persistent hypertension (BP supine >130/80 mmHg, SBP upright >110 mmHg)
or				
amlodipine	5mg q.d.	5mg	5-10mg	
or				
metyrosine	250mg t.i.d.	250-500mg	750-2000mg	
metoprolol ER	50mg q.d.	50mg	50-200mg	Add-on in case of tachycardia (HR supine >80bpm, HR upright >100bpm). Preferably be started after sufficient preparation with α-adrenergic receptor blockade (≥3-4 days)
or				
propranolol	20mg t.i.d.	20mg	20-240mg	
or				
atenolol	25mg q.d.	25mg	25-100mg	
high sodium chloride diet	≥15 grams	-	-	Restoration of intravascular volume depletion; prevention of preoperative orthostatic hypotension and postoperative hypotension
and				Diet should be started >7-14 days before surgery
saline 0.9% i.v.	2L /24h	-	-	Intravenous saline should be started 24h before surgery

Berends et al. JCEM 2020

FDA category C/D (not teratogenic, possible harm to the fetus)

Probably less harmful than catecholamine excess

Determinants of outcome



However, better APGAR scores after surgery during pregnancy than with pheochromocytoma in situ (score <7 in 2.6% vs 25.6%)

Bancos et al. Lancet Diabetes Endocrinol 2021; Langton et al. BJOG 2021

Surgical resection of PPGL

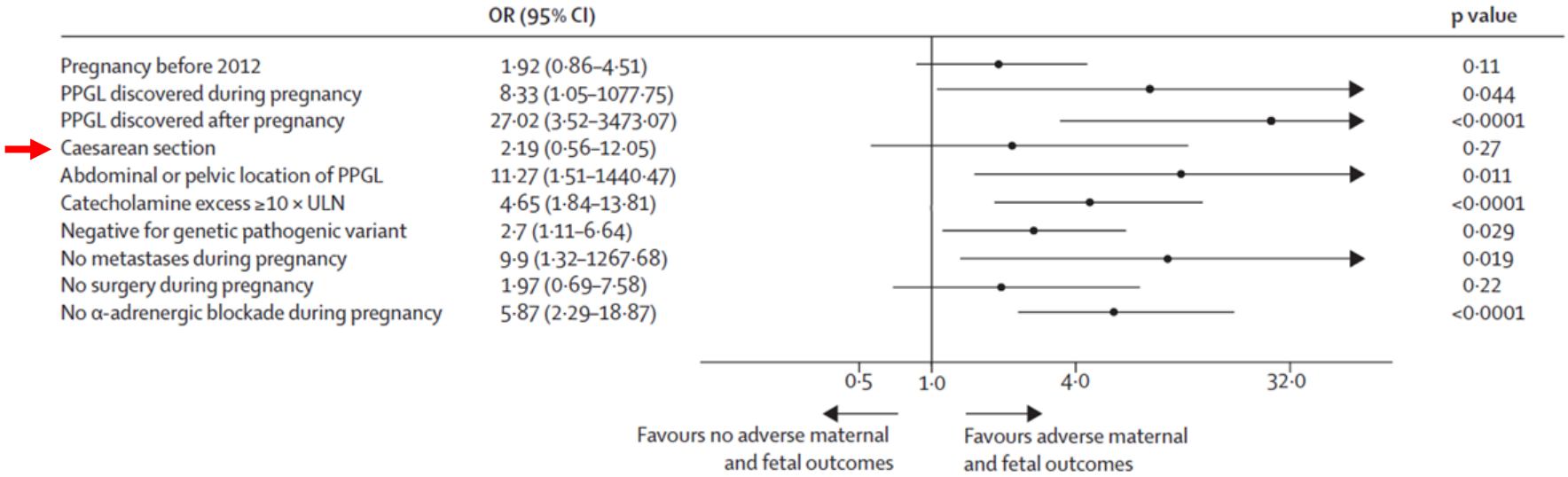
Surgery for PPGL (n=231)	
During pregnancy	42 (18%)
Gestation week	20 (10–35)
After pregnancy	161 (70%)
Weeks post partum (n=151)	8 (0–224)
No surgery	28 (12%)

Surgery is preferrably performed <24 w when organogenesis completed and the uterus still permits accessibility

When > 24 w: postpone until after delivery

Bancos et al. Lancet Diabetes Endocrinol 2021; Van der Weerd et al., Eur J Endocrinol 2017

Determinants of outcome



Bancos et al. Lancet Diabetes Endocrinol 2021

Delivery

Caesarean section	146 (59%)
Gestation week	36 (25–41)
Vaginal	76 (31%)
Gestation week	38 (28–41)
Unknown, live birth	3 (1%)
Gestation week	38 (36–39)
Emergent induced vaginal	1 (<1%)
Gestation week	20†
Elective abortion	8 (3%)
Gestation week	9 (3–22)
Miscarriage or intrauterine fetal loss	11 (4%)
Gestation week, median (range)	18 (8–37)
Pregnancy ongoing	2 (1%)
Gestation week	25, 26†
Autopsy	2 (1%)
Gestation week	28, unknown†

Caesarian section was performed in most cases, however, natural labour is probably safe in appropriately selected patients

Bancos et al. Lancet Diabetes Endocrinol 2021

Conclusion

Recommendation

6.2 We recommend that patients with PPGLs should be evaluated and treated by multidisciplinary teams at centers with appropriate expertise to ensure a favorable outcome. In particular, patients should be referred to such centers should there be pregnancy, metastatic disease, or issues concerning the complexity or difficulty in biochemical diagnosis; localization; performance, and interpretation of genetic testing; preoperative preparation; surgical treatment; and follow-up. (Ungraded recommendation)

Endocrine Society Clinical practice guideline. Lenders et al. J Clin Endocrinol Metab 2014

Acknowledgements

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Adrenal disorders

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Anouk van Berkel, Margo Dona, Nike Stikkelbroeck, Jaap Deinum, Jacques Lenders
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Pathology
Benno Kusters
Medical genetics
Arjen Mensenkamp
Nuclear medicine
Martin Gotthardt, Marcel Janssen
Urology
Hans Langenhuijsen
Otolaryngology
Dirk Kunst, Henri Marres

ens@t
European Network for the Study of Adrenal Tumours

Endo-ERN

the PARADIFFERENCE foundation

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COST is supported by the EU Framework Programme Horizon 2020

Funded by the European Union

Mercedes Robledo

How to deal with variants of unknown significance?

How to deal with variants of unknown significance?

Mercedes Robledo

Spanish National Cancer Research Centre (CNIO)

The development of technologies capable of sequencing the exome, or even the genome of an individual, has improved the ability to diagnose disease genetically. However, one of the problems associated with the implementation of these technologies is the identification of variants of uncertain significance even in genes for which an association with a specific disease has been demonstrated, and also the identification of incidental findings in genes apparently not associated with the disease of interest.

Variants of uncertain significance are a recurrent problem in genetic diagnosis, and laboratories offering such services must be prepared to give a clear interpretation of the meaning of the variant. For this purpose, there are numerous databases that provide information on the frequency of a variant in different populations, as well as bioinformatics tools designed to predict the effect of a change according to different algorithms. However, the *in silico* results need to be functionally validated, as the correct classification of a variant has consequences for the clinical follow-up not only of the index case of a family, but also of the relatives carrying the genetic change.

During the class we will analyse concrete examples of families in which we have found variants of uncertain significance, we will review public database websites where we can find relevant information, as well as the type of information provided by the most commonly used bioinformatics tools. The class aims to provide guidance on the steps to follow in a laboratory to classify a variant of uncertain significance.

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How to deal with variants of unknown significance?

Mercedes Robledo, PhD
mrobledo@cnio.es

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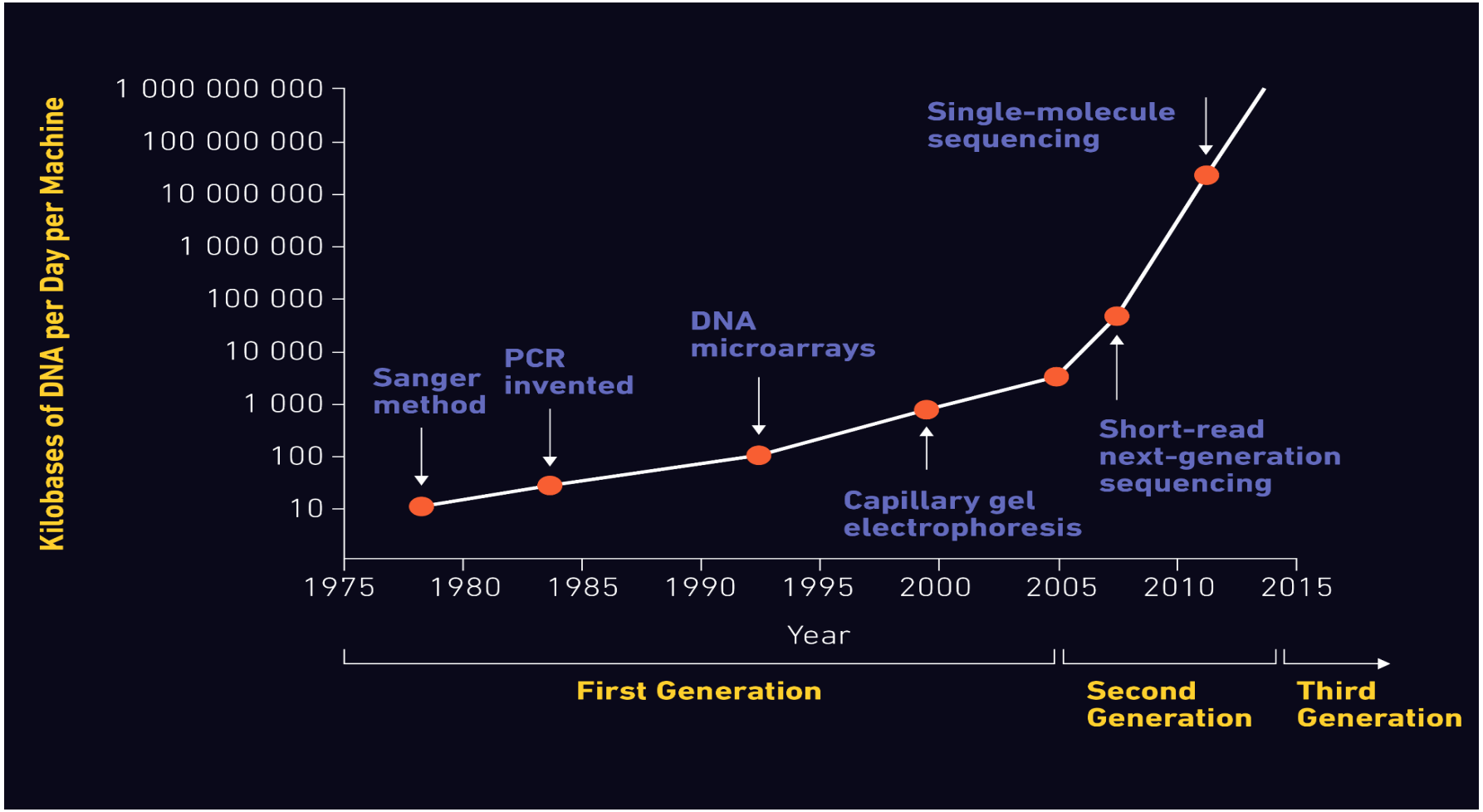

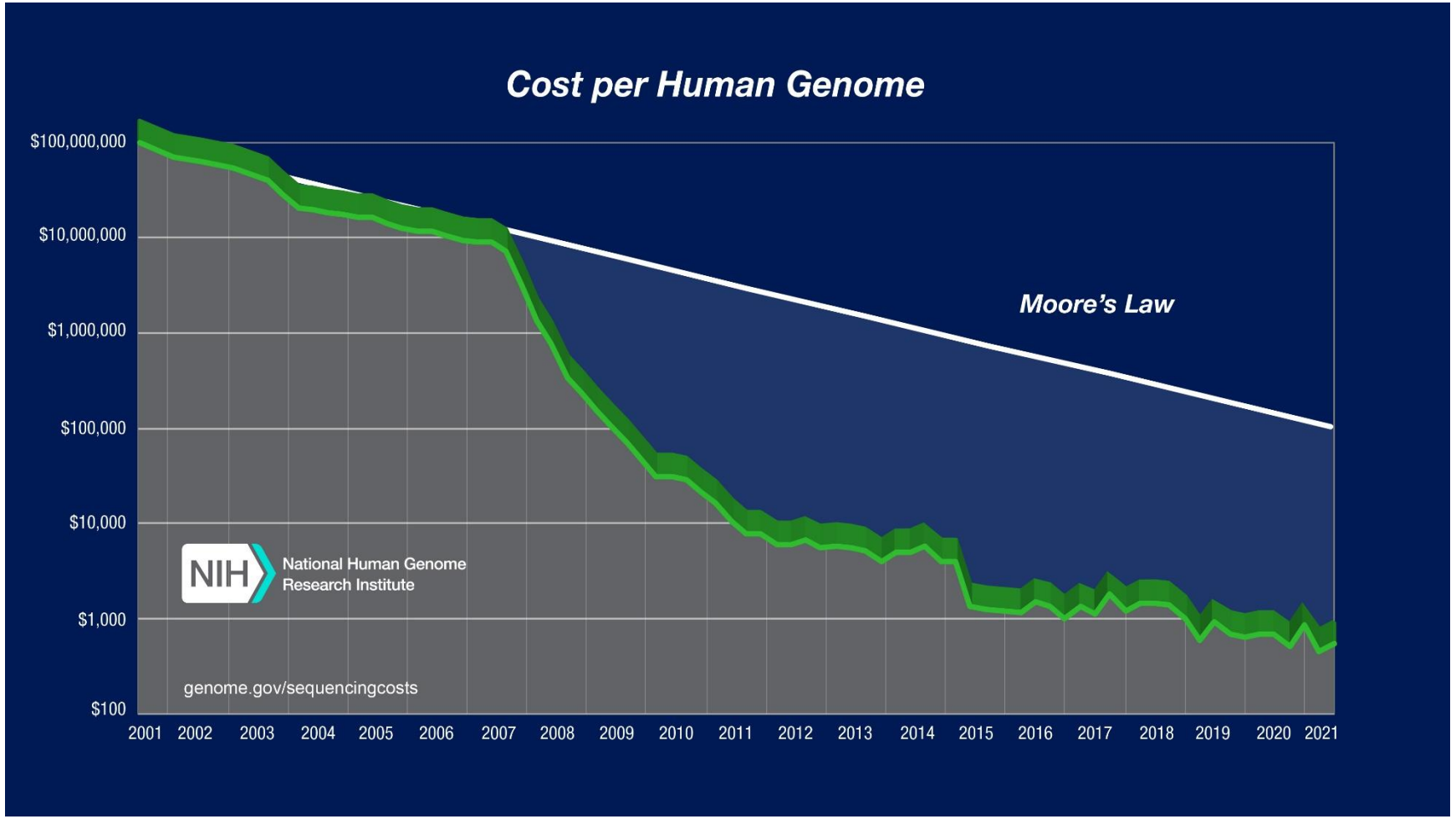

Wetterstrand KA. DNA Sequencing Costs: Data from the NHGRI Genome Sequencing Program (GSP) Available at: www.genome.gov/sequencingcostsdata.

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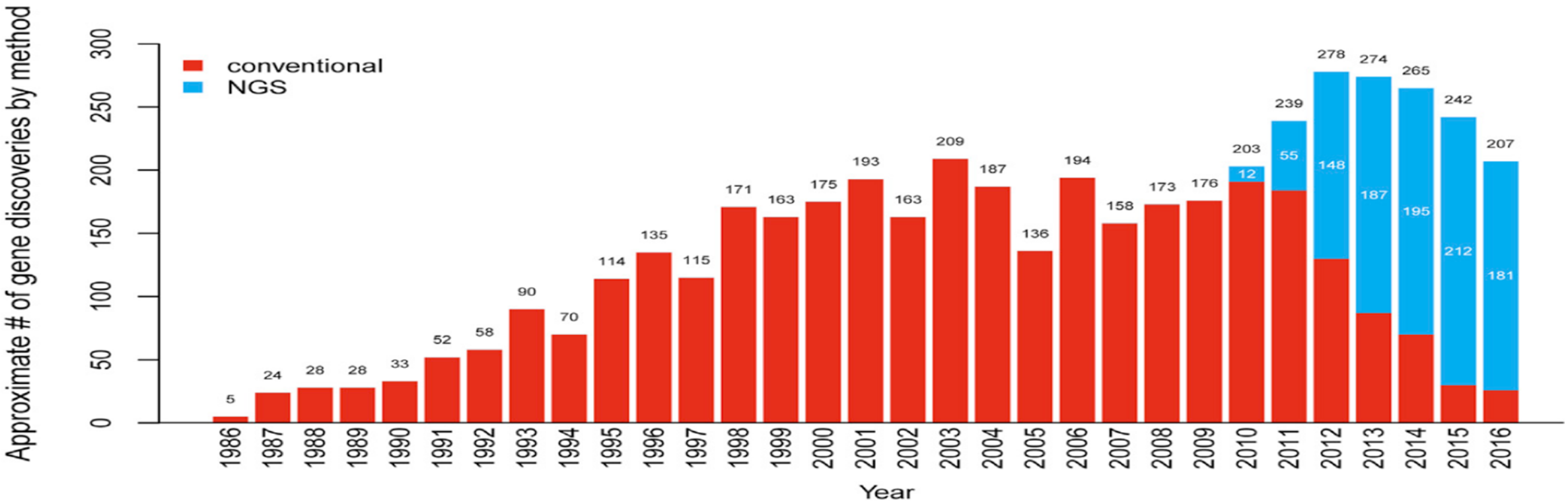

Danielsson K, et al. *Expert Rev Mol Diagn. 14:469–487, 2014;* **Makrythanasis P, and Antonarakis SE.** *Clin. Genet. 84: 422–428, 2013;* **Jessica X. Chong, et al**. *AJHG 97:199–215, 2015;* ***Boycott et al.*** *Am J Hum Genet. 100:695, 2017*

Advantages:

It is posible to check simultaneosly all the genes related to a specific disease (panels).

To follow an agnostic strategy (whole exome sequencing or whole genome sequencing).

Many applications (cause of a disease, progression related mutations, treatment resistance, etc.)

Disadvantages:

There are regions poorly covered (false negative).

To find variants of unknown significance.

VUS → What it means?

A **variation** in a genetic sequence **for which the association with disease risk is unclear**. Also called unclassified variant, variant of unknown significance, and VUS.

Are VUS common?
Close to 20% of genetic tests identify a VUS.

Some tests examine only a handful of genes associated with cancer at a time, while others analyze hundreds of genes, or even WES and WGS.

The more genes you look at, the more variants of uncertain significance you'll find.

Quality check — Coverage and deep

Variant meaning analysis — Allele frequency; *In silico* prediction; DB

Validation — Sanger sequencing; MLPA

Results report — International Nomenclature and Variant Interpretation Guidelines

Genetic counselling

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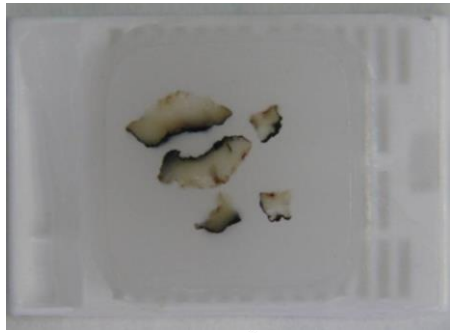
Quality check

Coverage and deep

FFPE tissue



Targeted NGS

List of genetic variant Interpretation

Gene	Filters	Alt Variant Freq	Read Depth	Alt Read Depth	Conseq	Sift	PolyPhen	HGVSc	HGVSp
FH	PASS	58,59	11778	6901	Missense	tolerated(0.09)	benign(0.368)	c.952C>A	p.His318Asn
HRAS	PASS	23,97	1961	470	Missense	deleterious(0.02)	benign(0.125)	c.182A>G	p.Gln61Arg
NF1	LowDP	45,96	892	410	Frameshift			c.519delT	p.Asp173Glu*fs*Ter5
NF1	LowDP	21,28	343	73	Missense	deleterious(0.01)	possibly_damaging (0.647)	c.2125T>C	p.Cys709Arg
NF1	PASS	52,92	8490	4493	Frameshift			c.6854_6855insT	p.Asn2286Gln*fs*Ter21
NF1	PASS	50,28	1770	890	Stop			c.7909C>T	p.Arg2637Ter
NF1	LowDP	39,51	2131	842	Splice			c.5609+1G>A	
RET	LowDP	50,37	538	271	Synonymous			c.1941C>T	c.1941C>T(p.=)
RET	LowDP	40,61	3580	1454	Missense	deleterious(0)	probably_damaging(1)	c.2753T>C	p.Met918Thr
VHL	PASS	4,68	1240	58	Missense	deleterious(0.01)	probably_damaging(0.994)	c.494T>G	p.Val165Gly

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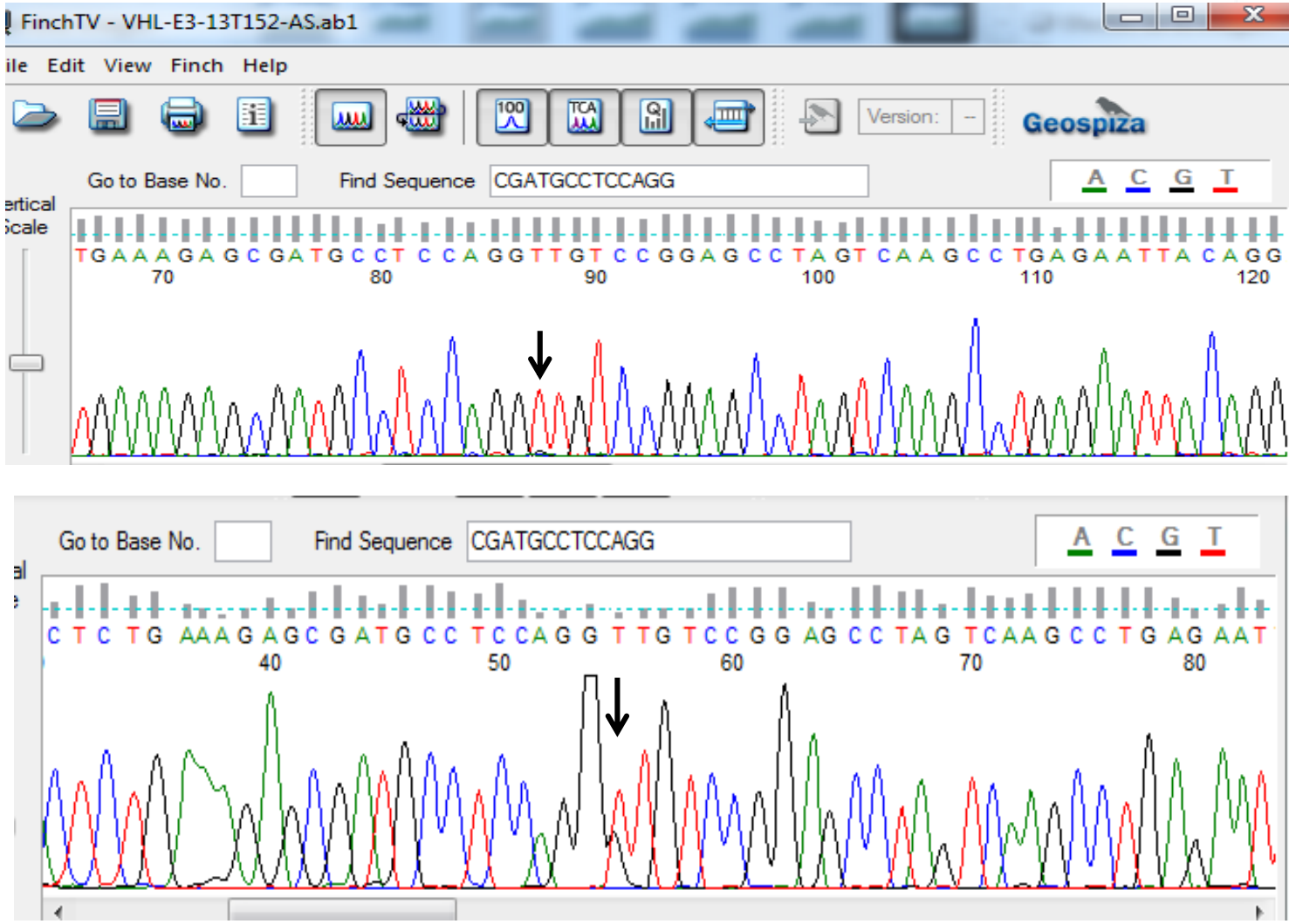

The importance of appropriate material selection

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Rules for classifying variants

	Benign			Pathogenic		
	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
Population Data	MAF is too high for disorder *BA1/BS1* **OR** observation in controls inconsistent with disease penetrance *BS2*			Absent in population databases *PM2*	Prevalence in affecteds statistically increased over controls *PS4*	

Richards S, et al. ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med. 2015 May;17(5):405-24.*

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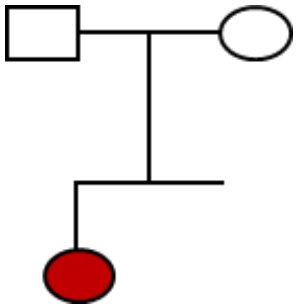
Blood



MTC

Variant meaning analysis

Allele frequency
In silico prediction
DB

p.R114H / rs76397662 (RET)

Population Frequencies

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
East Asian	204	19952	1	0.01022
South Asian	6	30614	0	0.0001960
European (non-Finnish)	9	128314	0	0.00007014
African/African American	0	24966	0	0.000
Latino/Admixed American	0	35430	0	0.000
Ashkenazi Jewish	0	10356	0	0.000
European (Finnish)	0	24914	0	0.000
Other	0	7218	0	0.000
XX	111	128880	1	0.0008613
XY	108	152884	0	0.0007064
Total	**219**	**281764**	**1**	**0.0007772**

gnomAD browser https://gnomad.broadinstitute.org

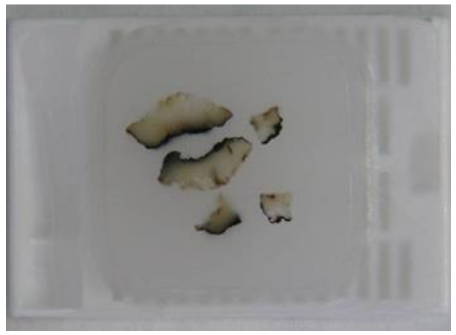
ClinVar → https://www.ncbi.nlm.nih.gov/clinvar/

ClinVar is a **freely accessible**, public archive of reports of the **relationships among human variations and phenotypes**, with supporting evidence.

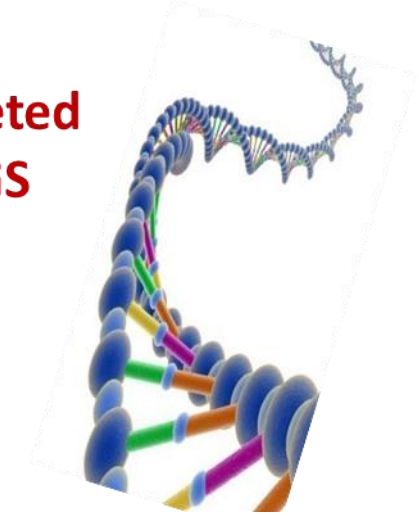
NM_020975.6(RET):c.341G>A (p.Arg114His)

Interpretation:	**Conflicting interpretations of pathogenicity Benign(7);Likely benign(1);Uncertain significance(1)**
Review status:	★☆☆☆ criteria provided, conflicting interpretations
Submissions:	11 (Most recent: Jul 4, 2021)
Last evaluated:	Nov 12, 2020
Accession:	VCV000013947.14
Variation ID:	13947
Description:	single nucleotide variant

FFPE tissue



Targeted NGS



Medullary Thyroid Carcinoma

Up to **50%**→ *RET* somatic mutation

Around **20%** of somatic mutation involving *RAS*-family members

To check as many DB as possible. There are DB focussed on specific phenotypes, while in others it is possible to find information from many genes. Some examples:

https://www.lovd.nl/
https://varsome.com/
https://arup.utah.edu/database/ → MEN2
https://ftp.ncbi.nlm.nih.gov/pub/clinvar/ClinGen/expert_panels/InSiGHT/ → Lynch síndrome
https://brcaexchange.org/ → Brest cancer

PubMed → critically analyse whether there is sufficient evidence to classify a variant as pathogenic or probably pathogenic.

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	Benign		Pathogenic			
	Strong	**Supporting**	**Supporting**	**Moderate**	**Strong**	**Very Strong**
Population Data	MAF is too high for disorder *BA1/BS1* **OR** observation in controls inconsistent with disease penetrance *BS2*			Absent in population databases *PM2*	Prevalence in affecteds statistically increased over controls *PS4*	
Computational And Predictive Data		Multiple lines of computational evidence suggest no impact on gene /gene product *BP4* Missense in gene where only truncating cause disease *BP1* Silent variant with non predicted splice impact *BP7*	Multiple lines of computational evidence support a deleterious effect on the gene /gene product *PP3*	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before *PM5* Protein length changing variant *PM4*	Same amino acid change as an established pathogenic variant *PS1*	Predicted null variant in a gene where LOF is a known mechanism of disease *PVS1*

Richards S, et al. ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med. 2015 May;17(5):405-24.*

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Shorting intolerant from tolerant https://sift.bii.a-star.edu.sg/
PolyPhen-2 (Polymorphism Phenotyping v2) http://genetics.bwh.harvard.edu/pph2/
Mutation Taster https://www.mutationtaster.org/
ESEfinder http://krainer01.cshl.edu/tools/ESE2/
RESCUE-ESE Web Server http://hollywood.mit.edu/burgelab/rescue-ese/
EX-SKIP https://ex-skip.img.cas.cz/

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	Benign		Pathogenic			
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Functional Data	Well-established functional studies show no deleterious effect *BS3*		Missense in gene with low rate of benign missense variants and path. missenses common *PP2*	Mutational hot spot or well-studied functional domain without benign variation *PM1*	Well-established functional studies show a deleterious effect *PS3*	

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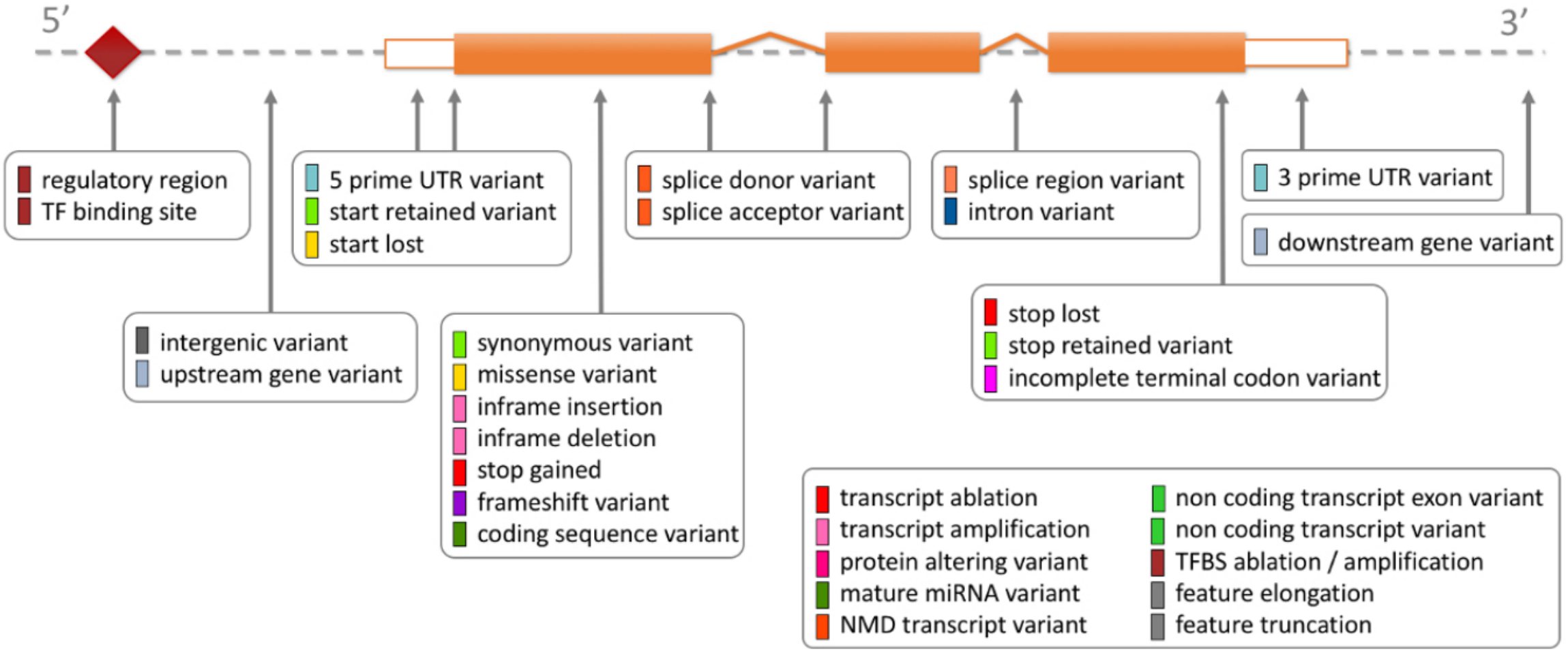
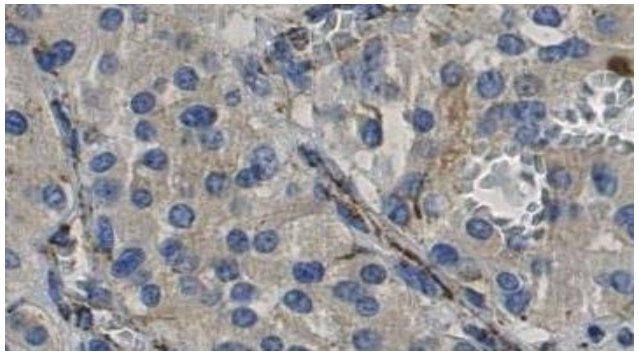


For some genes, complementary assays are available to help in interpretation

If pathogenic → SDHA IHC → no expression



SDHA (low penetrance)

There are variants that appear in low frequency in control population.

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Aim

Prevalence

Clinical features

Sample size

N=756

Variant found in blood:

- 6 intronic variants
- 3 synonymous genetic variant
- **5 missense**

ENS@T consortium

Wurzburg
Munich
Dresden
Rotterdam
Liège
Paris
Florence
Padova
Madrid

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VUS	cDNA Protein	dbSNP ID ExAC	Protein function prediction	3D structural prediction and annotation
V-1	c.8C>T p.S3F	Not described	5 as disease-causing 4 as benign	NA
V-4	c.310A>G p.R104G	Not described	10 as disease-causing	4 as non-stable 1 as stable
V-7	c.389A>G p.Q130R	Not described	3 as disease-causing 7 as benign	3 as non-stable 2 as stable
V-8	c.478G>A p.V160M	rs138541865 T: 0.01649%; 0 hom.	7 as disease-causing 2 as possibly disease-causing 1 as benign	5 as non-stable
V-13	c.766G>A p.A256T	rs147655350 T: 0.003320%; 0 hom.	9 as disease causing 1 as benign	6 as non-stable

Predictors used: *SIFT, Polyphen (HDIV), Polyphen (HVAR), LRT score, Mutation Taster, Mutation Assessor, fathmm-MKL, PROVEAN, MetaSVM, MetaLR*

Predictors used: *PoPMuSiCv33.1, ERIS, CUPSAT, I-Mutant v3.0, MAESTRO, INPS-3D*

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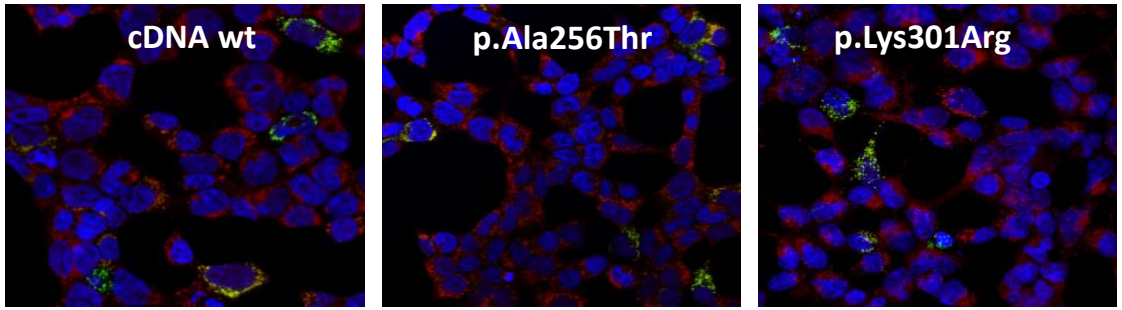
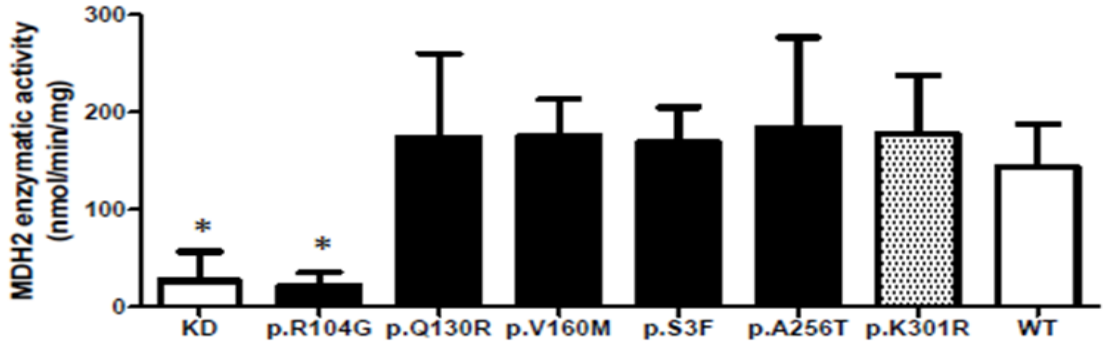
In the absence of access to a sample of the patient's tumour, functional assays need to be designed to demonstrate the pathogenic effect of the genetic change.

Up to 5 functional assays were used



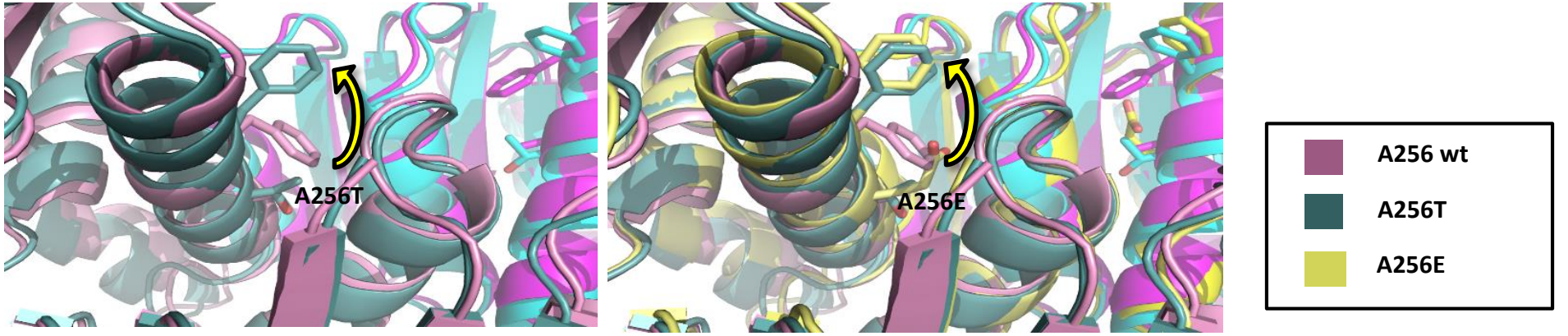
MDH2
MitoTraker – mitochondria label
Hoetchst 33342 - nuclei

1- Immunoflorescence assay to assess the localisation of MDH2



2- Enzymatic assay to assess MDH2 activity

3- Molecular dynamics simulation to predict conformational changes



- VUS is a challenge for laboratories
- Databases are available that give relevant information to interpret a variant.
- It is essential to consult as many databases as possible.
- In a high percentage of cases it is necessary to design appropriate functional assays to evaluate the effect of the change.

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Joakim Crona

PPGL cases with variants of unknown significance

PPGL cases with challenging genetics

Joakim Crona

Uppsala Universitet, Sweden

Pheochromocytoma and Paraganglioma (PPGL) may be the most heritable tumor seen in adults. A large number of disease causing genes have been described and linked to both distinct and more diffuse clinical presentations. As a result, all PPGL patients are currently recommended genetic testing. Still, many challenging aspects remain, some of these will be highlighted by this case presentation: what recommendations can be made to syndromic patients after curative treatment of a PPGL? At what age should genetic testing be offered to kids in families with confirmed genetic mutations? Is the interpretation of genetic test result written in stone or can a genetic variant be re-classified from benign to pathogenic, or vice versa? And finally, how can we approach a case that carries a high risk of having an underlying genetic cause, but where no germline mutation can be found in known PPGL genes? These cases are presented from the perspective of a clinician seeing PPGL patients in his/her routine clinical practice. The aim is to generate a discussion of how to apply the current state of the art in the management of PPGL patients with complicated genetics.

COST Harmonisation
Adrenal Tumor Master Class

PPGL cases – the genetic aspect

Joakim Crona
Clinical Oncologist, Associate Professor
Uppsala Universitet, Sweden

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Första fallet – Case 1

- 29 years old woman
- *SDHB*-family Arg90*
- Sister Pheochromocytoma 10y, dead 22y
- Father dead, unknown reason
- Grandmother, renal cell carcinoma
- Healthy son 8y, carrier of *SDHB* Arg90*

Patient/tumor characteristics	
Previous/concurrent illness	No
Family history	Yes
Metanephrine nmol/L (<0,3)	NA
Normetanephrine nmol/L (<0,6)	NA
Tumor type	Unilateral head neck paraganglioma, skull base
Tumor size	12mm
SDHB immunohistochemistry	NA

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Andra fallet – Case 2

- 27 years old male
- Hypertension and palpitations
- Laparoscopic surgery
- Disease free

Patient/tumor characteristics	
Previous/concurrent illness	No
Family history	No
Metanephrine nmol/L (<0,3)	3,5
Normetanephrine nmol/L (<0,6)	6,3
Tumor type	Unilateral pheochromocytoma
Tumor size	100mm
SDHB immunohistochemistry	Positive

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Tredje fallet – Case 3

- 30 years old male
- Back pain + sensory loss
- MRI: large paravertebral tumor with spinal cord compression
- Emergency surgery with spine decompression
- 68Ga-DOTATOC-PET: Extensive bone metastasis, thoracic/paravertebral lesion with 2 morphological components (pos/neg)

Patient/tumor characteristics	
Previous/concurrent illness	No
Family history	Cousin with head neck paraganglioma (age unknown)
Metanephrine nmol/L (<0,3)	0,2
Normetanephrine nmol/L (<0,6)	25
Metoxytyramine nmol/L (<0,2)	0,8
Tumor type	Metastatic paraganglioma
Tumor size	Unknown
SDHB immunohistochemistry	Unknown

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Summary

- Follow-up and screening protocols (Amar et al. *Nat Rev End* 2021)
- Re-classification of genetic variants
- Approach to a high risk patient without genetic findings?

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Fatima Al-Shahrour

Meet the expert: Omic platforms. What do you need to achieve robust results?

Meet the expert: Omics platforms. What do you need to achieve robust results?

Fátima Al-Shahrour

Bioinformatics Unit, Spanish National Cancer Research Centre (CNIO)

Precision Oncology (PO) is already revolutionizing healthcare and will play a dominant role in the future of cancer therapy. PO integrates tumor multi-omic profiles and data that reflect the course of the disease, lifestyle and environment to guide clinical decisions during cancer patient journey such as prevention, diagnosis and treatment. Bioinformatics analyses are essential to identify patients who will benefit from treatment based on their molecular profile, and to tailor chemotherapeutic regimens accordingly.

The aim of the talk is to describe the computational pipeline for the analysis and interpretation of Next-Generation Sequencing (NGS) data such as exome sequencing or targeted panels that are commonly used in the clinic. We will address the implementation of large-scale genomic sequencing in clinical practice and the computational strategies for the analysis of NGS data, limitations and future challenges and with a particular emphasis on the interpretation of the results, selection of biomarkers of drug response and afford opportunities to match therapies with the characteristics of the individual patient's tumor. An exercise will be used to illustrate the principles of how genetics influence led to refining diagnoses and personalized treatment of cancer disease.

cost
EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY
Funded by the Horizon 2020 Framework Programme of the European Union

cnio CENTRO NACIONAL DE INVESTIGACIONES ONCOLÓGICAS

Meet the expert: Omics platforms. What do you need to achieve robust results?

COST Harmonisation Adrenal Tumor Masterclass

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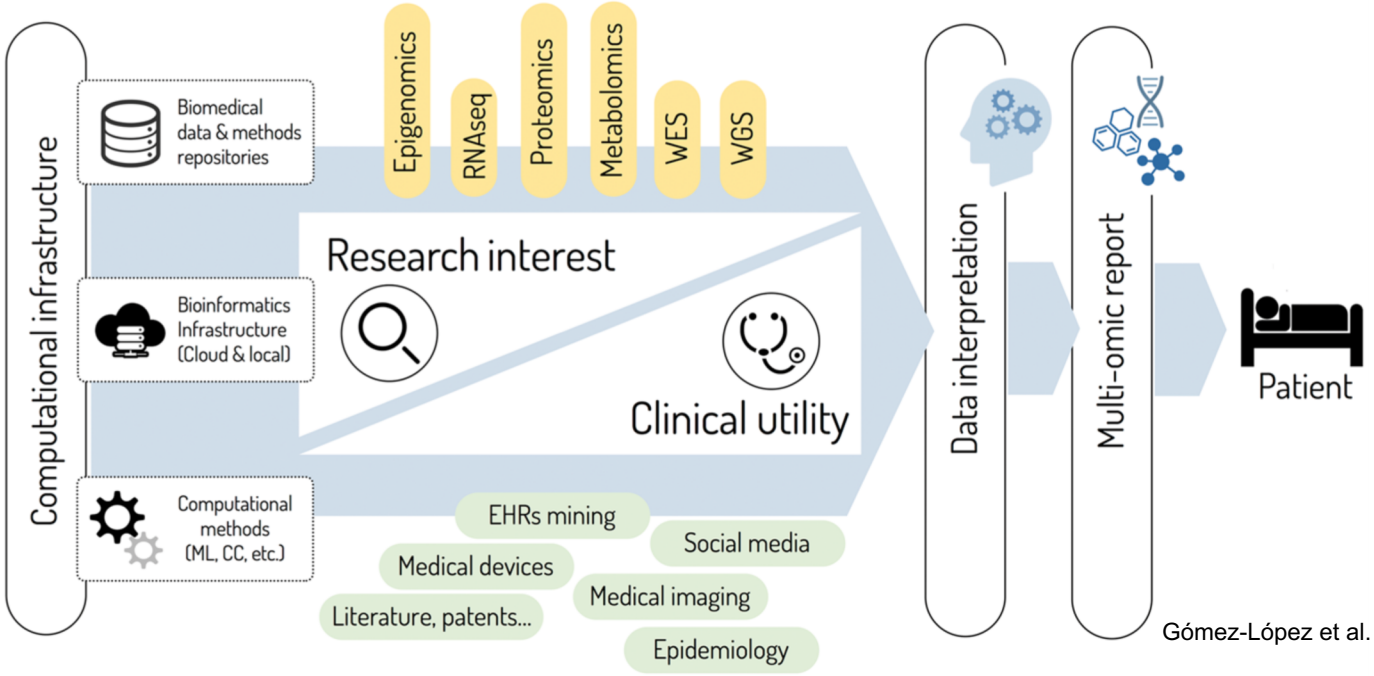
Spanish National Cancer Research Centre (CNIO)

Madrid

@BU_CNIO https://bioinformatics.cnio.es/

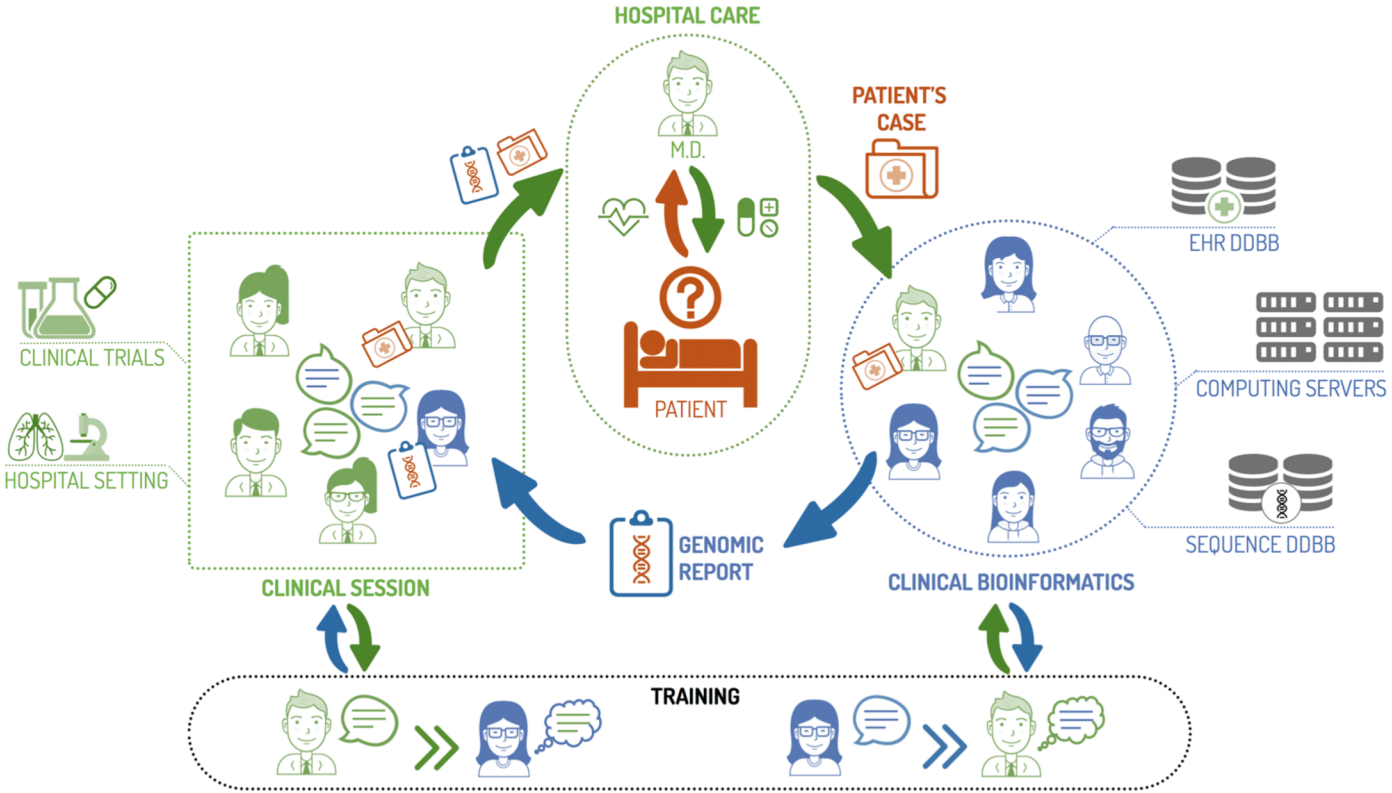
Precision medicine workflow

Precision oncology uses an **individual's genetic profile and individual information to guide decisions** made in regard to the prevention, diagnosis, and treatment of cancer including also other patient´s (citizen) data: lifestyle (diet, habits, physical exercise..), population data, monitoring, devices...



Gómez-López et al. Brief Bioinformatics 2017

Multidisciplinary team

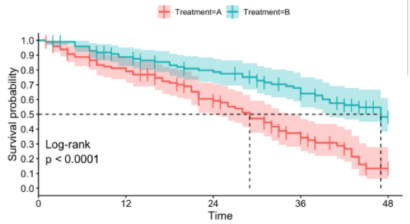


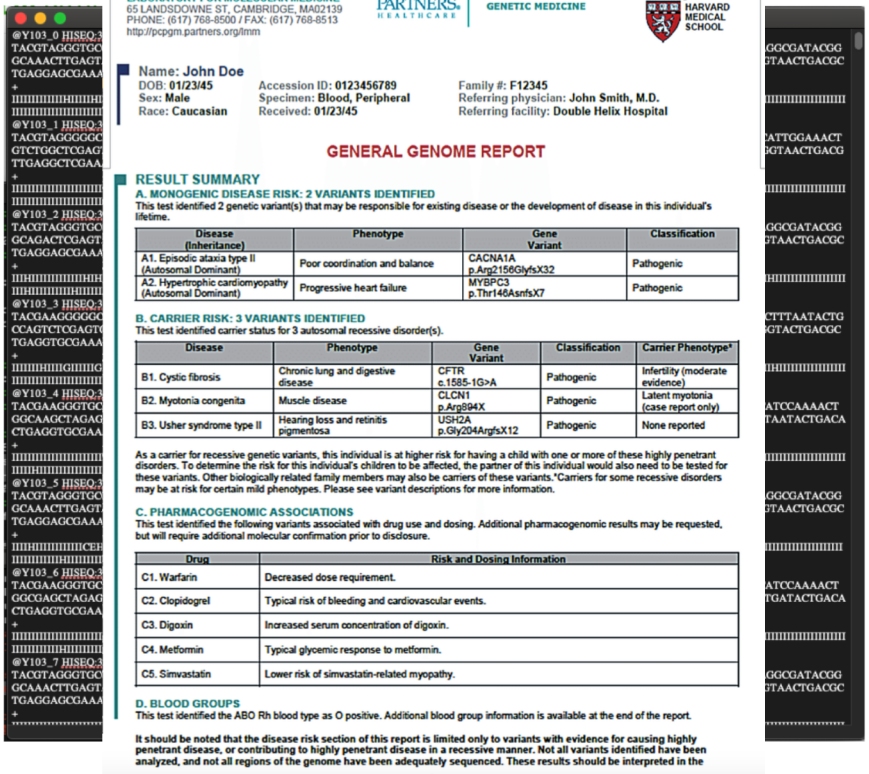
Gómez-López et al. Brief Bioinformatics 2017

PM challenges in Cancer

- Cancer **therapeutic options are still very limited and most patients acquire resistance** to the treatment.
- Now the TCGA/ICGC have defined cancer molecular landscape for many tumor types, a challenge remains to **associate molecular alterations with current therapies in the appropriate clinical context**.
- It is critical to **identify and understand the molecular landscape for each patient** beyond the tumoral type.
- Still a need for **new biomarkers for patients' stratification.**
- Molecular alterations **to identify drug responders and non-responders**.
- **Rational** in cancer treatments (surgery, RTX, combinations, sequential, etc).

Data integration for clinical decision making





LABORATORY FOR MOLECULAR MEDICINE
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http://pcpgm.partners.org/lmm

PARTNERS HEALTHCARE | CENTER FOR PERSONALIZED GENETIC MEDICINE | A teaching affiliate of: HARVARD MEDICAL SCHOOL

Name: John Doe
DOB: **01/23/45** Accession ID: **0123456789** Family #: **F12345**
Sex: **Male** Specimen: **Blood, Peripheral** Referring physician: **John Smith, M.D.**
Race: **Caucasian** Received: **01/23/45** Referring facility: **Double Helix Hospital**

GENERAL GENOME REPORT

RESULT SUMMARY

A. MONOGENIC DISEASE RISK: 2 VARIANTS IDENTIFIED
This test identified 2 genetic variant(s) that may be responsible for existing disease or the development of disease in this individual's lifetime.

Disease (Inheritance)	Phenotype	Gene Variant	Classification
A1. Episodic ataxia type II (Autosomal Dominant)	Poor coordination and balance	CACNA1A p.Arg2156GlyfsX32	Pathogenic
A2. Hypertrophic cardiomyopathy (Autosomal Dominant)	Progressive heart failure	MYBPC3 p.Thr146AsnfsX7	Pathogenic

B. CARRIER RISK: 3 VARIANTS IDENTIFIED
This test identified carrier status for 3 autosomal recessive disorder(s).

Disease	Phenotype	Gene Variant	Classification	Carrier Phenotype*
B1. Cystic fibrosis	Chronic lung and digestive disease	CFTR c.1585-1G>A	Pathogenic	Infertility (moderate evidence)
B2. Myotonia congenita	Muscle disease	CLCN1 p.Arg894X	Pathogenic	Latent myotonia (case report only)
B3. Usher syndrome type II	Hearing loss and retinitis pigmentosa	USH2A p.Gly204ArgfsX12	Pathogenic	None reported

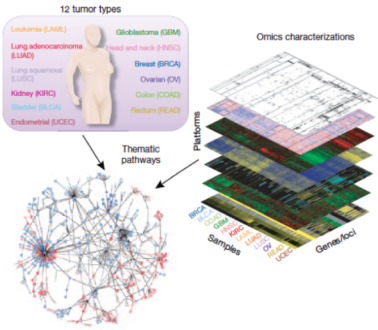
As a carrier for recessive genetic variants, this individual is at higher risk for having a child with one or more of these highly penetrant disorders. To determine the risk for this individual's children to be affected, the partner of this individual would also need to be tested for these variants. Other biologically related family members may also be carriers of these variants. *Carriers for some recessive disorders may be at risk for certain mild phenotypes. Please see variant descriptions for more information.

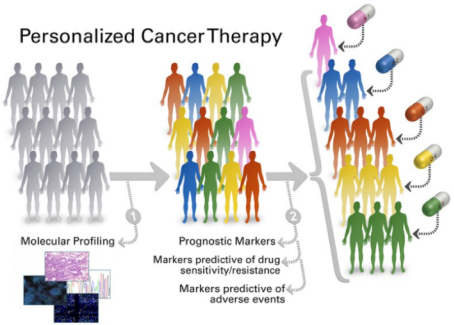
C. PHARMACOGENOMIC ASSOCIATIONS
This test identified the following variants associated with drug use and dosing. Additional pharmacogenomic results may be requested, but will require additional molecular confirmation prior to disclosure.

Drug	Risk and Dosing Information
C1. Warfarin	Decreased dose requirement.
C2. Clopidogrel	Typical risk of bleeding and cardiovascular events.
C3. Digoxin	Increased serum concentration of digoxin.
C4. Metformin	Typical glycemic response to metformin.
C5. Simvastatin	Lower risk of simvastatin-related myopathy.

D. BLOOD GROUPS
This test identified the ABO Rh blood type as O positive. Additional blood group information is available at the end of the report.

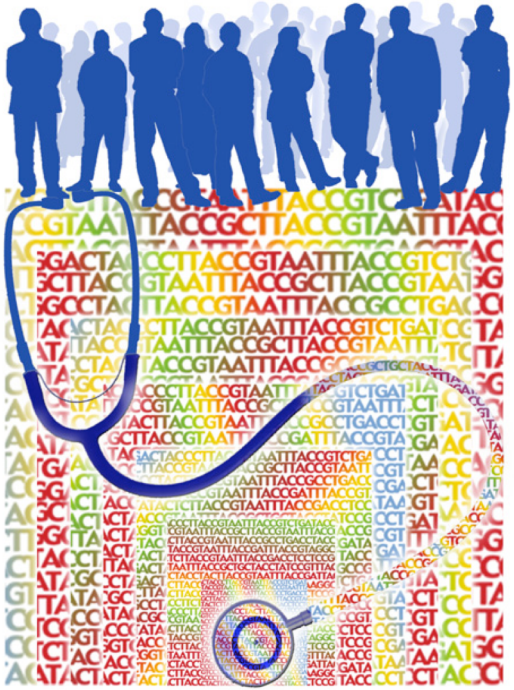
It should be noted that the disease risk section of this report is limited only to variants with evidence for causing highly penetrant disease, or contributing to highly penetrant disease in a recessive manner. Not all variants identified have been analyzed, and not all regions of the genome have been adequately sequenced. These results should be interpreted in the



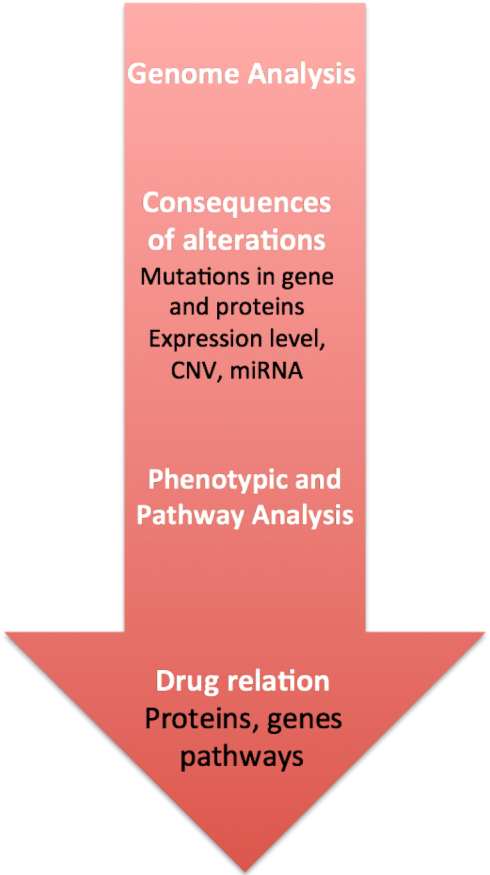


Precision Medicine: what do we need?

- Fast and cheap new **technologies.**
- International collaborative expert teams.
- **Databases** linking data to known information; methods to store, make accessible, query and interrogate them in sensible ways.
- **Predictive methods** to assess biological sense.
- Ability to automate and speed up the analysis through faster **algorithms** and **computational power.**
- **Funding** to analyze the information, provide it in a meaningful way and store it secure and permanently.
- An **ethical, social and legal background.**



Precision Medicine: what do we need?



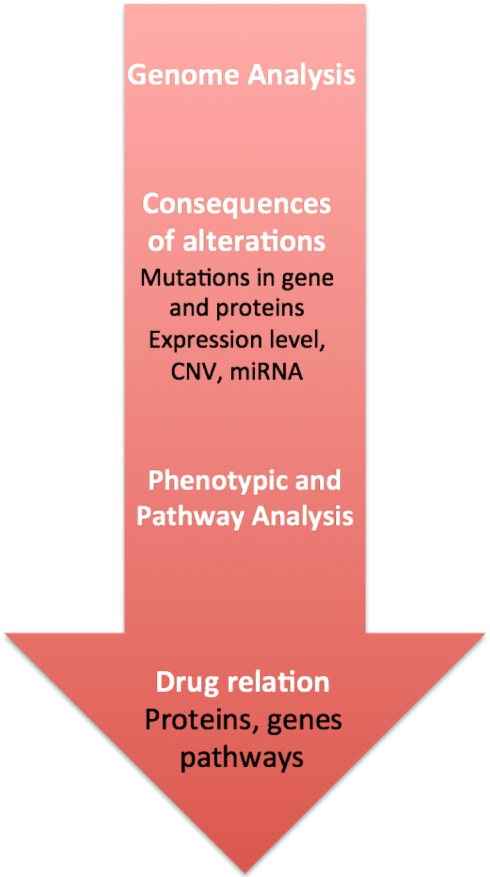
Developer (Bioinformatician):
Easy to maintain, modular, easy to expand with external methods, scalable, evaluable in continuous mode, robust software

Designer (Comp. Biologist):
Solve the scientific issues, understand the underlying problems, create new methods, access larger data sets

User (Clinician):
fast, accurate, robust, easy to understand and easy to interrogate at each step

Adapted from A. Valencia

Precision Medicine: what do we need?

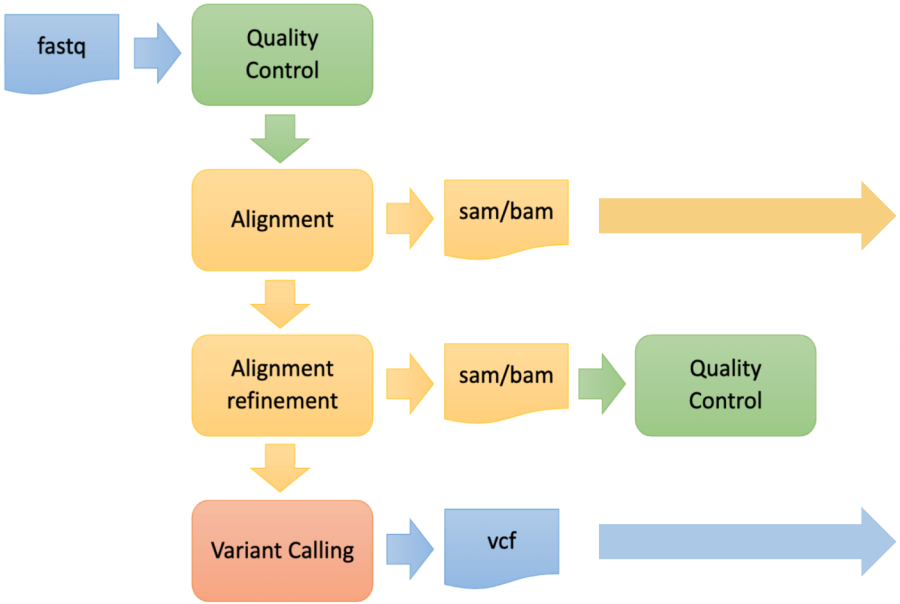


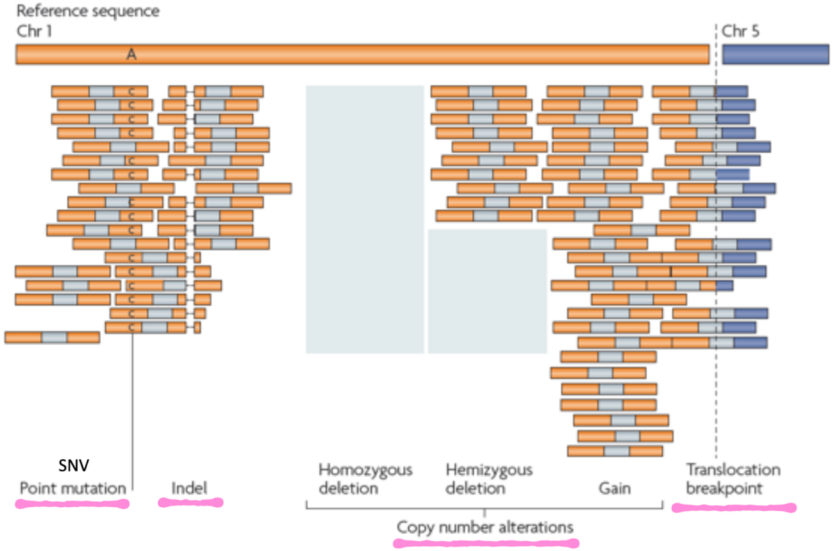
NGS, GWAS and others	**Evolving technology and software**
Protein function prediction, splicing, TF and miRNA binding	**Pending scientific issues.** **- Function prediction** **- consequences of mutation** **- etc**
Pathway integration, Gene control networks, Protein interaction networks	**Use of protein networks to map complex disease genes in functional pathways**
Extraction of Mutations-disease symptoms - drugs	**Text and DB mining**

Adapted from A. Valencia

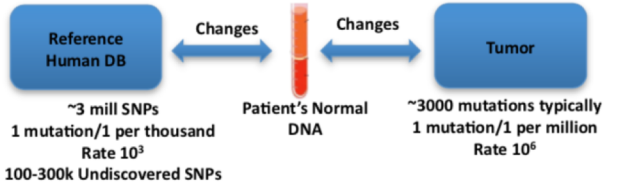
1. Big data in precision oncology
2. Methods for variant analysis (SNVs)
3. Methods for drugs prioritization
4. IA, data sharing…

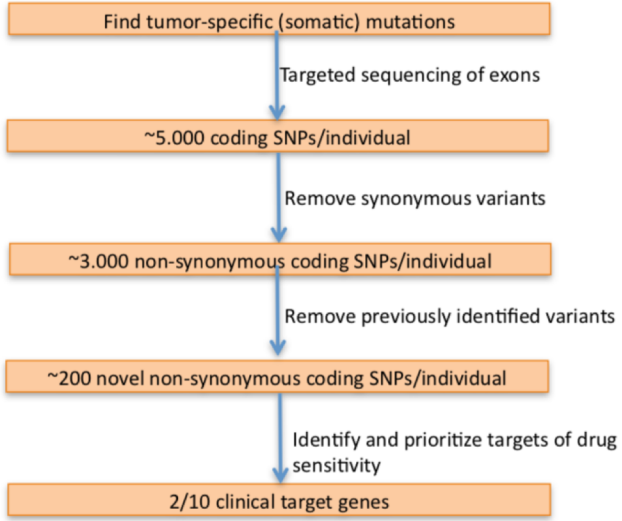
DNA-seq for detection of point genomic variants (SNVs)





Workflow analysis for SNVs



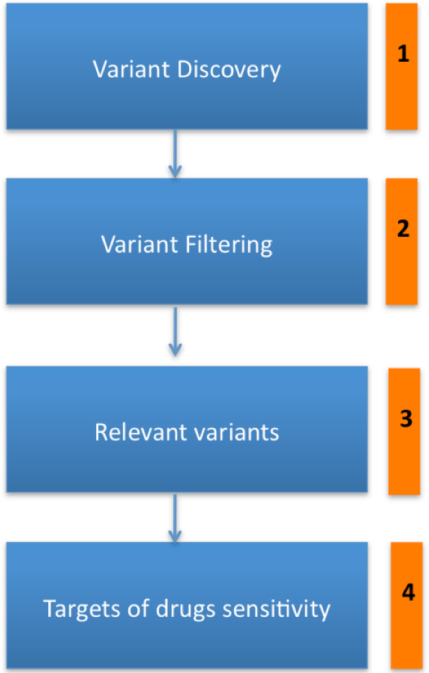


Detect tumor-specific somatic mutations.

Discard variants that not segregate with disease and they dont have a functional impact.

Prioritizate variants based on their functional effect & their association with the disease.

Successfully link genomic features with clinical applications.



Gonzalez-Perez A et al. Nat Meth 2013; 10(8):723-9; Ding L et al. Nat Rev Genet. 2014;15(8):556-70.

1. VARIANT DISCOVERY

Methodologies

- Mapping and alignment algorithms (BLAT, BWA, Bowtie, BFAST, GEM, MuTect, SomaticSniper…).
- Public and home-made pipelines for NGS analysis (GATK, Varca)
- Sequencing technologies are affordable. < 1000 € / ~1week.

Limitations

- Lack of technology standards (genomics, informatics, emerging technologies)
- Mapping and alignments algorithms produce different results
- Whenever a novel variant is identified, it will still have to be verified due to the this false positive
- Indels are difficult to detect

Challenges

- Processing large scale genomic data
- Decrease the error rate (~1 error/100Kb means 30.000 errors per genome).
- Detection of rare and novel variants will require increased confidence in the variant call.
- New algorithms for calling indels
- Improve QC metrics

WHAT IS CRUCIAL IN VARIANT DISCOVERY?

- For clinical practices, the use of **gold standard methods** and **reproducible analysis** are mandatory.
- The analysis is based on the comparison against the reference genome:

A single consensus sequence for the whole genome. It was built up from a high quality set of representative samples of the species (from different populations).
It is the first-line comparison during analysis.

By **Genome Reference Consortium (GRC)** (http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/)

- **Human assemblies (Versions):**
  - **GRCh37/hg19** : former version. Released in 2012. It is still used for analysis.
  - **CRCh38/hg38** : current version (Sep. 2017). Released in 2014. More accurate, comprehensive (includes Haplotypes) and sophisticated.

We must **keep consistency in the Genome Reference Version** through the variant analysis.

- We must know what **regions along the genome were sequenced** in the experiment, that is, the sequencing library.

2. VARIANT FILTERING

Methodologies:

- Gene structure: Elimination of synonymous/non-coding variant.
- Control variants: Elimination of dbSNP, HGMD, HapMap, dbVAR, DGV variants, 1000 Genomes Project.
- Computational methods to predict deleterious missense variants. The prediction algorithms input features generally include amino acid sequence, protein structure and evolutionary information: (SIFT, PolyPhen, MutationAssessor, Condel, SNPEffect, SNPs3D, FIREDB, FireStar: validated annotations of binding sites and analysis of mutations at the 3D level).

Limitations:

- GWAS are providing new insights, but only a limited number of variants have been characterized, and understanding the functional relationship between associated variants and phenotypic traits is difficult.
- Prediction methods do not provide any information about the pathophysiology of the diseases and so experimental tests are required to validate genetic predictions

Challenges:

- To filter or not to filer? Optional
- Data management, retrieval and quality control.

3. PRIORITIZE VARIANTS (GENES)

Methodologies:

- Prioritize variants predicted as damaging.
-Literature. Annotation: UCSC, Ensembl, Cancer Genes, OMIM, Text-mining. COSMIC
- Reference Cancer Genome (by recurrence): ICGC and TCGA projects.
- Evolutionary conservation: Prioritize variants highly conserved across species.
- Integration of genomic data resources: Expression, CNVs, Proteomics,
- Guilty-by-association: Genes involved to the biological process of interest.
- Pathway level: Identification of significantly altered gene sets and/or pathways using KEGG or other DBs (PARADIGM, Dendrix).

Limitations:

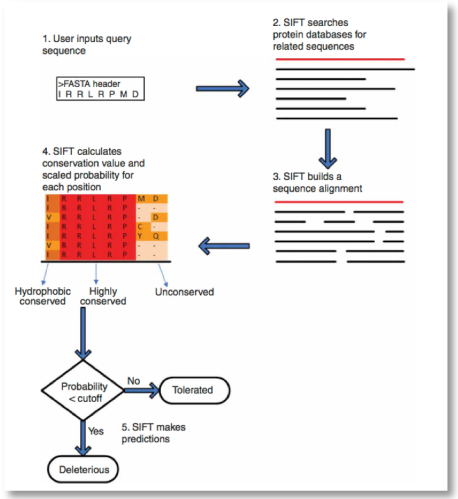
- Understanding of genomic variation data is limited and complex.
- There aren't tool for disease prediction.
- Different tools but they aren't integrated in a useful and guided pipeline.
- New methods are needed to evaluate the impact of insertion, deletion and synonymous variants.
- Predict the impact of non-coding variants affecting (regulatory regions and splicing sites).

Challenges:

- To manage large quantities of pre-processed data.
- Interpreting the functional effect and the impact of genomic variation.
- Integrating systems data to relate complex genetic interactions with phenotypes.
- Analyze biomedical DBs to build relationship between diseases, genes, mutations, drugs and pathways.

VARIANT FUNCTIONAL PREDICTION ALGORITHMS

- SIFT (http://sift.jcvi.org/)
- PROVEAN (http://provean.jcvi.org/)
- Variant Effect Predictor (http://www.ensembl.org/info/docs/variation/vep/index.html)
- Mutation Assesor (http://mutationassessor.org/)
- ANNOVAR (http://www.openbioinformatics.org/annovar/)
- SnpEff (http://snpeff.sourceforge.net/)
- Condel (http://bg.upf.edu/condel/home)
- Polyphen2 (http://genetics.bwh.harvard.edu/pph2/)
- Mutation Taster (http://www.mutationtaster.org/index.html)
- CanPredict (http://www.cgl.ucsf.edu/Research/genentech/canpredict/)
- PicMit (http://www.biocomputing.it/picmi/index.php)
- FireStar (http://firedb.bioinfo.cnio.es/Php/FireStar.php)
- MAPP (http://mendel.stanford.edu/SidowLab/downloads/MAPP/index.html)
- Varietas (http://kokki.uku.fi/bioinformatics/varietas/index.php)
- Ensembl SNP Effect tool (http://www.ensembl.org/info/website/upload/index.html#Consequence)
- SNPs3D (http://www.snps3d.org/)
- PantherPSEC (http://www.pantherdb.org/tools/csnpScoreForm.jsp)
- MTBAN(http://mtban.kaist.ac.kr/)
- CADD (https://cadd.gs.washington.edu/)



Mahmood, K., et al. Hum Genomics. 2017.

1. Big data in precision oncology
2. Methods for variant analysis (SNVs)
3. Methods for drugs prioritization
4. IA, data sharing…

4. DRUGGABLE TARGETS

Methodologies:

- Drug-single gene association or sometimes pathways.
- Literature
- Cancer Cell line Encyclopedia and Sanger Resources.

Limitations:

- one-variant, one-phenotype approach is inefficient.
- Gene-gene interactions
- Environmental interactions
- There aren't integration with any external knowledge sources or inform the biology behind the interactions.
- Lack of common technology platforms to enable the sharing of information and transfer to clinical application.

Challenges:

- Developing methods to integrate drug sensitivity with genomic data.
- Developing methods that combine multiple data sources and multi-factor predictions.
- Translating these discoveries into medical practice.
- Tools for predicting drug–target or drug–gene interactions will be essential.
- Prospective gene-stratification hypotheses need to be generated for future trials and will require new bioinformatics methods.
- Lack of common vocabularies

Treatment prioritization methods according to variants

Tool	Cit.	Year	User interface	Output	Online demo	Source code	Data integration	Automatic KB update
ANNOVAR	[14]	2010	File based	Annotation			Materialized	Individual
SnpEff/ClinEff	[15]	2012	File based	Annotation		✓	Materialized	Bundled
AnalyzeGenomes	[16]	2014	File based/ query based	Result set	✓		Materialized	Individual
GATK Funcotator	[17, 18]	2015	File based	Annotation		✓	Materialized	Bundled
MyVariant.info	[19]	2016	Query based	Result set	✓		Materialized	Individual
Ensemble Variant Effect Predictor (VEP)	[20]	2016	File based	Annotation	✓	✓	Materialized	Bundled
CanProVar	[21, 22]	2017	Query based	Result set	✓		Materialized	Unknown
PathOS	[23]	2017	File based	Report	✓	✓	Materialized	Bundled
Houston Methodist Variant Viewer (HMVV)	[24]	2017	File based	Report		✓	Materialized	Bundled
MTB-Report	[25]	2018	File based	Report		✓	Materialized	Unknown
Smart Cancer Navigator	[26]	2018	Query based	Report	✓	✓	API based	API based
Clinical and Genomic Information System (CGIS)	[27]	2018	File based	Report	✓		Materialized	Unknown
PanDrugs	[28]	2018	File based/ query based	Report	✓		Materialized	Unknown
PREDICT Variant Information System (VIS)	[29]	2018	Query based	Result set	✓		Materialized	Individual
Sequence Variant Identification and Annotation Platform (SeqVItA)	[30]	2018	File based	Annotation		✓	Materialized	Bundled
Precision Medicine Knowledgebase (PreMedKB)	[31]	2019	Query based	Report	✓		Materialized	Unknown
Pathogenicity of Mutation Analyzer (PathoMAN)	[32]	2019	File based/ query based	Result set	✓		Materialized	Unknown
Variant Interpretation for Cancer (VIC)	[33]	2019	File based	Annotation		✓	Materialized	Bundled
Translational Genomics expert (TGex)	[34]	2019	File based/ query based	Report	✓		Materialized	Unknown
PAS	[35]	2020	Query based	Result set	(✓)		Materialized	Individual
AML Variant Analyzer	[36]	2020	File based	Report	✓	✓	Materialized	Bundled
Open Custom Ranked Analysis of Variants Toolkit (OpenCRAVAT)	[37]	2020	File based	Annotation		✓	Materialized	Individual
VICC Meta-Knowledgebase (VICC MetaKB)	[38]	2020	Query based	Result set	✓	✓	Materialized	Individual
MIRACUM-Pipe	[39]	2020	File based	Report		✓	Materialized	Individual
Molecular Tumor Board (MTB) Portal	[40]	2020	File based/ query based	Report	✓		Materialized	Individual
VarStack	[41]	2020	Query based	Result set	✓		Materialized	Unknown

Borchert et al. Briefings in Bioinformatics 2021.

CNIO Bioinformatics Unit

https://bioinformatics.cnio.es/

- ✓ **Knowledge-driven hypothesis generation** for cancer treatment
- ✓ Bioinformatics + "omics" to propose therapies

PANDRUGS

https://www.pandrugs.org/

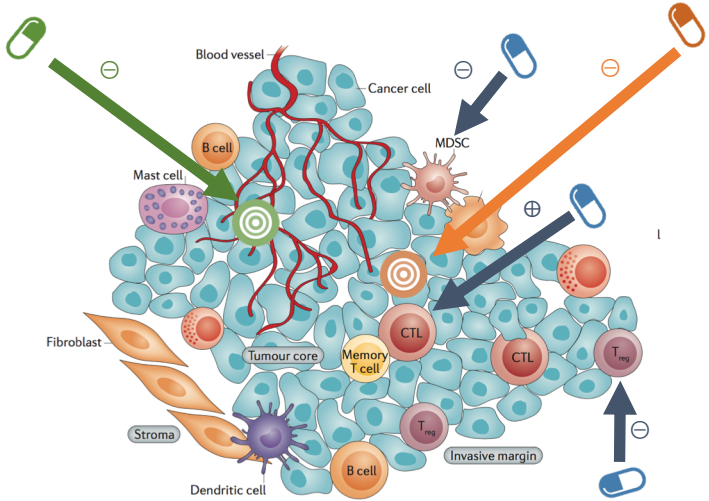
- **GENOMICS**
- Antitumoral *in silico* prescription
- Biological + clinical relevance scoring
- Input: Gene lists, VCF (Web, API)

Piñeiro-Yañez E *et al. Genome Med.* 2018
Piñeiro-Yañez E *et al. Cancers.* 2019
Fdez-Navarro P *et al. BMC Cancer.* 2019
Goldman M *et al* (PCAWG). *Nature Communications.* 2019

DREIMT

https://www.dreimt.org/

- **TRANSCRIPTOMICS**
- Drug screenings - hypothesis generation
- Drug prioritization to target immune cells
- Input: Expression signatures (Web, API)

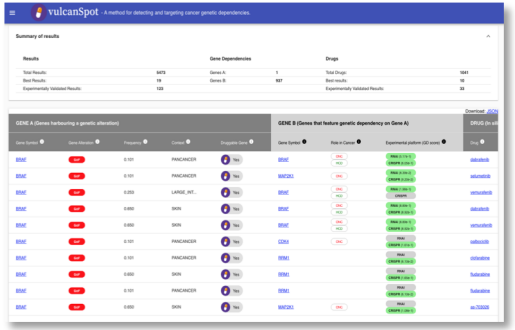


Troule et al.. *Bioinformatics* 2020

vulcanSpot

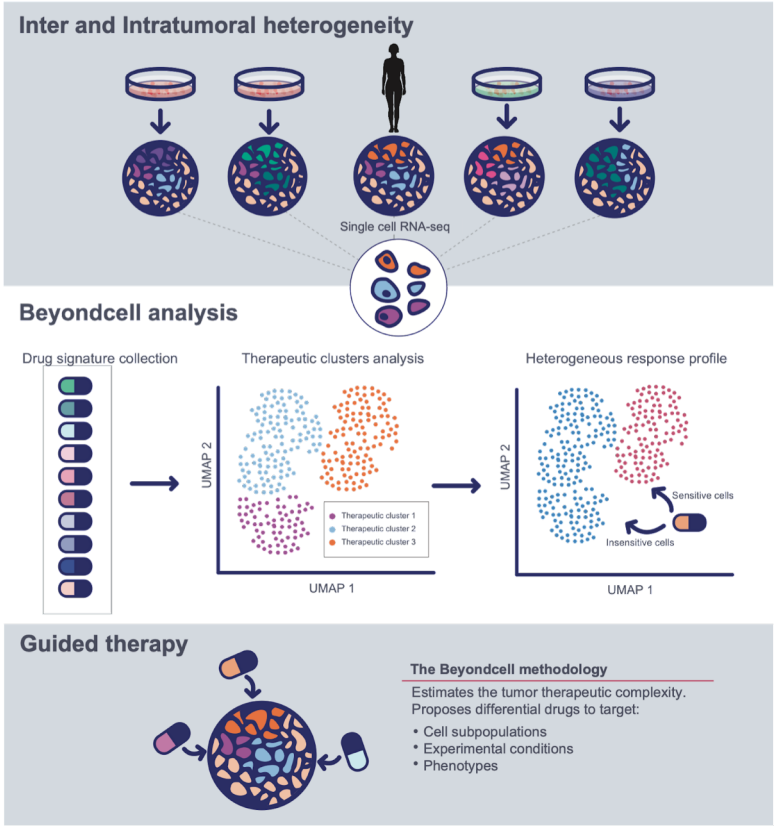
http://www.vulcanspot.org/

- **GENOMICS-TRANSCRIPTOMICS**
- Antitumoral *in silico* prescription
- Genetic dependencies identification
- Input: HUGO name (Web, API)



Perales-Patón J et al. *Bioinformatics* 2019

Detecting tumour and TME subpopulations with distinct drug response using single cell data

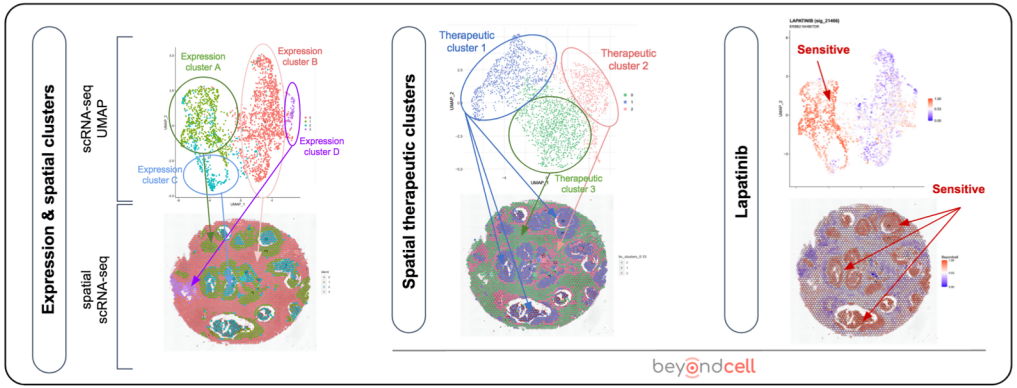


Fustero-Torre *et al*. *Genome Med.* 2021

Visit: https://gitlab.com/bu_cnio/beyondcell

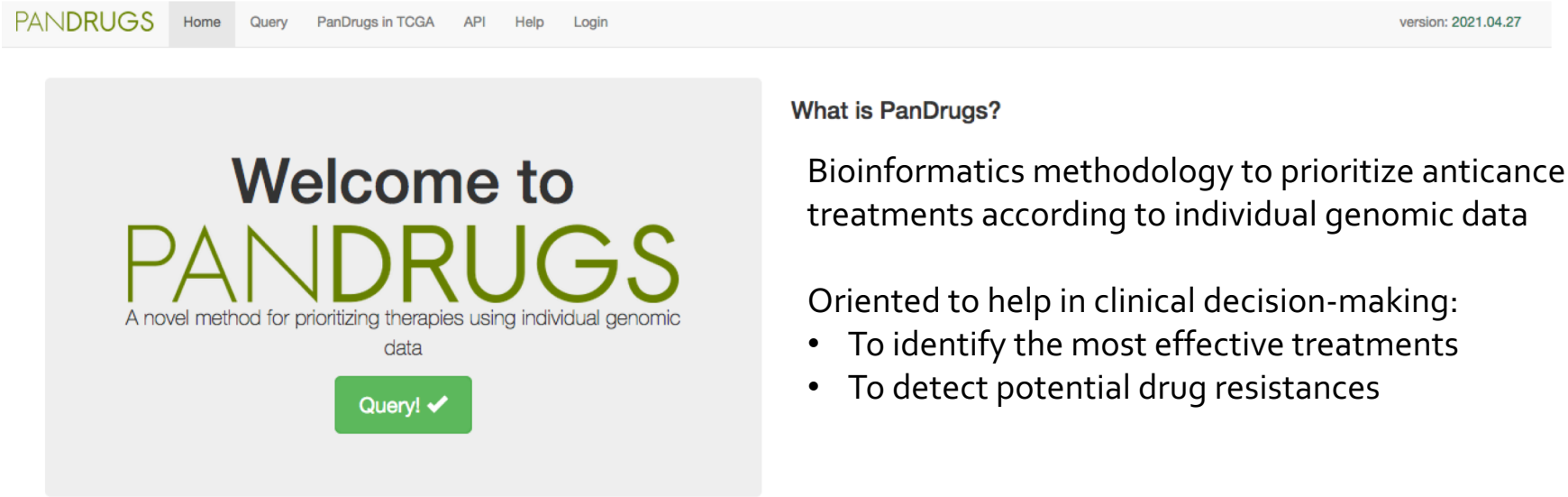
beyondcell

Drug targeting Tumour and TME using Spatial Single Cell transcriptomics



PanDrugs: *in silico* drug prescription tool

www.pandrugs.org



Bioinformatics methodology to prioritize anticancer drug treatments according to individual genomic data

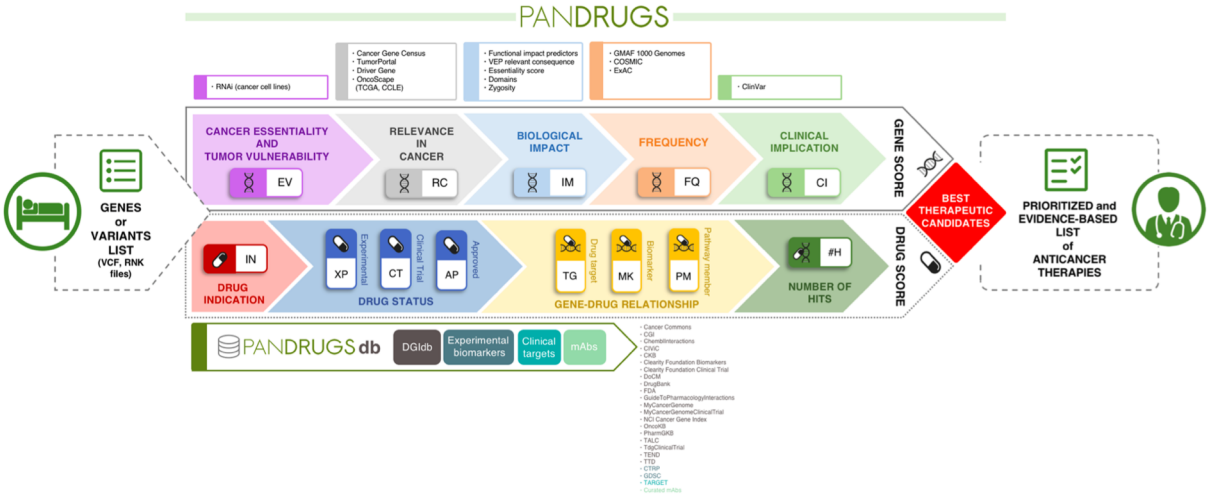
Oriented to help in clinical decision-making:

- To identify the most effective treatments
- To detect potential drug resistances

Piñeiro-Yáñez E, Reboiro-Jato M et al. Genome Medicine. 2018

Prioritizing drugs from variant lists: PanDrugs

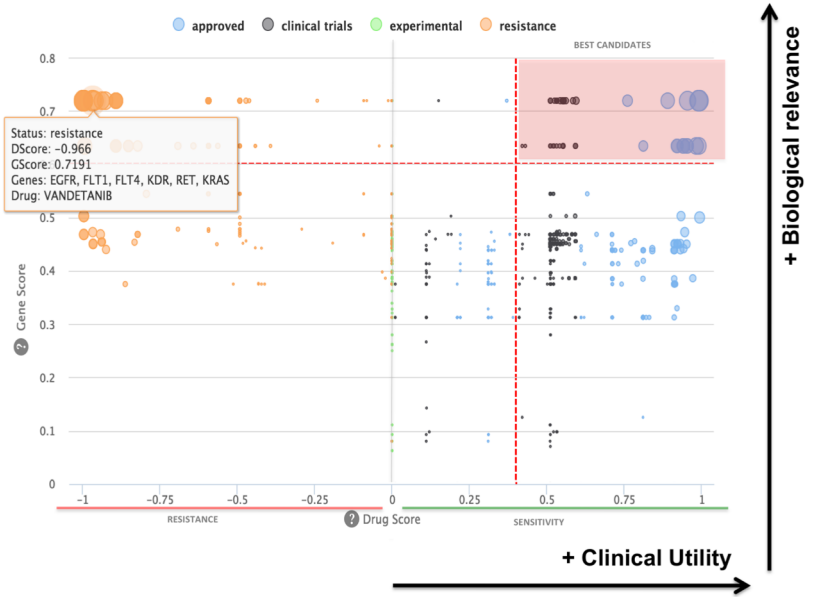
PanDrugs prioritizes <u>the best therapeutic candidates</u> according to individual genomic data in cancer patients. PanDrugs includes the largest pharmacogenomics database currently available and a scoring system to prioritize treatments



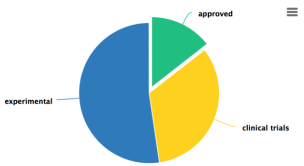
https://www.pandrugs.org/

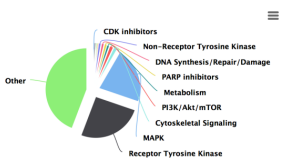
Piñeiro-Yañez E *et al. Genome Med*. 2018; Piñeiro-Yañez E *et al. Cancers.* 2019; Fdez-Navarro P *et al. BMC Cancer*. 2019; Goldman M *et al. Nature (PCAWG)*. 2020.

Prioritizing treatments from variant lists: PanDrugs



Actions	Gene(s)	Drug	Family	Source(s)	Drug status	Type of therapy	Interaction	DScore	GScore
+	EGFR	ERLOTINIB	Receptor Tyrosine Kinase	CC CFB CFCT DB MCG PGKB TALC TCGA TEND TDD	Approved for lung	Targeted therapy	T⊣D	0.9180	0.9398
+	BRAF	VEMURAFENIB	MAPK	CC CFB CFCT MCG MCGCT PGKB TALC TCGA TDD	Approved for skin	Targeted therapy	T⊣D	0.9180	0.7687
+	EGFR	CETUXIMAB	Receptor Tyrosine Kinase	CC CFB DB MCG TALC TEND TDD moAb	Approved for colon and rectum	Targeted therapy	T⊣D	0.9170	0.9398

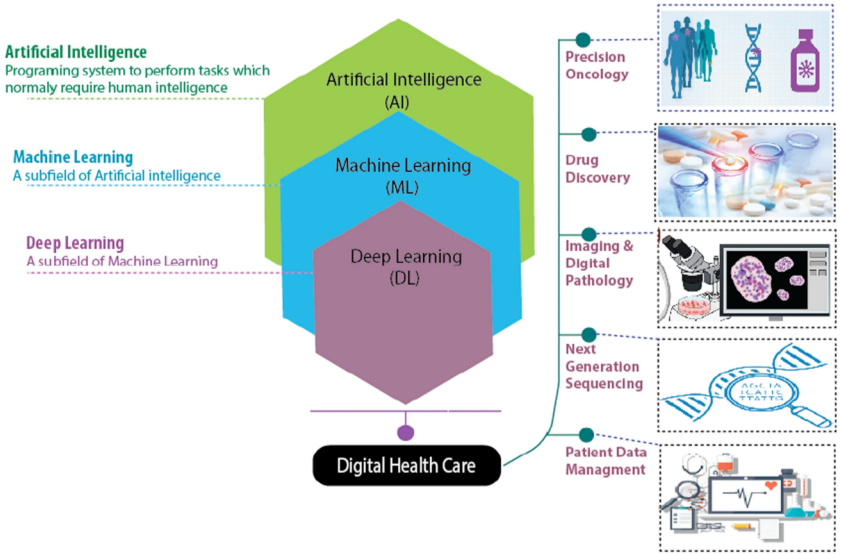


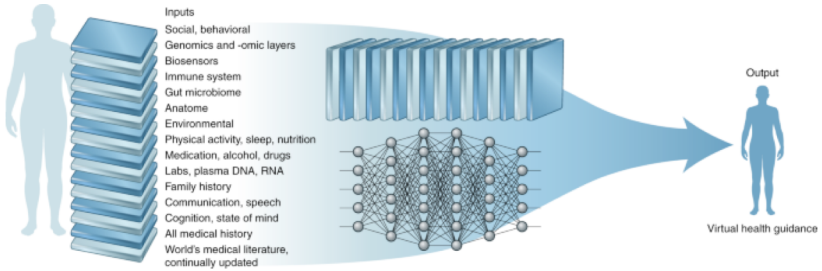


https://www.pandrugs.org/

1. Big data in precision oncology
2. Methods for variant analysis (SNVs)
3. Methods for drugs prioritization
4. IA, data sharing…

Artificial intelligence in precision oncology





Data	Applications	Output
• Genomics • Histopathology Images • Electronic Medical Records	• Diagnosis • Treatment • Screening and Prevention • Prognosis and survival • Classification • Drug design • Clinical trials	• Improving early detection and survival rates • Finding right treatments • Accelerating drug discovery and development • Reducing errors • Faster, more affordable and accesible

Rajpurkar et al. Nature Medicine. 2022; Greener, J. G., et al. Nat Rev Mol Cell Biol, 2022; Iqbal et al. Cancer Cell. 2021; Tran et al. Genome Medicine 2021; Denny, J. C., & Collins, F. S. Cell, 2021.

Applying deep-learning algorithms to healthcare 2021

Method of the year 2022: Protein folding problem

Prospective and retrospective clinical Trials

AI approaches in medicine have been limited by the (un)availability of large, commonly structured datasets

Precision oncology and Pharmacogenomics

Integrating spatial gene expression and breast tumour morphology via deep learning

Bryan He[1], Ludvig Bergenstråhle[2], Linnea Stenbeck[2], Abubakar Abid[3], Alma Andersso Åke Borg[4], Jonas Maaskola[2]✉, Joakim Lundeberg[2]✉ and James Zou[1,3,5,6]✉

ARTICLE

A deep learning model to predict RNA-Seq expression of tumours from whole slide images

Benoît Schmauch[1✉], Alberto Romagnoni[1,4], Elodie Pronier[1,4], Charlie Saillard[1], Pascale Maillé[2,3], Julien Calderaro[2,3], Aurélie Kamoun[1], Meriem Sefta[1], Sylvain Toldo[1], Mikhail Zaslavskiy[1], Thomas Clozel[1], Matahi Moarii[1], Pierre Courtiol[1,5] & Gilles Wainrib[1,5✉]

Data and text mining

DeepSynergy: predicting anti-cancer drug synergy with Deep Learning

Kristina Preuer[1], Richard P. I. Lewis[2], Sepp Hochreiter[1], Andreas Bender[2], Krishna C. Bulusu[2,3] and Günter Klambauer[1,*]

quality improving system for monitoring blind spots during esophagogastroduodenoscopy

Liu X, Nat Med. 2020 Sep;26(9):1364-1374.

The European roadmap

An european distributed infrastructure for life-science information (23 countries)

ROADMAP

Leveraging European infrastructures to access 1 million human genomes by 2022

Gary Saunders[1], Michael Baudis[2], Regina Becker[3], Sergi Beltran[4,5], Christophe Béroud[6,7], Ewan Birney[8], Cath Brooksbank[8], Søren Brunak[9,10], Marc Van den Bulcke[11], Rachel Drysdale[1], Salvador Capella-Gutierrez[12], Paul Flicek[8], Francesco Florindi[13], Peter Goodhand[14,15], Ivo Gut[4,5], Jaap Heringa[16], Petr Holub[13], Jef Hooyberghs[17], Nick Juty[18], Thomas M. Keane[8], Jan O. Korbel[19], Ilkka Lappalainen[20], Brane Leskosek[21], Gert Matthijs[22], Michaela Th. Mayrhofer[13], Andres Metspalu[23], Arcadi Navarro[24,25,26], Steven Newhouse[8], Tommi Nyrönen[20], Angela Page[15,27], Bengt Persson[28], Aarno Palotie[29], Helen Parkinson[8], Jordi Rambla[26], David Salgado[6], Erik Steinfelder[13], Morris A. Swertz[30], Alfonso Valencia[12,31], Susheel Varma[8], Niklas Blomberg[1] and Serena Scollen[1]*

To maximize the value of the genomic data generated, these data will need to be shared between institutions and across countries.

European research infrastructures are well-positioned to support the rapid implementation of widespread genomic data access.

https://elixir-europe.org/

Saunders, G. *et al*. *Nat Rev Genet* **26,** 778–9 (2019).

Use Case 1 - CANCER DIAGNOSIS

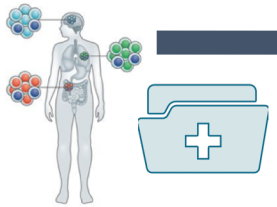
Patient CRUK0056
- 71-year-old woman. Chest X-ray showing a suspicious mass on the left lung.
- Biopsy, Surgical treatment and sequencing.
- Lung tumour tissue from this patient was sampled in 3 tumour regions (R1, R2, R3).

METHODS FOR DIAGNOSIS

Physical exam; Clinical biochemistry; Imaging; Biopsy; Surgical pathology; Anatomical Pathology, histology; Clinical genetics; NGS; Mining Electronic Health Record (EHR); AI; Literature...

DATA

Blood test biomarkers; SP and AP report; Images; Genetics & genomics variants (somatic and germline); EHR; AI predictive report...



Diagnosis:
- NSCLC (stage IB).
- 119 mutated genes.

GENOMIC REPORT

Best practices & Clinical guidelines

GOALS	Variant discovery and prioritization eg: GATK, VEP, etc	Variant interpretation Pathogenic variants eg: HGMD, ClinVar, etc Genomic report	Cancer driver genes eg: COSMIC, literature,..	AI prediction
CHALLENGES	- Benchmarking pipelines. - Standardised NGS pipelines. - Clinical guidelines (VF, artifacts)	- Standardized & harmonised DDBBs. - Common cancer reference resource for clinical decision-making. - Development of genomic reports. - Need EU Cancer Patient Digital Centre.	- Complete CDG list. - Tumours cohort analysis from large consortiums (ARGO, 1MG), . - Expand CDG detection methods - New functional exp data. - Integrative pheno-geno projects.	- Access, analysis and integration of EHR, images, genomic data... - Patient Digital Centre.

elixir

Trained bioinformaticians
Interoperable infrastructures (HL7 FHIR)
GA4GH standards (Beacon project, Phenopacket, SchemaBlocks...)
FAIR (Findable, Accessible, Interoperable, Reusable)

Use Case 2 & 3 - Patient Classification, prediction and enroll in clinical trial

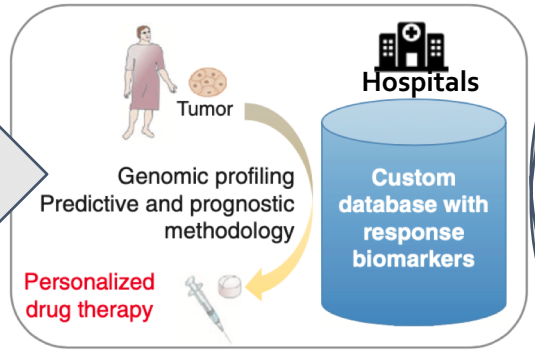
Cancer Mission Board Recommendations:

Create a **European Cancer Patient Digital Centre** where cancer patients and survivors can deposit and share their data for personalised care

CLINICAL ONCOLOGY DECISION MAKING

Data (genotype, phenotype, health care, longitudinal)

- **Electronic health Records**
- **Demographic data**
- **Environmental factors**
- **Genomic data**
- **Clinico-pathological data**
- **Full therapeutic regimen**
- **Evolution…**



TRANSLATIONAL RESEARCH

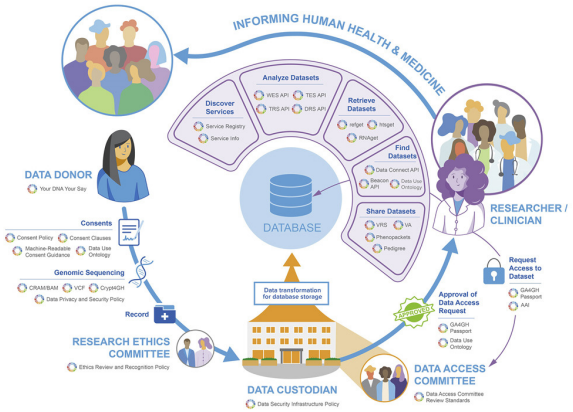
Scientific and clinical questions

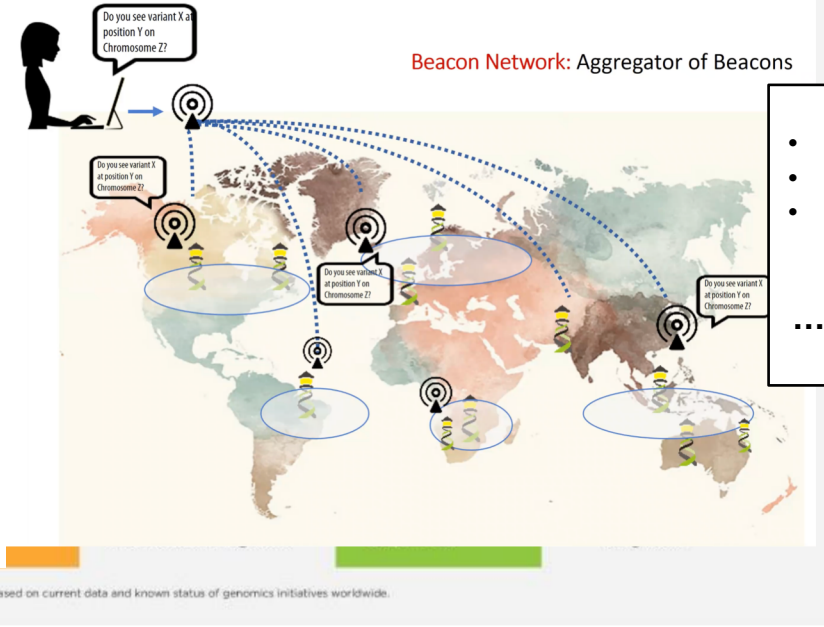
Web Service to upload a genomic profile and get response back

- Anonymized and custom annotated dataset.
- Correlation between genomic data (biology) and disease evolution.
- Multi-omics, clinico-pathological and treatment response data comparisons.
- Patient classification and prediction (origin, subtype, diagnosis, drug response, metastasis, survival, etc).

Data sharing for genomic medicine

Sharing data saves lives





- Sharing of expertise
- Submission to international DDBB
- Controlled Access

… keeping ethics and security

Saunders, G., et al. Nat Rev Genet, 2019; Rehm, H. L., et al. Cell Genomics, 2021.

CONCLUSIONS

- Precision oncology (PO) is not possible without **multidisciplinary teams** including experts in health, genetics and bioinformatics and the use of computational algorithms and bioinformatics methods.
- **Computational methods** for PO allow complex data to be processed, analysed, interpreted, compared and integrated to suggest individualized treatments and facilitate clinical decision-making.
- **Secure sharing** of clinical and multi-genomic data within the Health System is essential to enable PO.
- The main limitation of point variant interpretation and prioritisation methods is the **lack of optimal algorithms for predicting** the functional impact of variants on protein structure.
- **Treatment prediction methods** integrate lists of SNVs with information deposited in databases to facilitate clinical decision-making.
- **AI-based systems** require massive sets of structured data to be trained effectively.

1. Databases for genomics variants
2. Cancer Genomics portals
3. Tools for in silico drug prescription

Repositories and databases of genomic variants

- Genome variation and visualization
UCSC Genome Browser: genome.ucsc.edu
ENSEMBL: www.ensembl.org/info/genome/variation/
NCBI MapViewer : www.ncbi.nlm.nih.gov/mapview/map_search.cgi/
1000Genomes: http://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/
OMIM: http://www.omim.org
Human Genome Variation DB: https://gwas.biosciencedbc.jp/

- SNPs and haplotype data
dbSNP: http://www.ncbi.nlm.nih.gov/SNP/
HapMap website : www.hapmap.org
HapMap Genome Browser: www.hapmap.org/cgi-perl/gbrowse/gbrowse/
SNAP : www.broad.mit.edu/mpg/snap/
GWAS central: http://www.gwascentral.org/

- Somatic variants
Human Gene Mutation Database: http://www.hgmd.org
ClinVar: http://www.ncbi.nlm.nih.gov/clinvar
COSMIC: http://www.sanger.ac.uk/cosmic
ICGC data portal: https://dcc.icgc.org/
TCGA data portal: https://portal.gdc.cancer.gov/
HGVS: http://www.hgvs.org/mutnomen/recs-DNA.html
WHESS.db: http://genetics.bwh.harvard.edu/pph2/dbsearch.shtml
cBioPortal: http://www.cbioportal.org/public-portal
CCLE: http://www.broadinstitute.org/ccle
My Cancer Genome: http://www.mycancergenome.org
NHLBI Exome variant server: http://evs.gs.washington.edu/EVS/

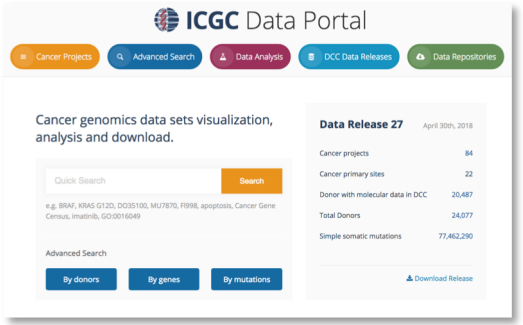
- Structural variants
TCGA Genomic Variants: projects.tcag.ca/variation/
UW Structural Variation db: humanparalogy.gs.washington.edu/
CNV Control Dd: http://gwas.biosciencedbc.jp/cgi-bin/cnvdb/cnv_top.cgi
dbVar: www.ncbi.nlm.nih.gov/dbvar/
Database of Genomic Variants (DGV): http://dgv.tcag.ca/dgv/app/home

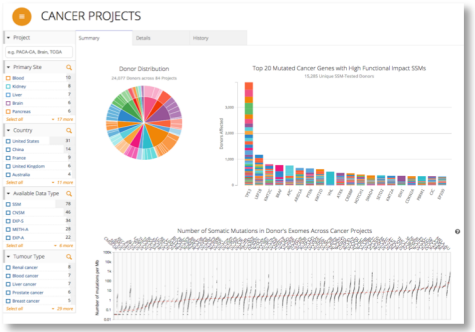
- Genome-Phenome interactions
dbGAP: https://www.ncbi.nlm.nih.gov/gap
EGA: https://www.ebi.ac.uk/ega/home

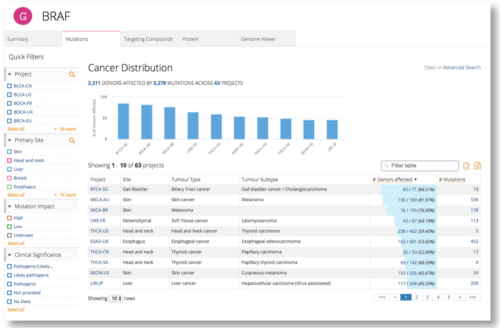
1. Databases for genomics variants
2. Cancer Genomics portals
3. Tools for in silico drug prescription

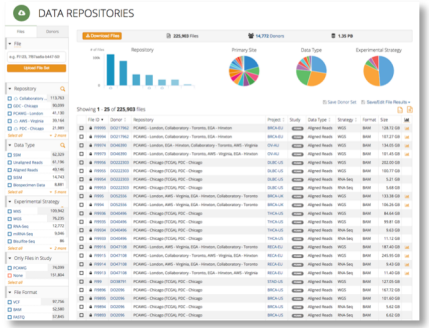
Cancer genomics portals: ICGC data portal

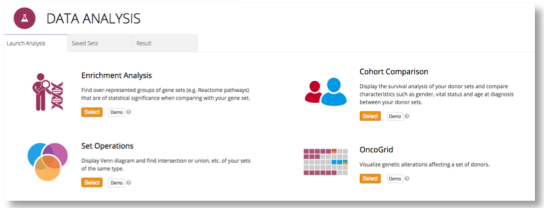
https://dcc.icgc.org/







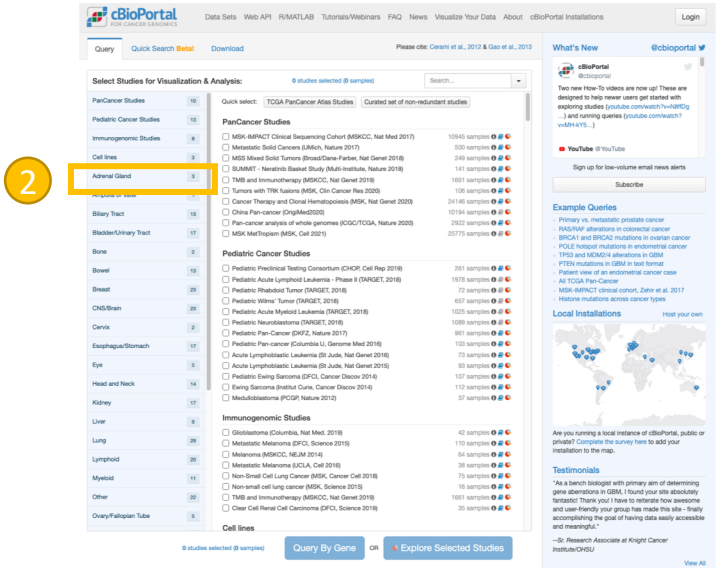


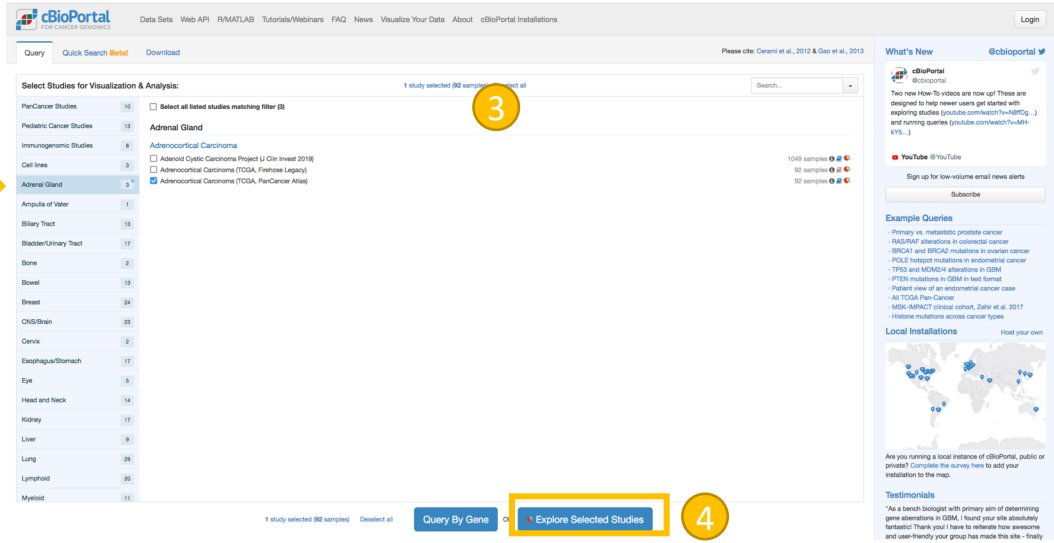


Zhang J et al. International Cancer Genome Consortium Data Portal--a one-stop shop for cancer genomics data. Database (Oxford). 2011:bar026.

Cancer genomics portals: cbioportal

1 https://www.cbioportal.org/





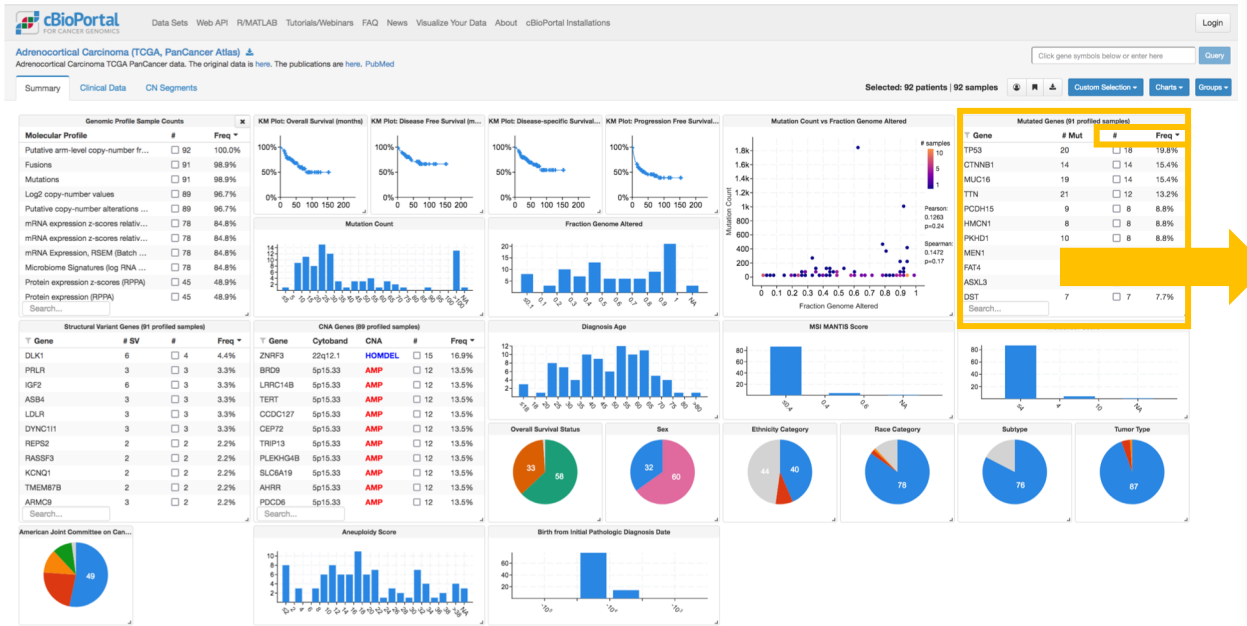
Gao J et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci. Signal. 2013

Cancer genomics portals: cbioportal

https://www.cbioportal.org/

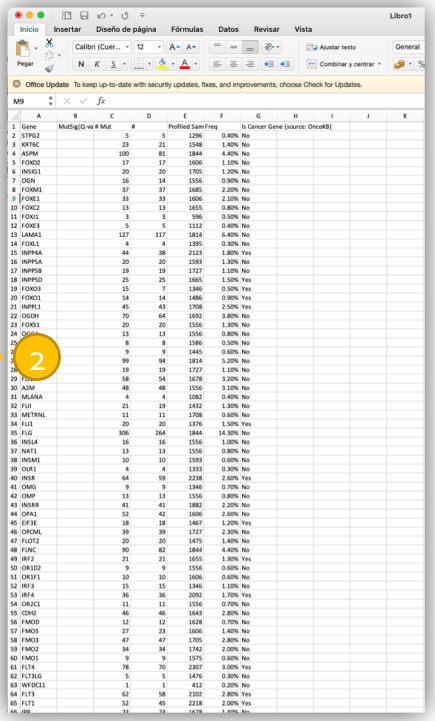
Adrenocortical Carcinoma TCGA PanCancer, 92 patients

1. Get mutations **from all patients**



List of mutations from all patients

2

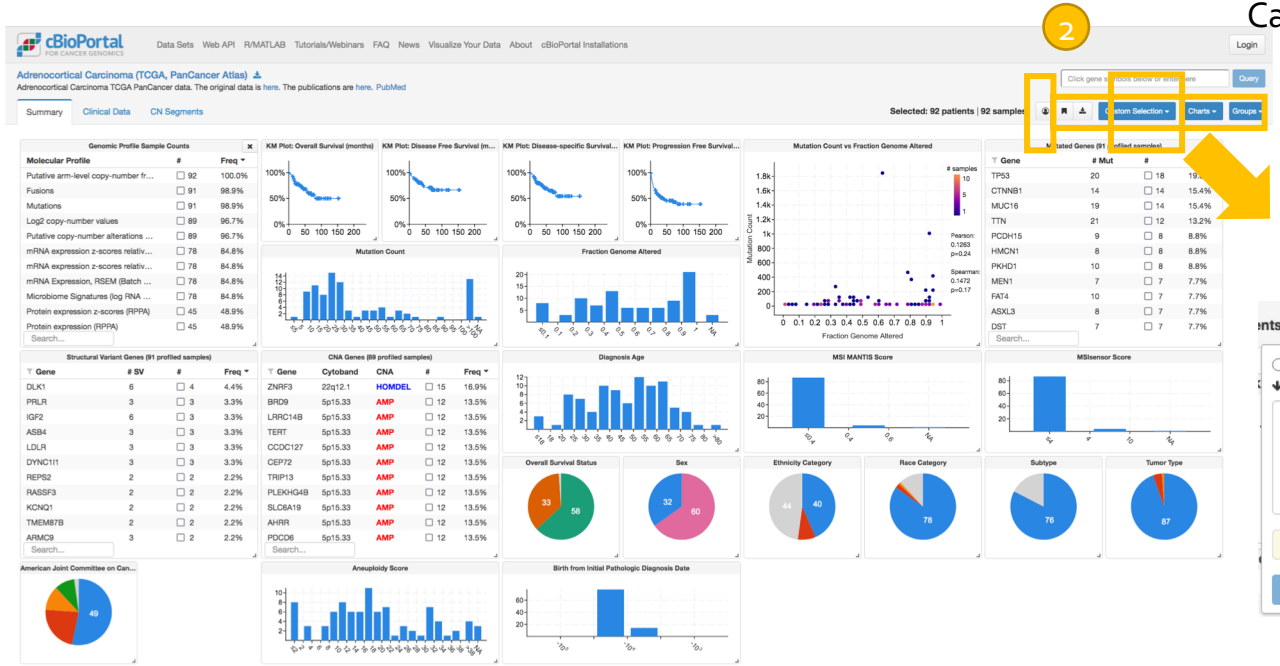


Cancer genomics portals: cbioportal

https://www.cbioportal.org/

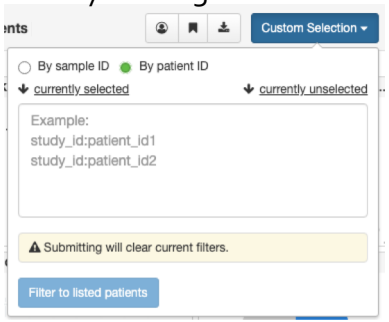
Obtain mutations **from 1 patient**

1. Selection menu



Case selected by cBioPortal
Patient TCGA-OR-A5J5

Alternatively:
Analyze using "Patient ID"



Cancer genomics portals: cbioportal

https://www.cbioportal.org/

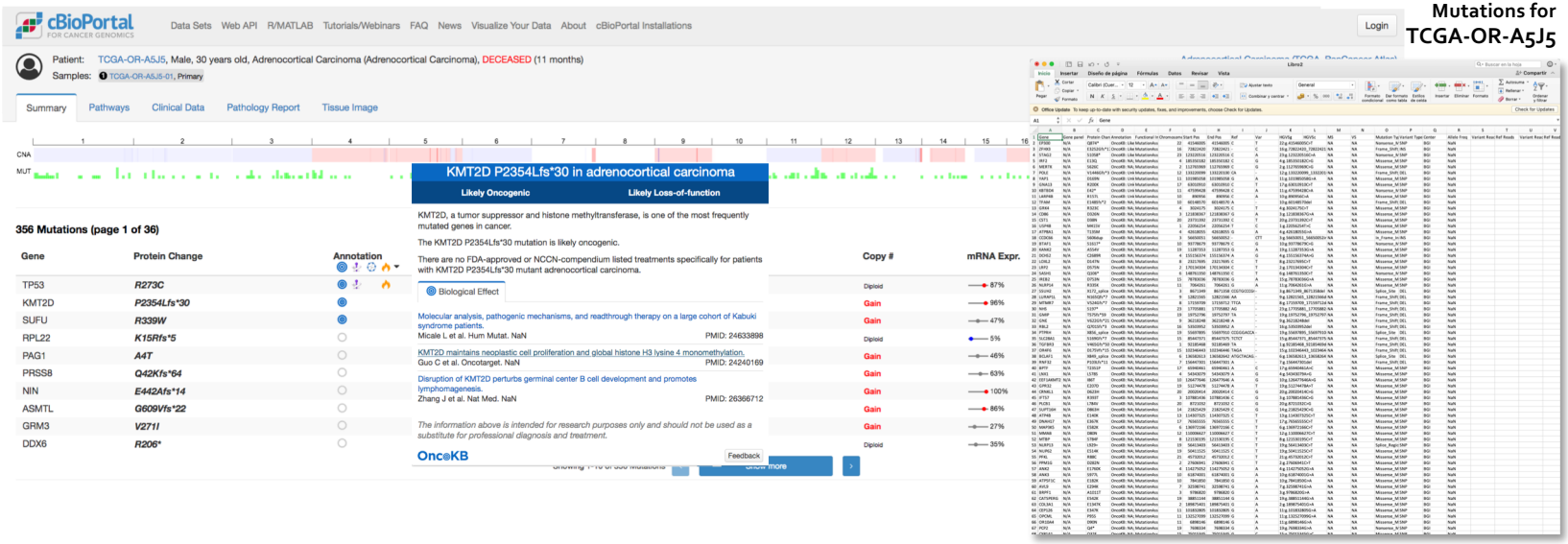
Obtain mutations **from 1 patient** (TCGA-OR-A5J5)

1

Download 356 mutations

2

Mutations information



Mutations for TCGA-OR-A5J5

There are no FDA-approved or NCCN-compendium listed treatments specifically for patient

1. Databases for genomics variants
2. Cancer Genomics portals
3. Tools for in silico drug prescription

Prioritizing drugs from variant lists: PanDrugs

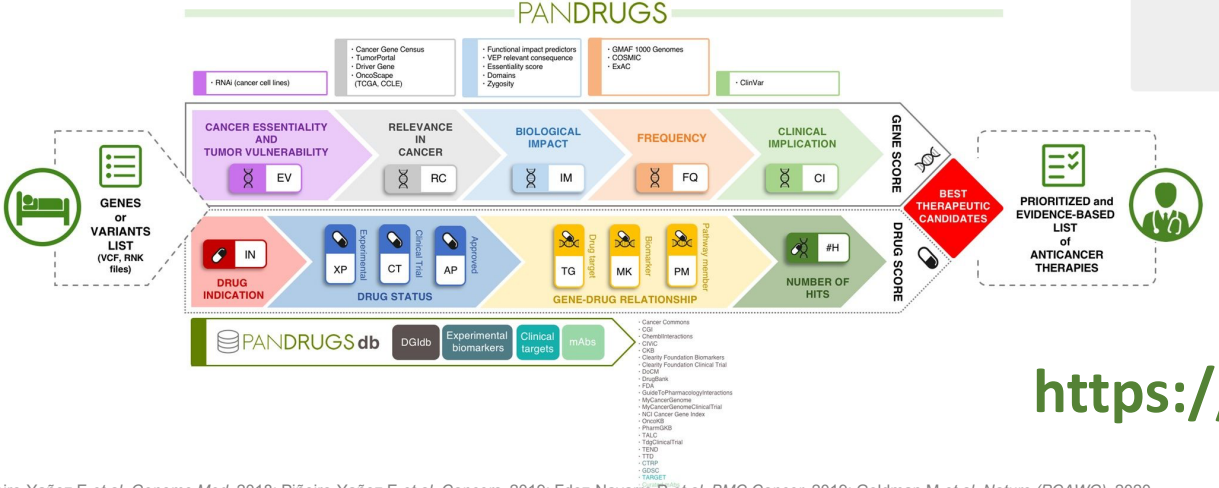
PanDrugs

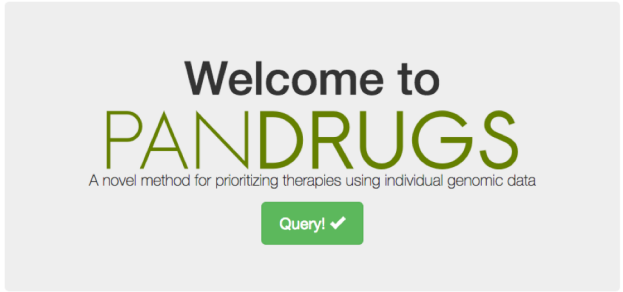
Integrates drug sensitivity with genomic data

24 data sources,

56044 drug-target associations

4703 genes and 9073 unique drugs





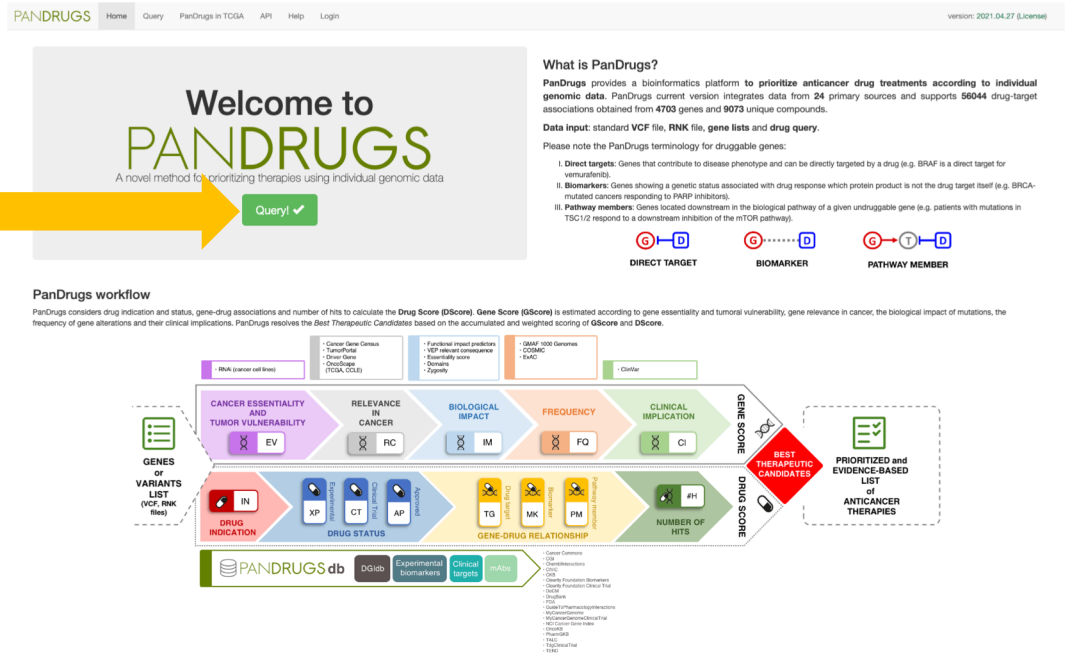
hands-on TRAINING

https://www.pandrugs.org/

Piñeiro-Yañez E *et al. Genome Med.* 2018; Piñeiro-Yañez E *et al. Cancers.* 2019; Fdez-Navarro P *et al. BMC Cancer.* 2019; Goldman M *et al. Nature (PCAWG).* 2020.

Prioritizing drugs from variant lists: PanDrugs

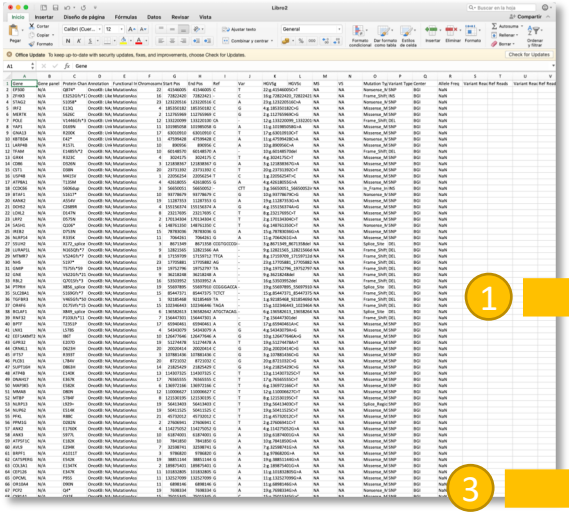
1. **https://www.pandrugs.org/**

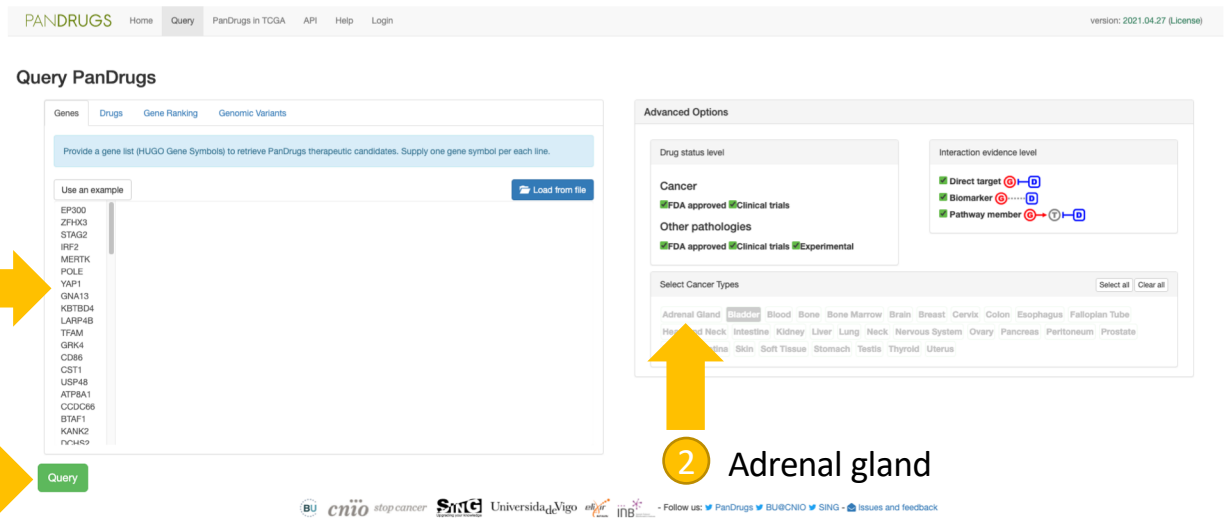


Prioritizing drugs from variant lists: PanDrugs

https://www.pandrugs.org/

Mutations for TCGA-OR-A5J5





Prioritizing drugs from variant lists: PanDrugs

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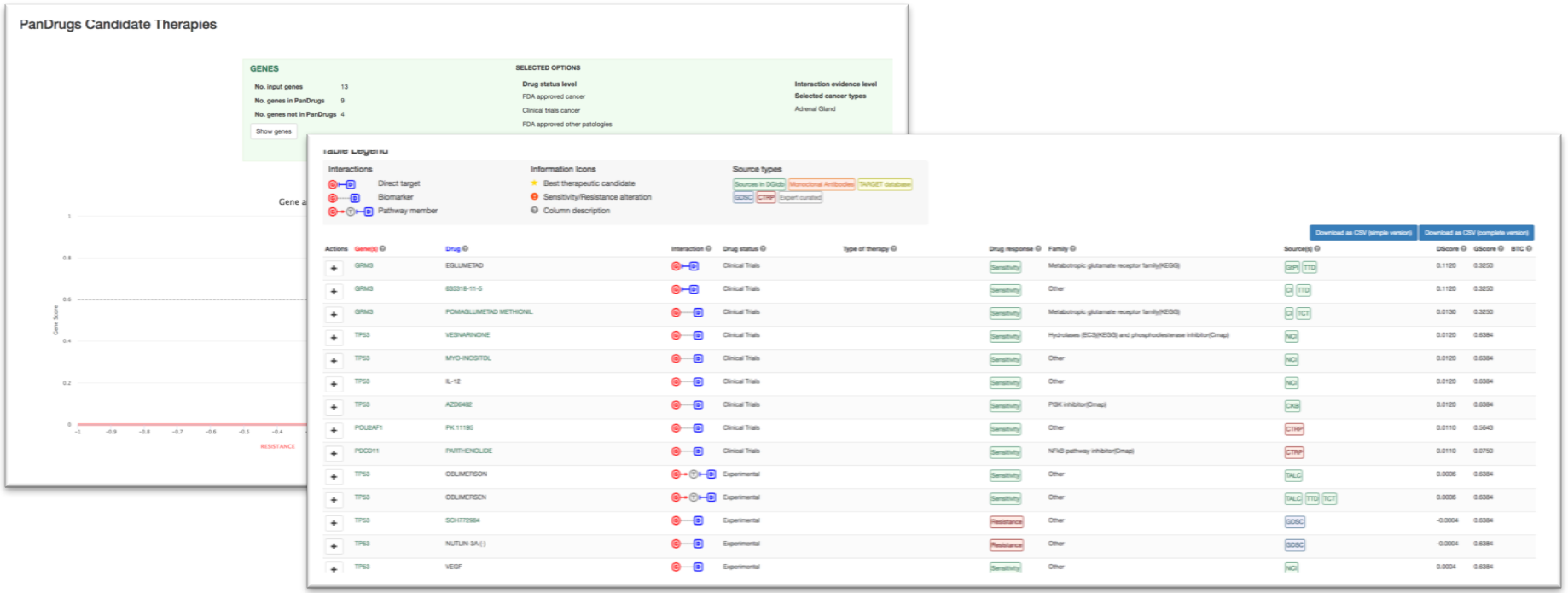
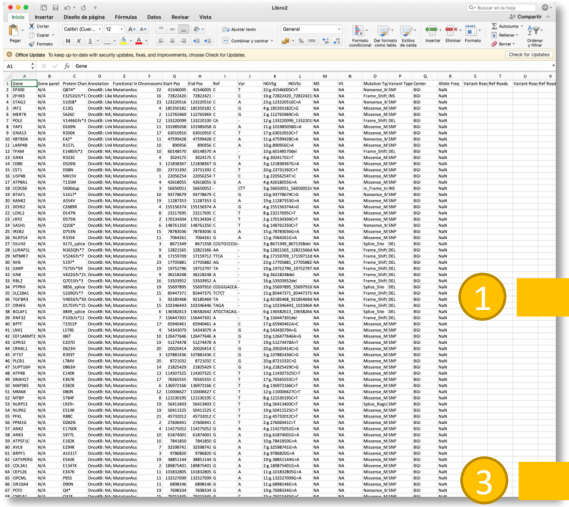


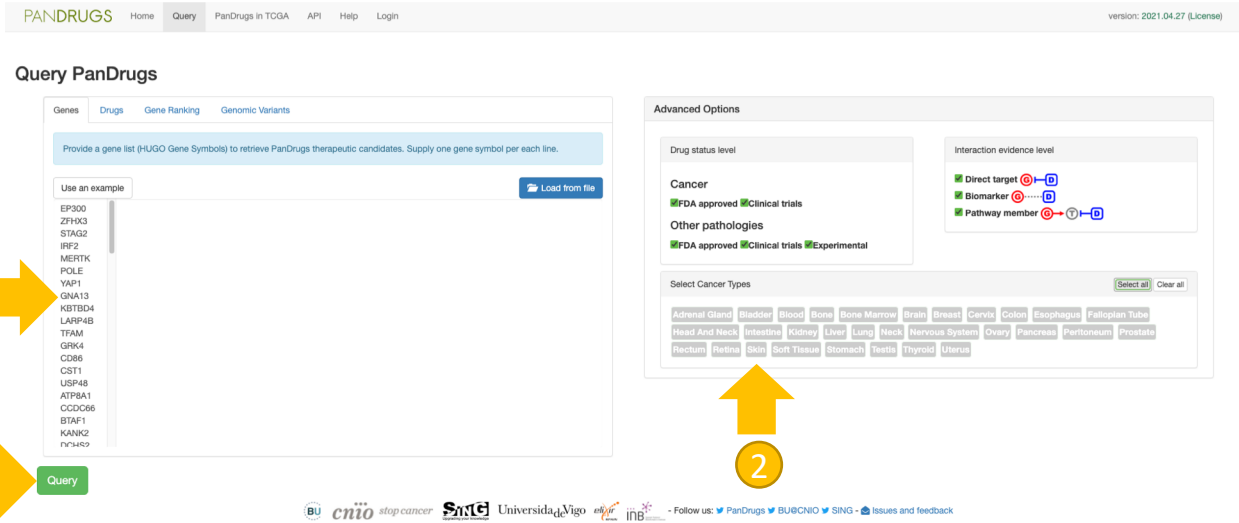
There are no FDA drugs approved for Adrenal tumours

Prioritizing drugs from variant lists: PanDrugs

https://www.pandrugs.org/

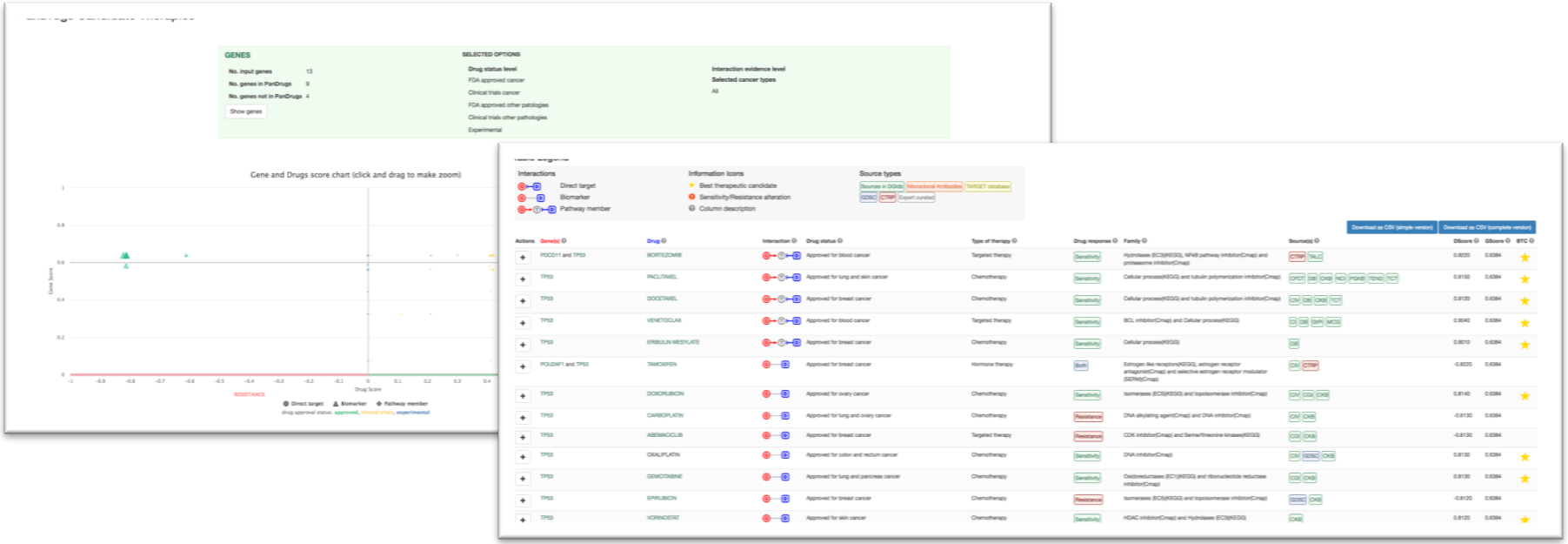
Mutations for TCGA-OR-A5J5





Prioritizing drugs from variant lists: PanDrugs

https://www.pandrugs.org/



Targeted therapy and Chemotherapy FDA-approved as tumor-agnostic treatment

Conclusions

- **The variants** (SNVs, indels and CNVs) described in the cancer genomics consortia **are accessible in public databases**.
- **ICGC data portal and cBioPortal** are two reference web tools that integrate somatic variants described in tumours, **allowing exploration and interpretation of variant lists**.
- cBioPortal allows downloading lists of variants associated with specific tumour types of interest and patients. In addition, you can upload customised lists to visualise your own data.
- The **clinical information** available in cancer genomics repositories **is very limited**.
- **PanDrugs** is a free and public drug prioritization tool that uses information available in public databases to return **a ranked list of personalised drugs to a list of variants**.
- **PanDrugs uses knowledge** to propose drugs directed against direct therapeutic targets, described biomarkers or members of molecular pathways associated with the input list of mutations.

Acknowledgements

@BU_CNIO https://bioinformatics.cnio.es/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Michael Tress

Comunidad de Madrid

FEDER Fondo Europeo de Desarrollo Regional
UNIÓN EUROPEA "Una manera de hacer Europa"

GOBIERNO DE ESPAÑA MINISTERIO DE CIENCIA E INNOVACIÓN

Instituto de Salud Carlos III

INB

elixir SPAIN

IMPaCT DATA

the PARADIFFERENCE foundation

GENCODE

cnio CENTRO NACIONAL DE INVESTIGACIONES ONCOLÓGICAS

SEBiBC Sociedad Española de Bioinformática y Biología Computacional

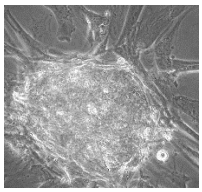
cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Funded by the Horizon 2020 Framework Programme of the European Union

Funded by the European Union

Michaela Luconi

Meet the expert: Organoids in adrenal tumor research



“Organoids in Adrenal Tumor Research”- Cost Masterclass March 2022

Michaela Luconi, PhD

Department of Experimental and Clinical Biomedical Sciences,
University of Florence, Italy

The Adrenal is a complex endocrine organ integrating the steroidogenic cortex and the neuroendocrine chromaffin medulla. The tuned interaction between the two components regulates the development, maintenance and functional activity of the entire gland in pathophysiological conditions. The currently available in vitro and in vivo cell and xenograft models of adrenal cancers have limits as they fail to mimic the 3D structure and complexity of the organ.

Novel models addressing the steroidogenic and chromaffin cell interactions and their architecture as well as the role of the microenvironment are needed for taking a significant step forward in adrenal cancer research and developing more efficacious and targeted therapies. The talk will covered the current in vitro cell and in vivo xenograft models for studying human adrenal tumors, in particular adrenocortical cancer, and how the development of innovative organoid models will impact the research in the field. Novel exciting data from the research on in vitro 3D organoids of human adrenals and their use for studying adrenal cancers and the tumor microenvironment interaction will be presented.

Organoids in Adrenal Tumor Research

Michaela Luconi, PhD

Dept. Experimental & Clinical Biomedical Sciences

University of Florence-Italy

michaela.luconi@unifi.it

March 25th 2022

23 - 25 March 2022

COST Harmonis@tion Adrenal Tumor Master Class

Harmonis@tion

cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Funded by the European Union

My presentation goal

Michaela Luconi

Need of 3D complex adrenal models to study adrenal tumors

Tumor should be addressed in its microenvironment complexity not as single tumor cells

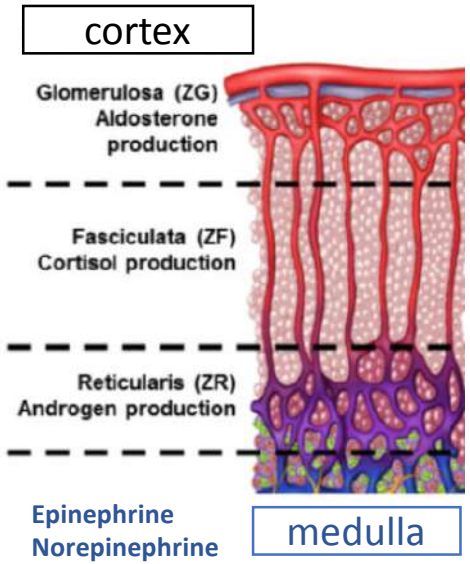
-spatial complexity (3D) & network of different type of cells and factors

Adrenals

-coexistance of 2 different endocrine organs

Steroid production (cortex)

Catecholamine production (medulla)



Phylogenesis of the Adrenals

Michaela Luconi

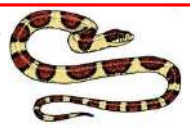
Cortex: mesodermic & Medulla: neuroectodermic

separated

Separate medullary nodules in cranial-caudal line & perirenal cortical mass

Medullary cordons & separate cortical cells + *Stilling cells* for hydrosaline balance

associated with the gonads

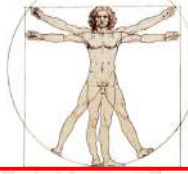


Medullary cordons & larger cortical mass associated with gonads

Medullary cordons & larger cortical mass associated with gonads

associated in an integrated glad with the kidney

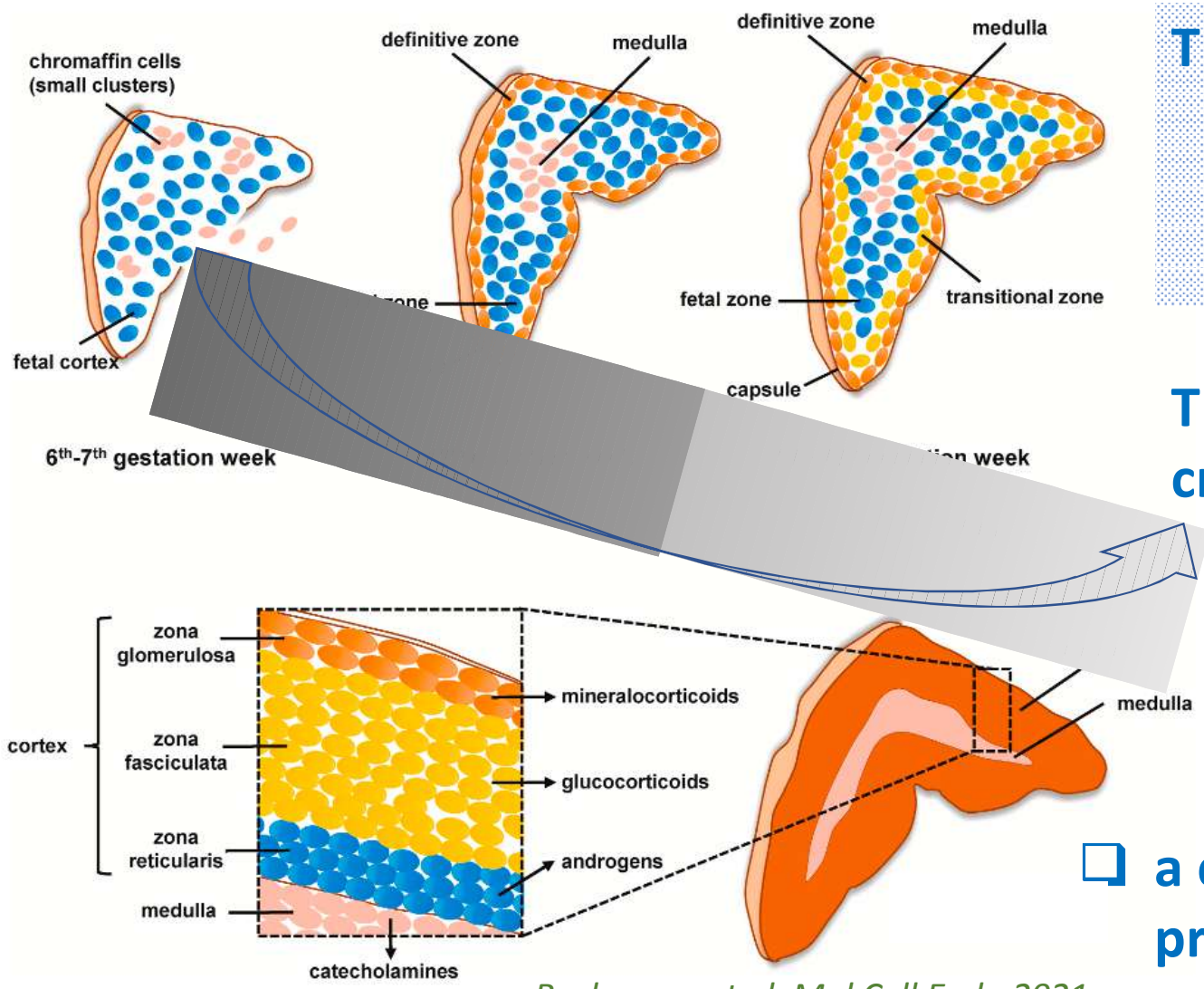
Integrated gland with peripheral cortex & central medulla associated with the kidney



Neonatal regression of the foetal cortex and substitution with adult cortex

Human adrenal development

Michaela Luconi



Bechmann et al. Mol Cell Endo 2021

The ontogenesis recapitulates the phylogenesis

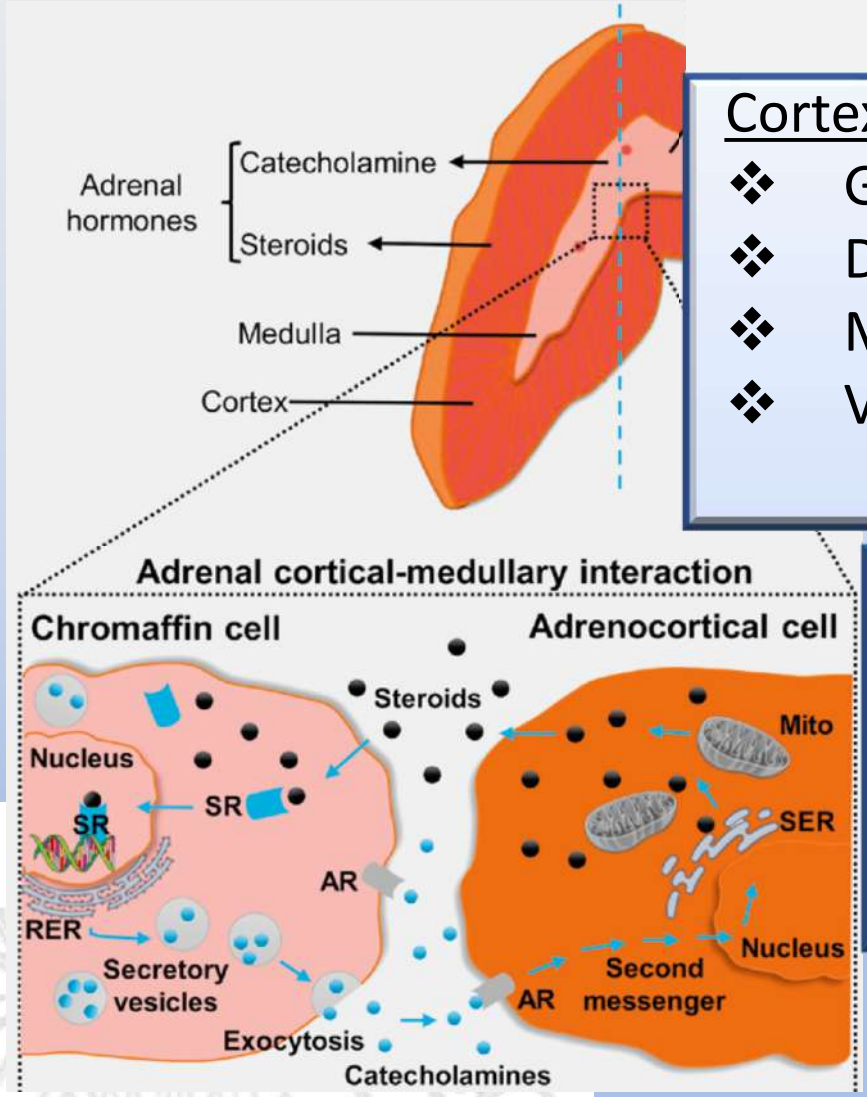
Ernst Haeckel

The journey: from the neural crest to the forming adrenal

- **a continuous differentiation process called zonation**

A strict relationship

Michaela Luconi



EMBRYOGENESIS & ADULT

Cortex-medulla

- Glucorticoids stimulate TH, PNMT, secretogranine
- DHEA/DHEAS control proliferation/survival
- MH Receptors enhances DOPA synthesis
- Vascularization: arterial plexus to the central vein capillaries from each Z

Medulla-cortex

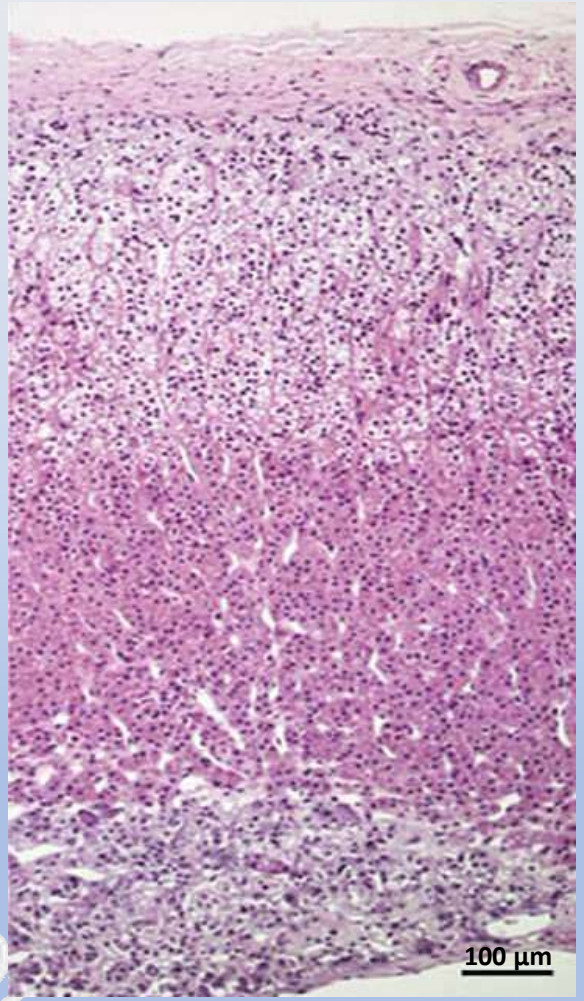
- Catecholamines
- Medullary neuropeptides *(galanin, NPY, VIP)*

stimulates steroid synthesis in all 3 Zs via specific R

Haase et al Endocr Dev 2011
Bechmann et al Mol Cell Endo 2021
Bechmann et al Horm Metab Res 2021

A strict relationship

Michaela Luconi



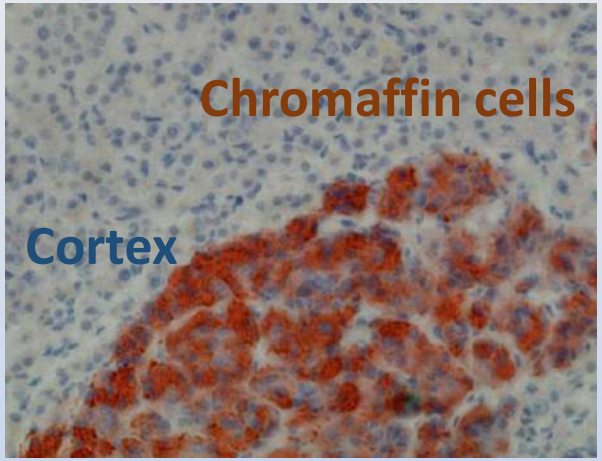
Capsule

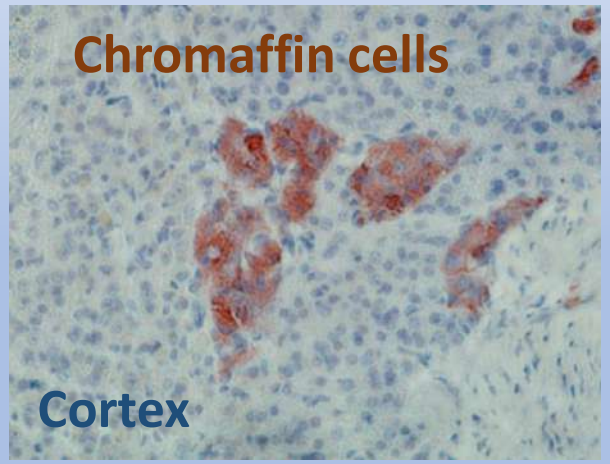
Zona Glomerulosa

Zona Fasciculata

Zona Reticularis

Medulla





Haase et al Endocr Dev 2011

Coordinated responses

Michaela Luconi

- ❑ Fight or Flight response - stress response
- ❑ Inflammatory response

- ✓ Innervation
- ✓ Vascularisation
- ✓ Paracrine-Hormonal factors

This integration and crosstalk between cortex and medulla must therefore be relevant also for cancer

WHICH MODELS ?

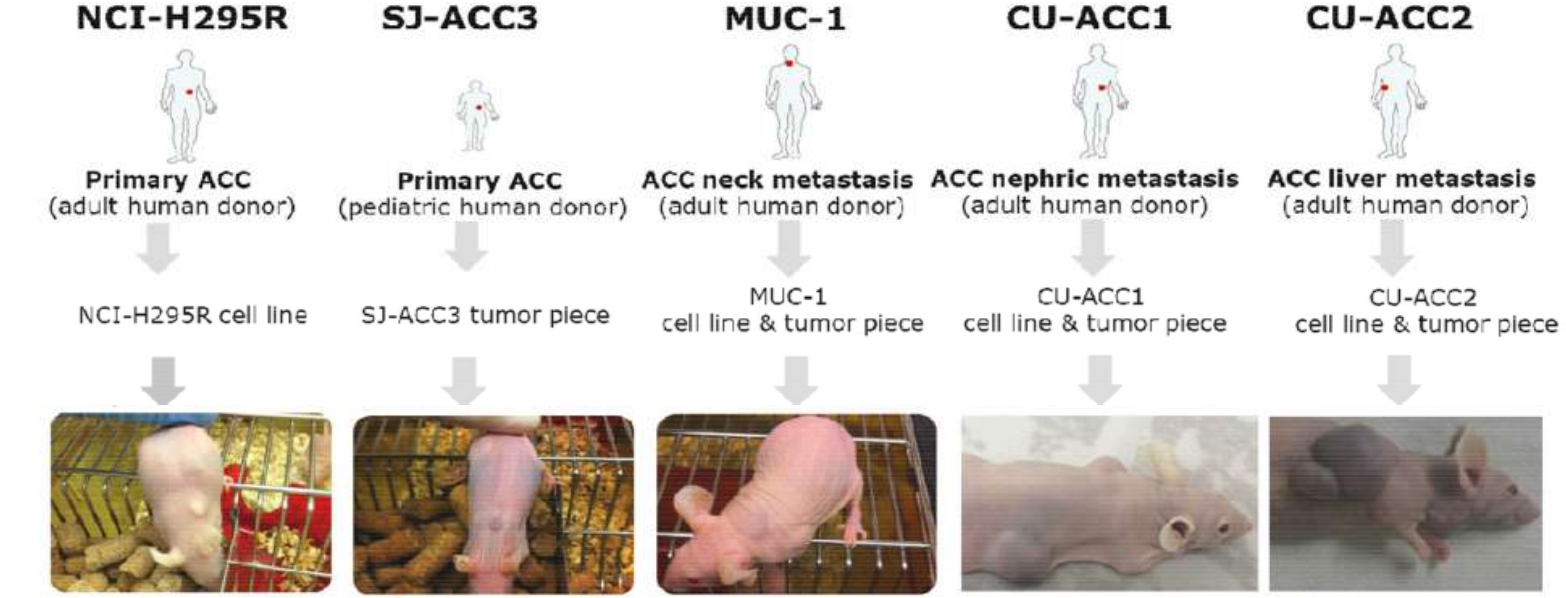
- ***In vivo: mouse xenografts***
- ***In vitro: 2D & 3D models***

In vivo mouse xenografts implanted with ACC

ACC cell types

Michaela Luconi

	Patient Age (y)	Patient Gender	Tissue Origin	SF-1 Status	Ki-67 Labeling Index	*TP53* Status	*CTNNB1* Status	Secr. Funct.	Ass. Syndromes
NCI-H295R	48	Female	Primary ACC	++	≈50 %	c.782+1_994–1del62740	p.S45P	F	Unknown
SJ-ACC3	11	Male	Primary pediatric ACC	++++++	≈33–60 %	p. G254C	WT		Li-Fraumeni
MUC-1	24	Male	Neck metastasis	+++	≈30 %	p.R210fs	WT	F	None
CU-ACC1	66	Female	Perinephric metastasis	+++	≈33–60 %	WT	p.G34R	F	
CU-ACC2	26	Female	Liver metastasis	+	≈33–60 %	p.G245S	WT	NF	Lynch (Loss of MSH2)



Pinto EM et al Curr Opin Endocr Metab Res 2019

Patient-derived xenografts

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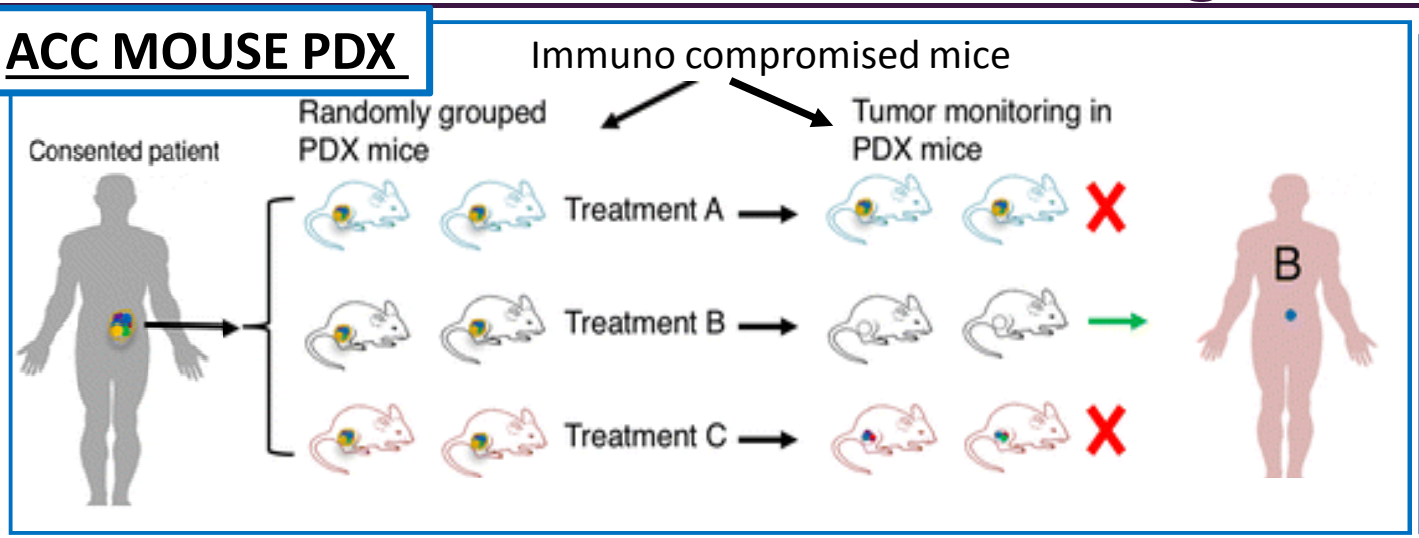
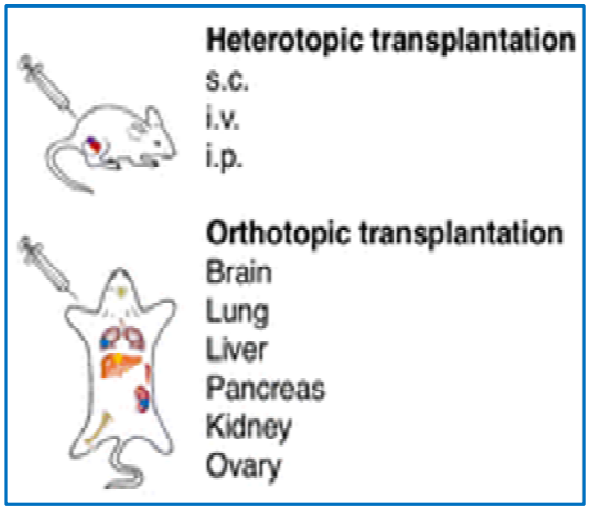
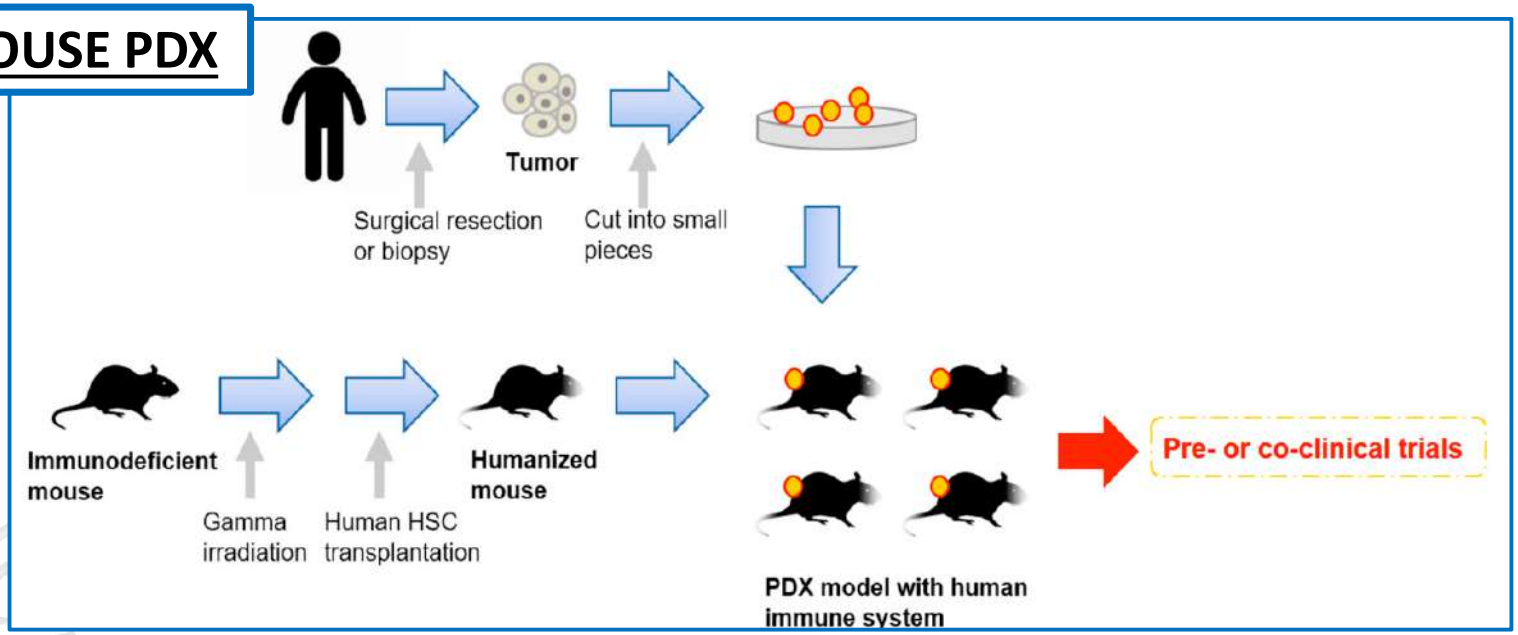
ACC MOUSE PDX



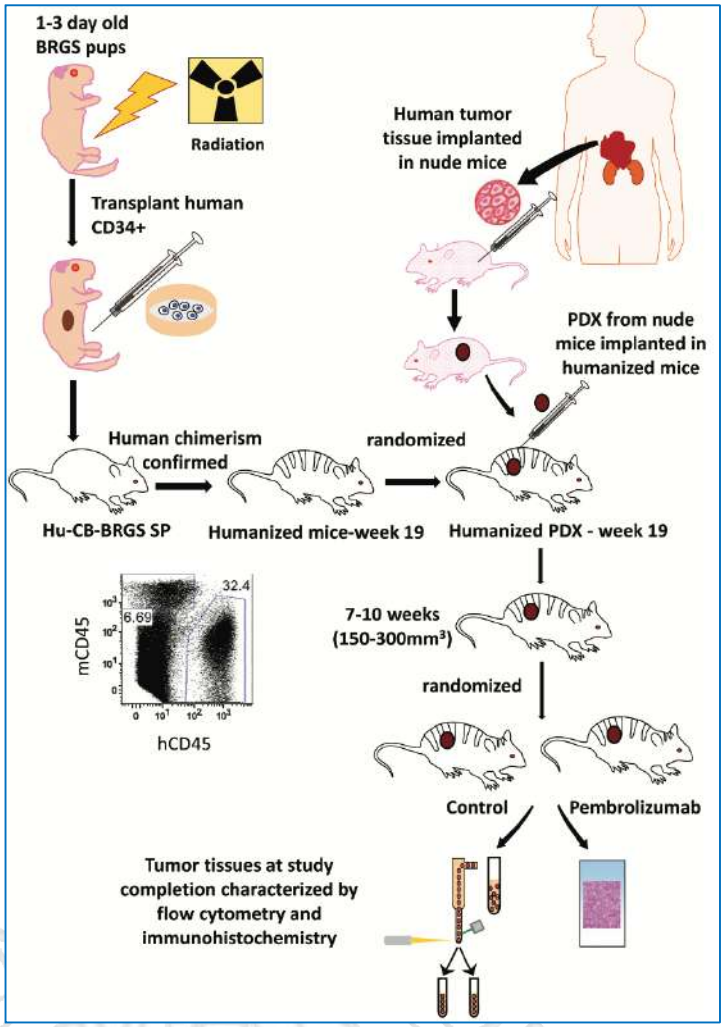


HUMANIZED ACC MOUSE PDX



Humanized ACC PDX mouse model

Michaela Luconi

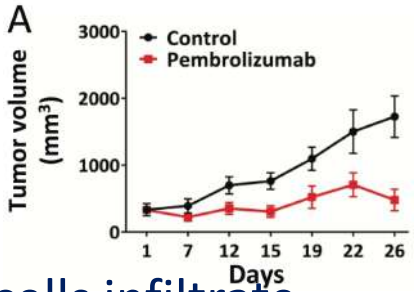


Development of an Adrenocortical Cancer Humanized Mouse Model to Characterize Anti-PD1 Effects on Tumor Microenvironment

J Clin Endocrinol Metab 2020, 105(1):26–42

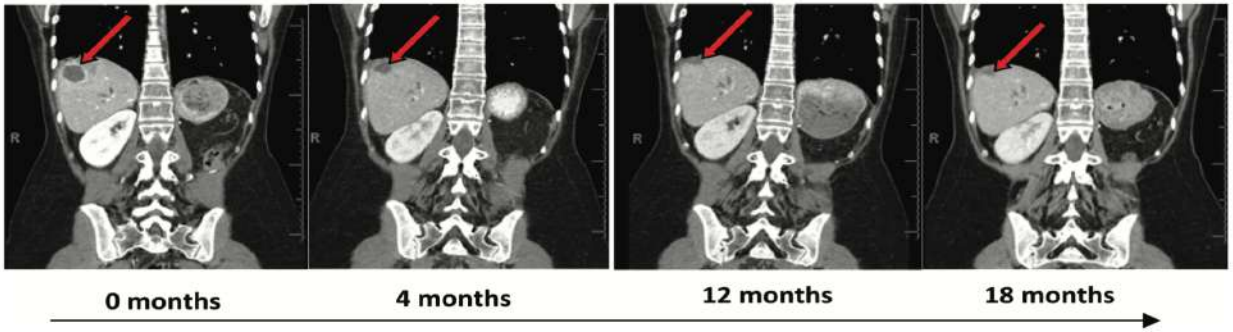
Julie Lang,[1,*] Anna Capasso,[2,*] Kimberly R. Jordan,[1] Jena D. French,[3] Adwitiya Kar,[3] Stacey M. Bagby,[2] Jacob Barbee,[1] Betelehem W. Yacob,[2] Lia S. Head,[2] Kenneth D. Tompkins,[3] Brian M. Freed,[1] Hilary Somerset,[4] Toshimasa J. Clark,[5] Todd M. Pitts,[2] Wells A. Messersmith,[2] S. Gail Eckhardt,[7] Margaret E. Wierman,[3,6] Stephen Leong,[2] and Katja Kiseljak-Vassiliades[3,6]

Tumor Growth in hPDX mouse



Pembrolizumab

- inhibits PDX tumor mass growth
- reduction in the size of target lesions
- no new sites of metastasis
- metastatic liver lesion abundant CD8+ T cells infiltrate

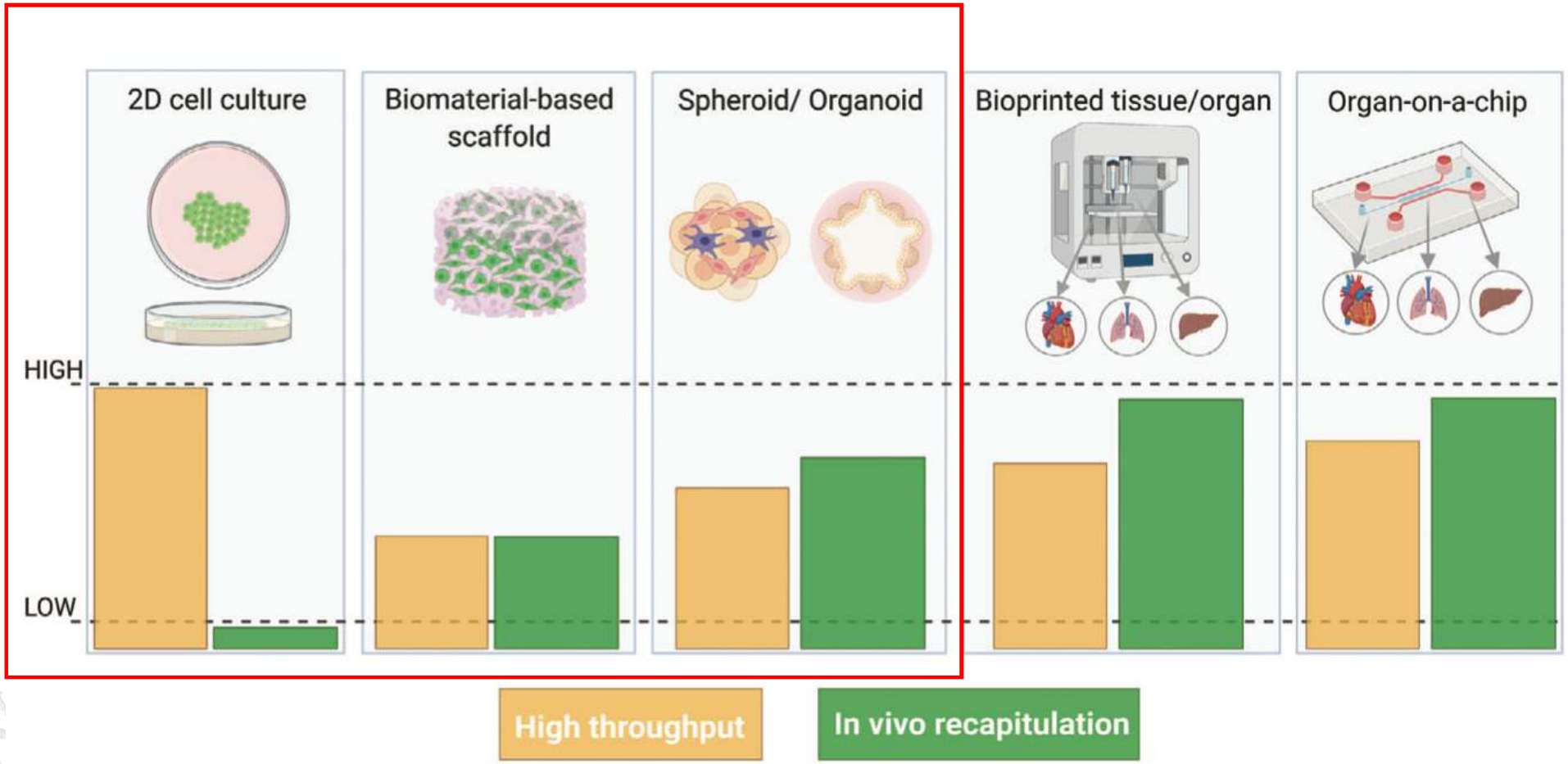


In vitro 2D and 3D cell models

3D in vitro models

Michaela Luconi

ADRENAL IN VITRO MODELS



Dellaquila et al Adv. Sci. **2021**, *8*, 2100798

COCULTURING CELL MODELS in 2D/3D
to study the microenvironment cell interaction with tumor cells

The adipose microenvironment & ACC

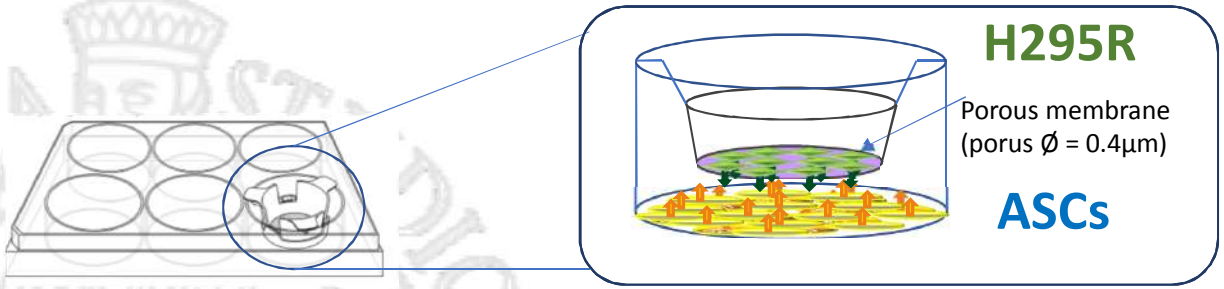
Michaela Luconi

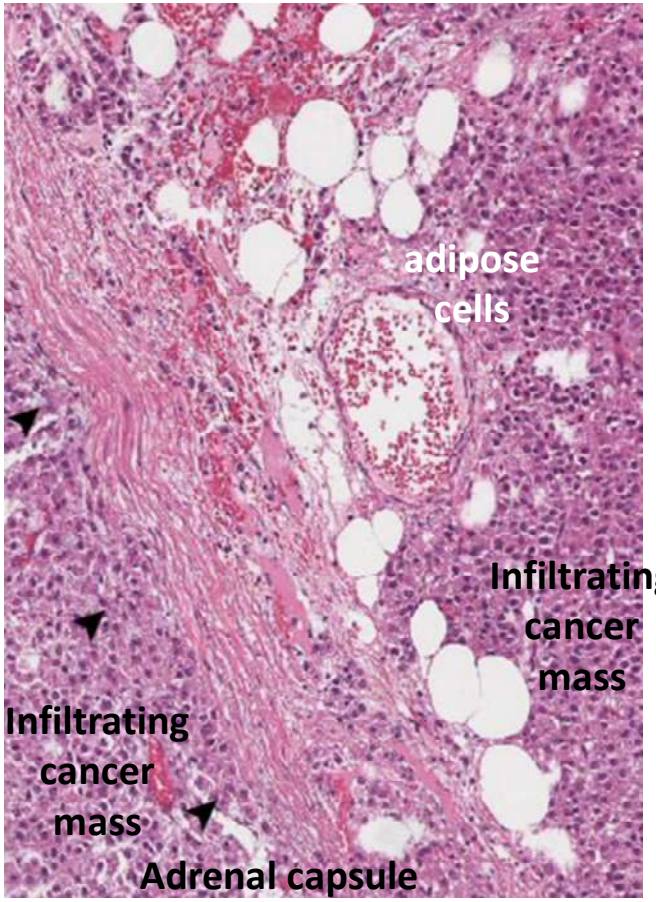
cancers *Cancers* **2019**, *11*, 1931; doi:10.3390/cancers11121931 MDPI

The Adipose Stem Cell as a Novel Metabolic Actor in Adrenocortical Carcinoma Progression: Evidence from an In Vitro Tumor Microenvironment Crosstalk Model

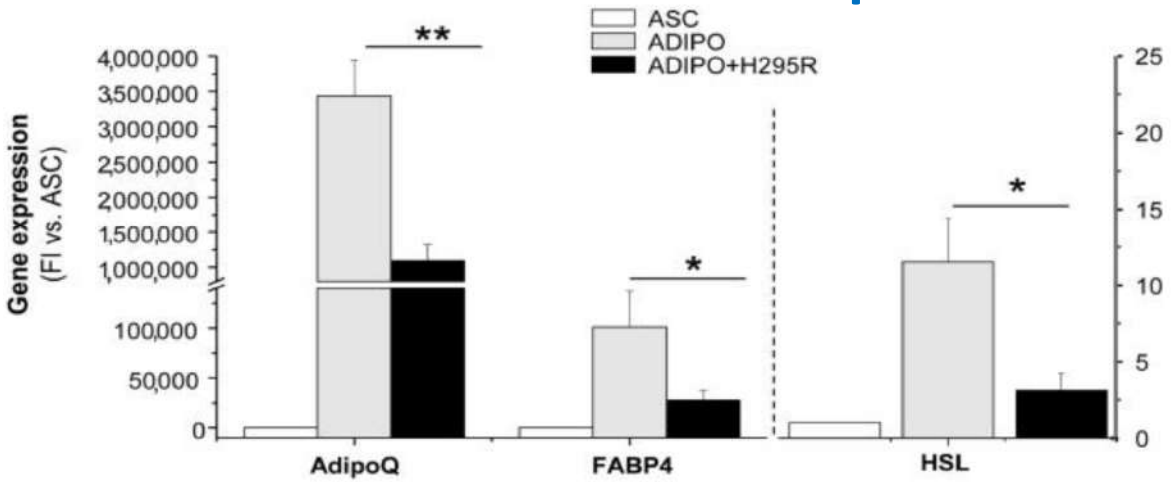
Roberta Armignacco [1,*,†], Giulia Cantini [1,†], Giada Poli [1], Daniele Guasti [2], Gabriella Nesi [3], Paolo Romagnoli [2], Massimo Mannelli [1] and Michaela Luconi [1,4,*]

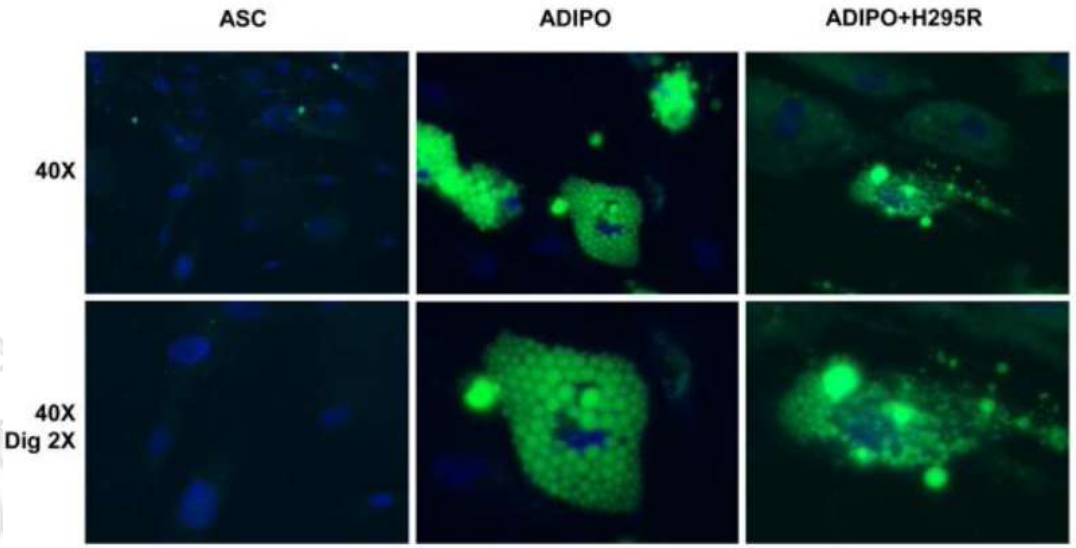
ROLE OF ADIPOSE MICROENVIRONMENT ON ACC INVASION THROUGH IN VITRO COCULTURING SYSTEM BETWEEN ACC H295R CELLS & ADIPOSE STEM CELLS ASCs



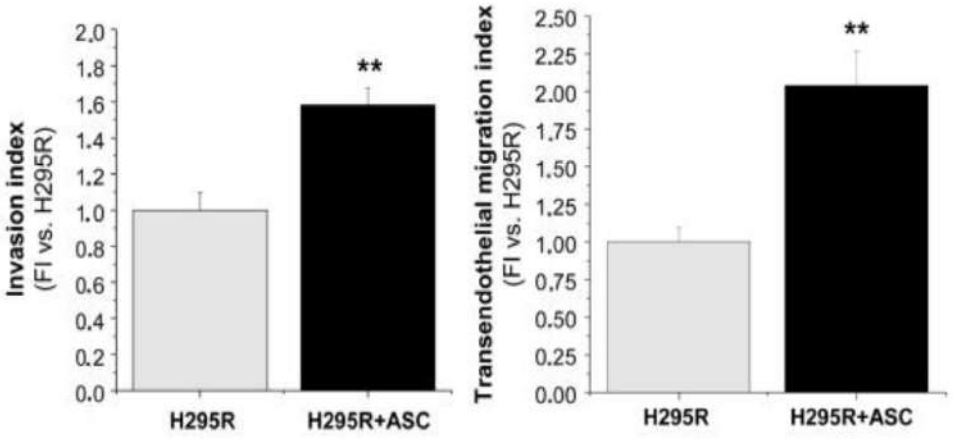


H295R cells inhibit adipose differentiation (ASCs to Adipocytes)





ASCs stimulates H295R invasion & trans-endothelial migration



Armignacco et al Cancers 2020

The tumor microenvironment could represent a novel target for anti-cancer therapies as it can modulate tumor behavior

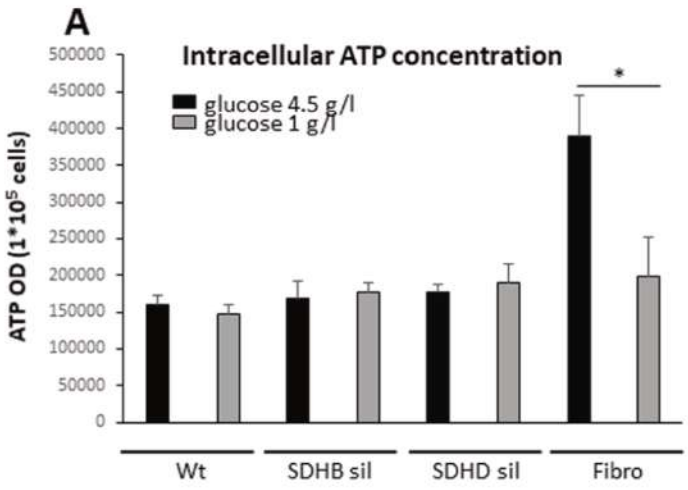
CAF and Pheochromocytoma

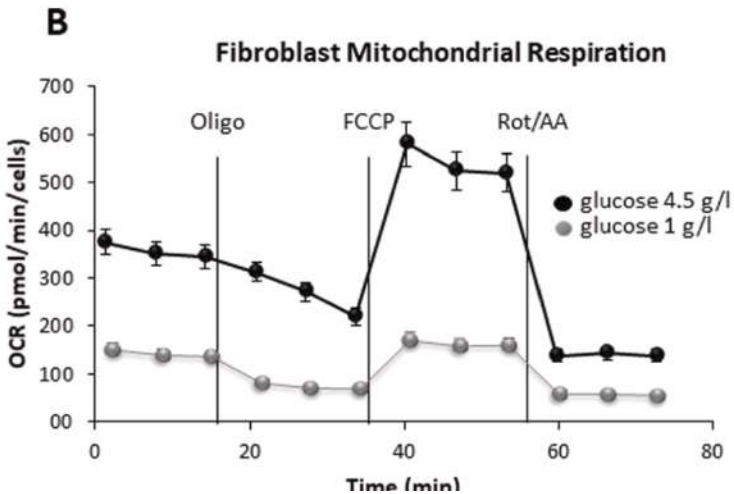
Molecular and Cellular Endocrinology 547 (2022) 111594

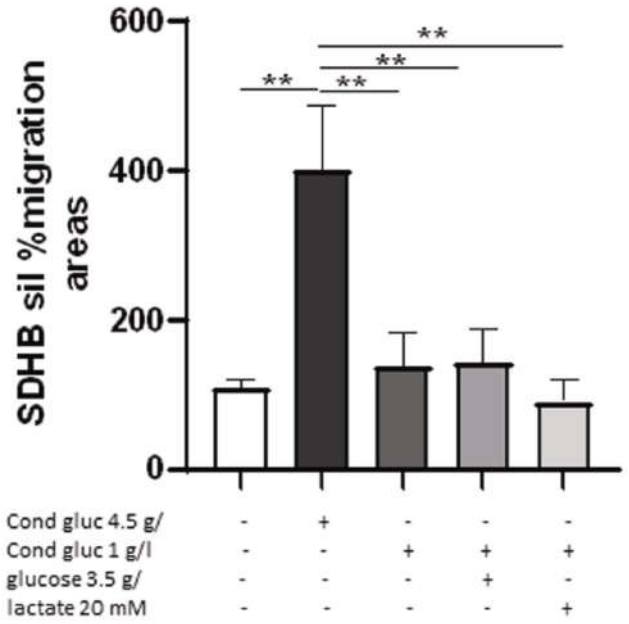
ELSEVIER

SDHB and SDHD silenced pheochromocytoma spheroids respond differently to tumour microenvironment and their aggressiveness is inhibited by impairing stroma metabolism

Serena Martinelli [a], Maria Riverso [a], Tommaso Mello [a], Francesca Amore [a], Matteo Parri [a], Irene Simeone [a], Massimo Mannelli [a], Mario Maggi [a], Elena Rapizzi [b,*]







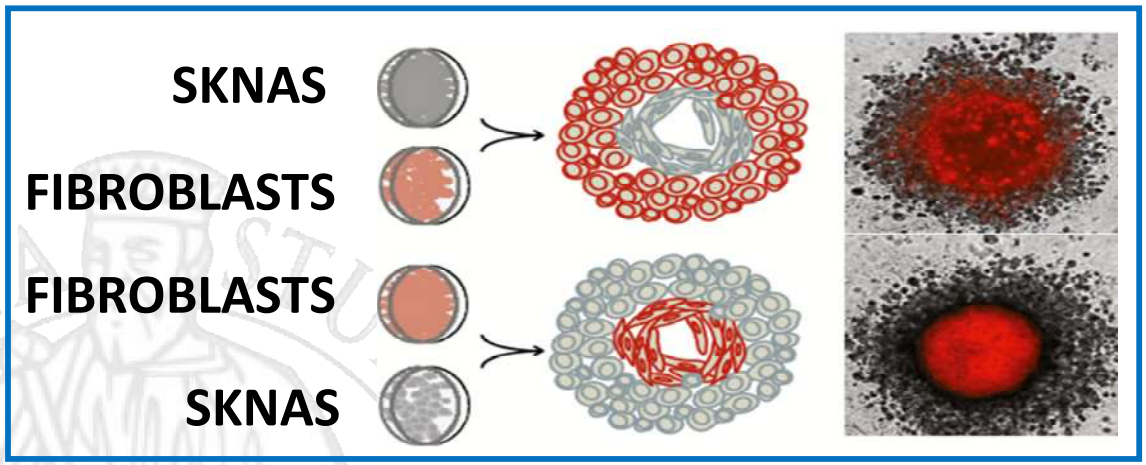
RESEARCH ARTICLE

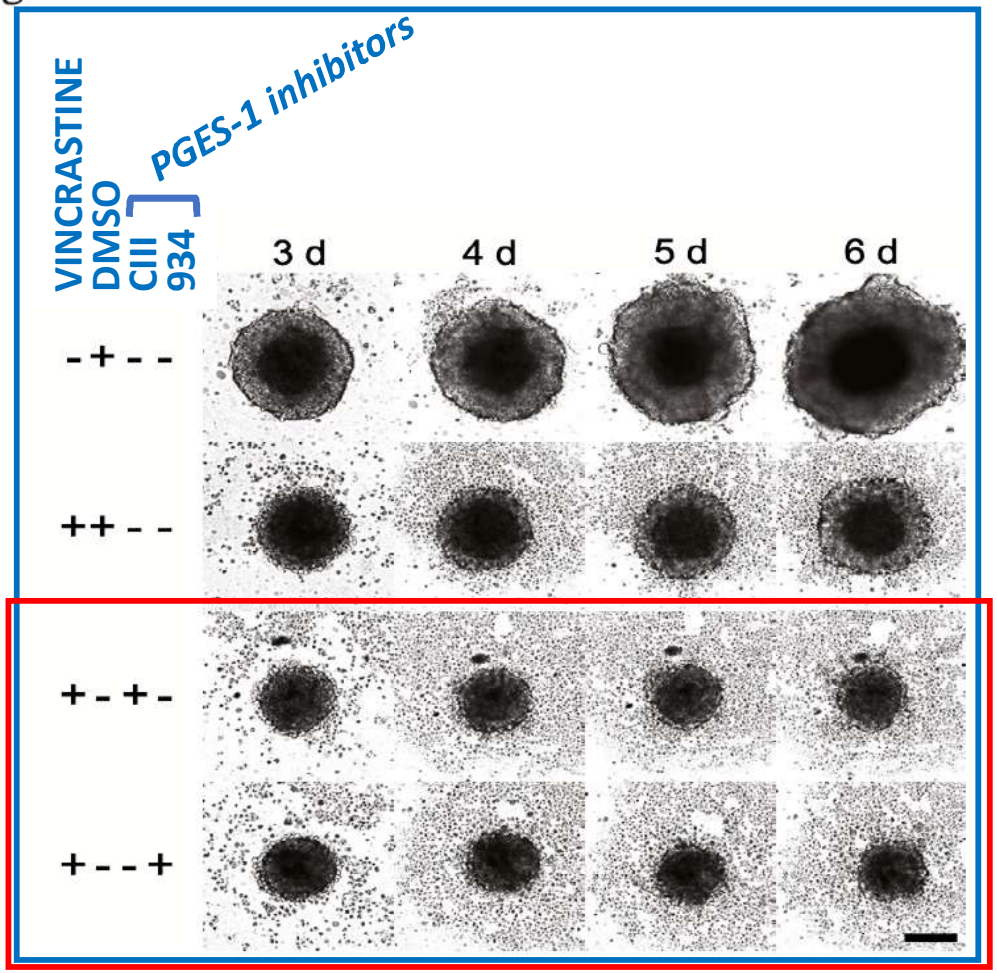
THE FASEB JOURNAL

Establishment of an in vitro 3D model for neuroblastoma enables preclinical investigation of combined tumor-stroma drug targeting

Anna Kock[1] | Filip Bergqvist[2] | Julia Steinmetz[2] | Lotta H. M. Elfman[1] | Marina Korotkova[2] | John Inge Johnsen[1] | Per-Johan Jakobsson[2] | Per Kogner[1] | Karin Larsson[2]

3D in vitro model mimicking the stroma of neuroblastoma, providing a tool to screen drugs targeting the tumor stoma in combination with established tumor cell-targeting therapies. Effect of stroma-targeting mPGES-1 inhibition in combination with tumor-targeting cytotoxic drugs vincristine



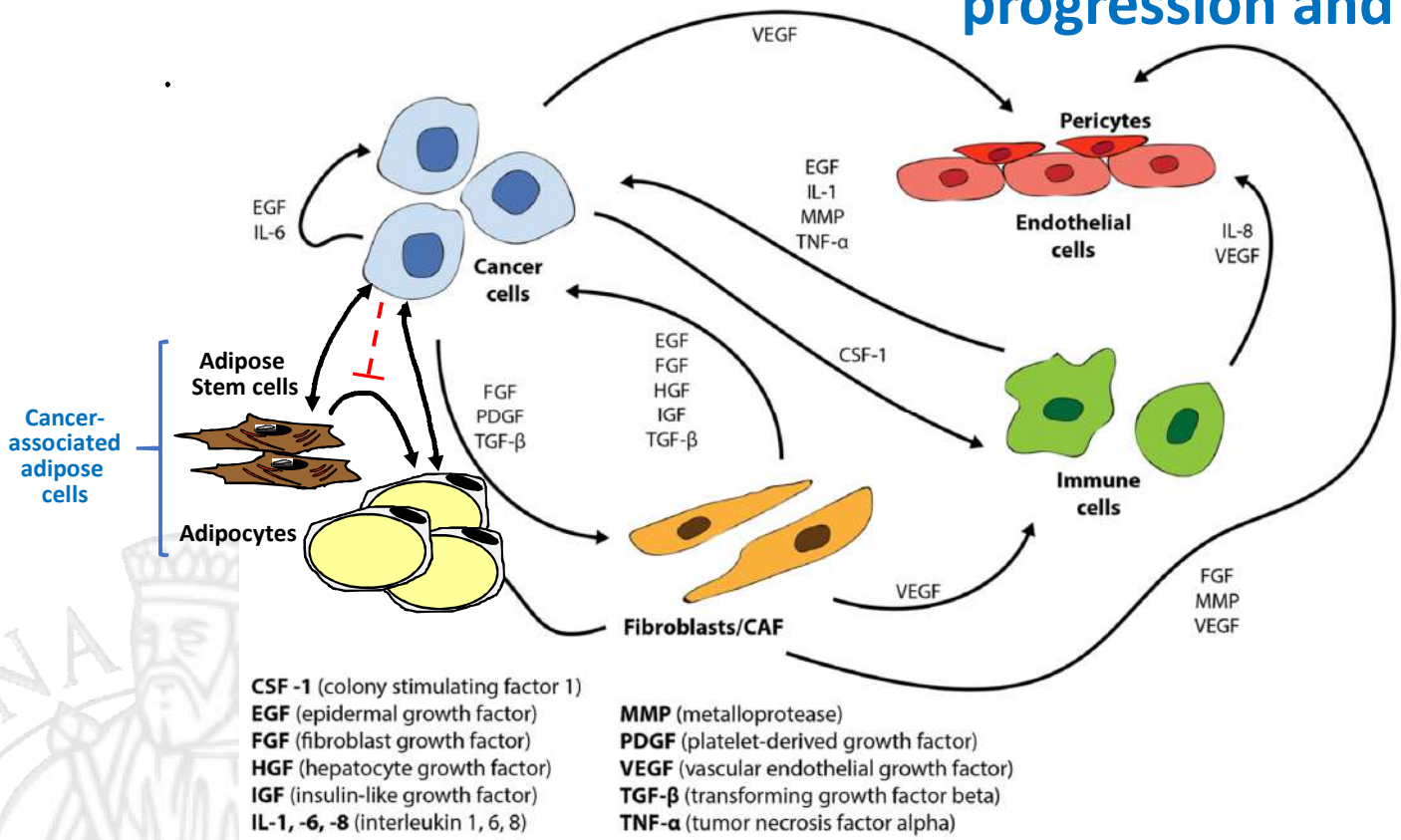


3D tumor spheroids as in vitro models to mimic in vivo human solid tumors resistance to therapeutic drugs

Biotechnology and Bioengineering. 2019;116:206–226.

Ana S. Nunes[1*] | Andreia S. Barros[1*] | Elisabete C. Costa[1*] | André F. Moreira[1] | Ilídio J. Correia[1,2]

Tumor microenvironment modulates tumor progression and response to chemotherapy



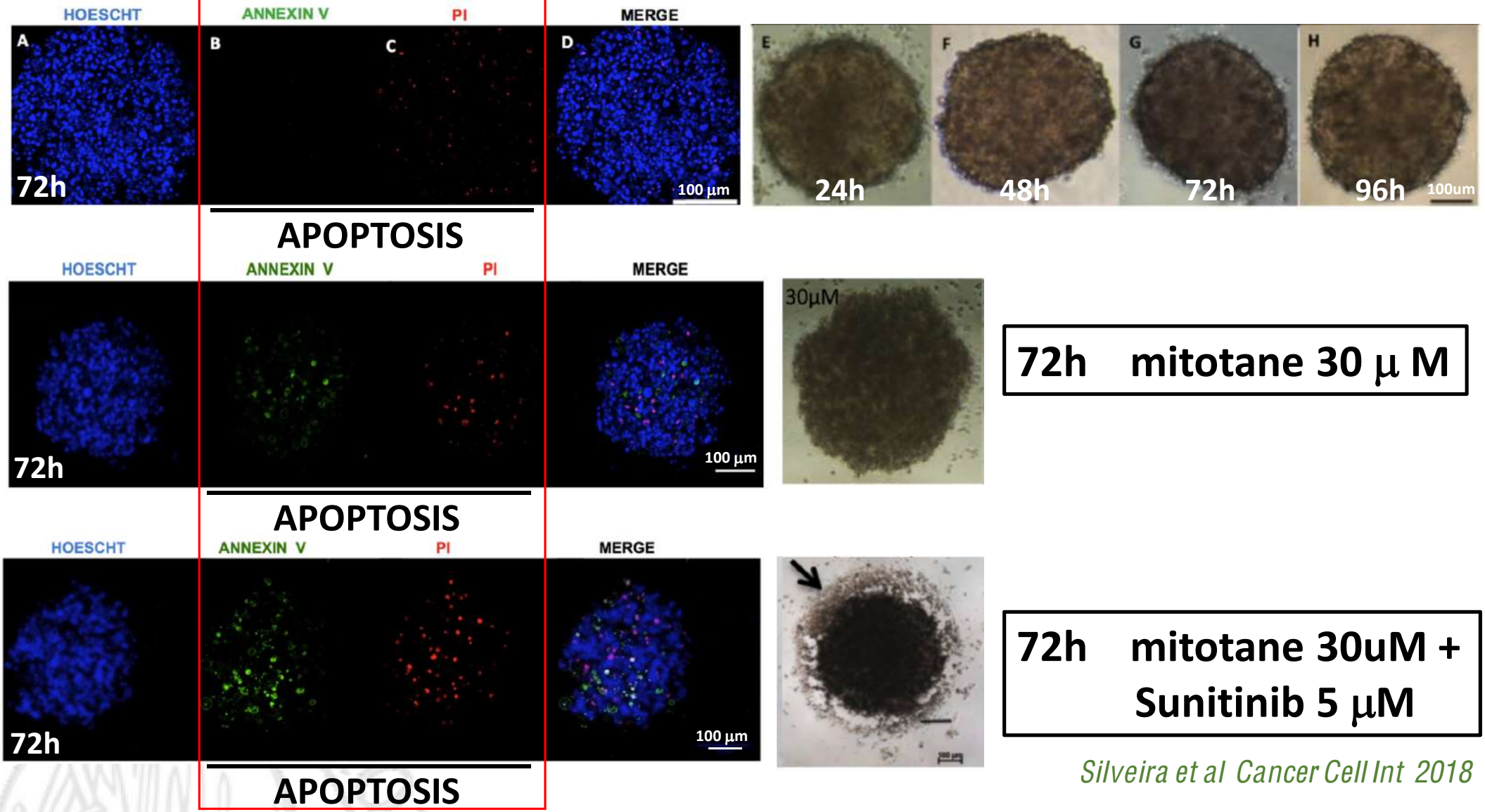
ADIPOSE CELLS
FIBROBLASTS
MACROPHAGES
IMMUNE CELLS
ENDOTHELIAL CELLS

CHEMOKINES
CYTOKINES
ADIPOKINES
GFs
ECM

modified from Nunes et al 2019

H295R Spheroids to test cytotoxicity

Michaela Luconi



72h mitotane 30 µ M

72h mitotane 30uM + Sunitinib 5 µM

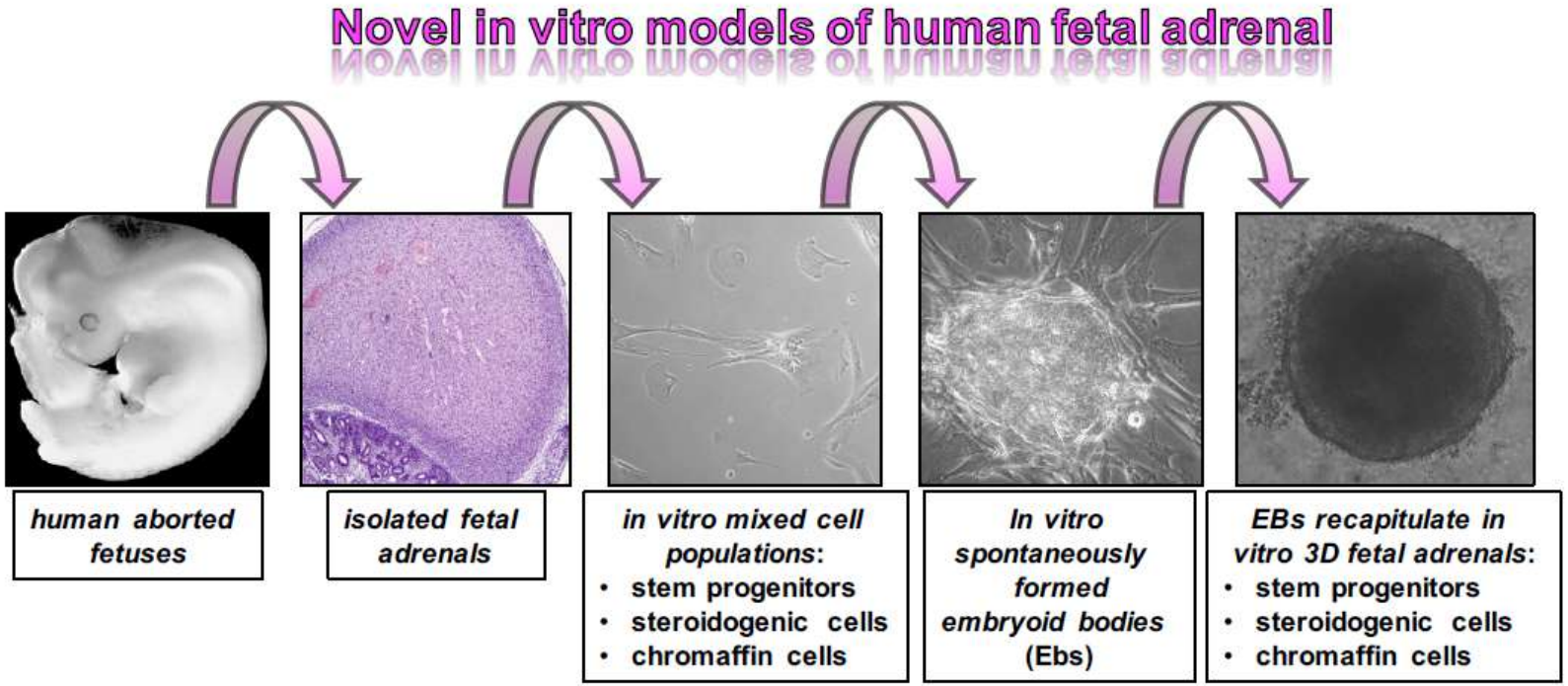
Silveira et al Cancer Cell Int 2018

Mono cellular type spheroids: non physiological conditions as they lack the interaction between the cortex and the medulla

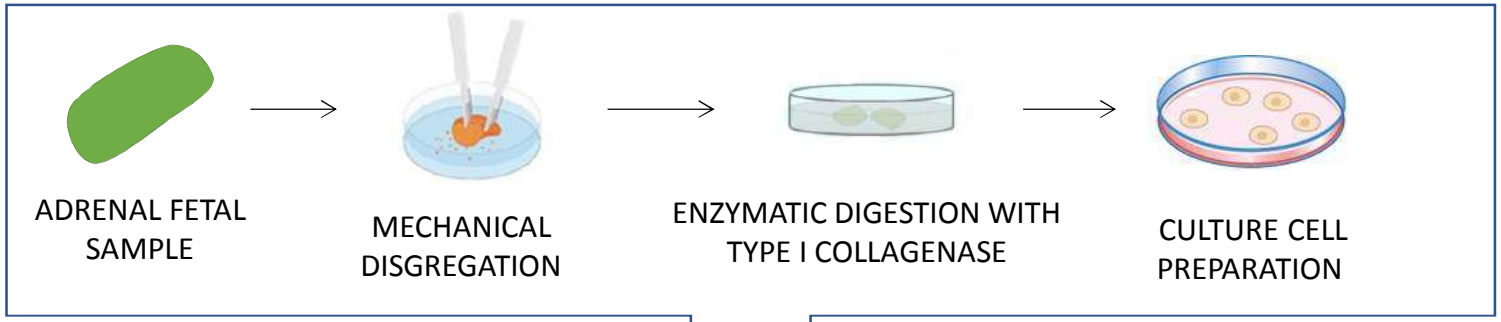
THE FASEB JOURNAL • RESEARCH • www.fasebj.org Vol. 33 February 2019

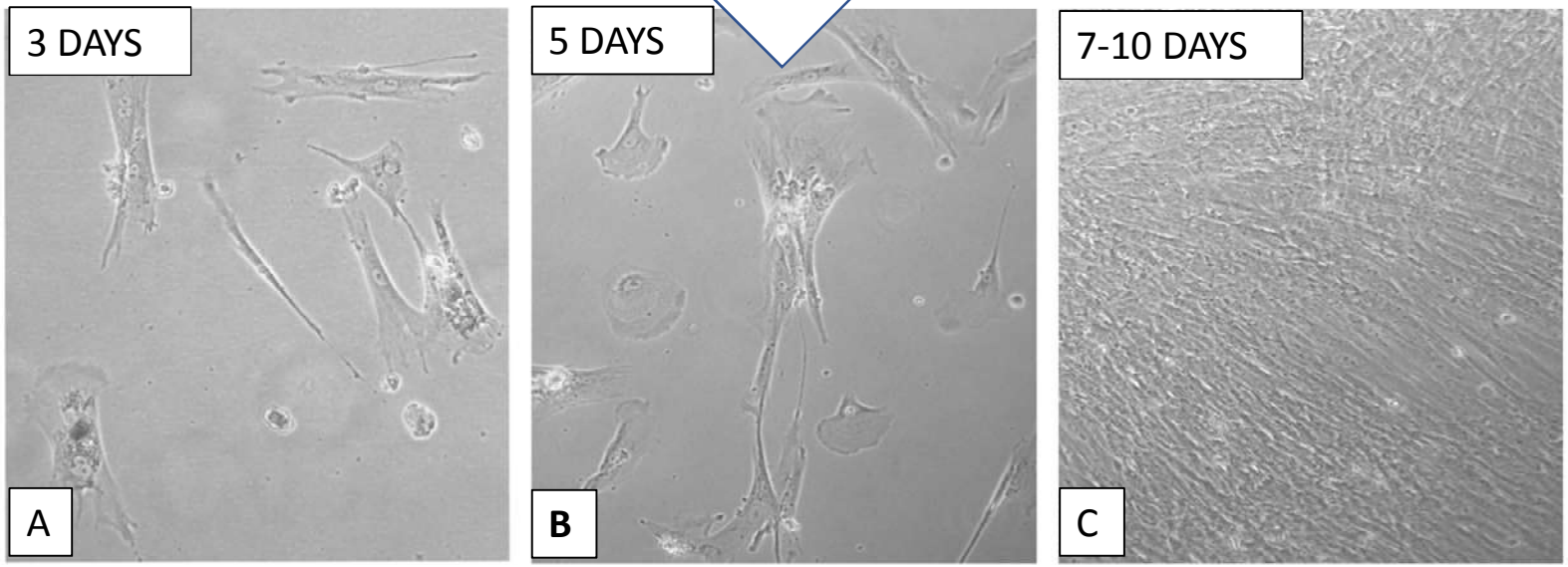
Human fetal adrenal cells retain age-related stem- and endocrine-differentiation potential in culture

Giada Poli,* Erica Sarchielli,† Daniele Guasti,† Susanna Benvenuti,* Lara Ballerini,‡ Benedetta Mazzanti,‡ Roberta Armignacco,* Giulia Cantini,* Matteo Lulli,§ Vasileios Chortis,¶ Wiebke Arlt,¶ Paolo Romagnoli,† Gabriella Barbara Vannelli,† Massimo Mannelli,* and Michaela Luconi*,1



Adrenal primary cell cultures derived from fetal adrenal material after legal voluntary abortion at 3 different times of gestation: 9-11-12 wks

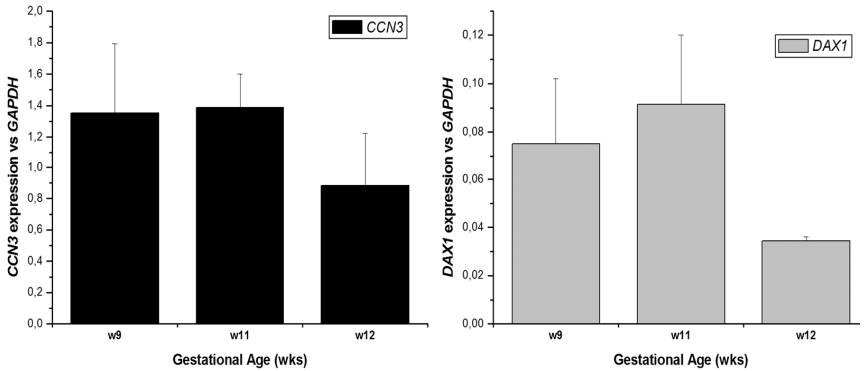




Poli et al FASEB J 2019

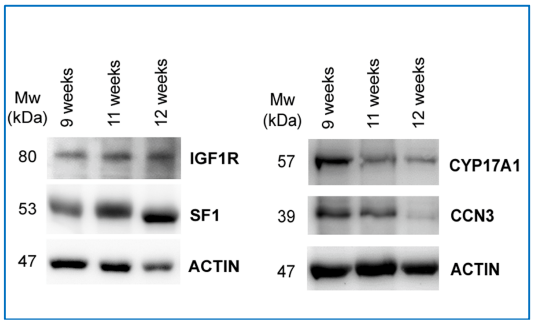
- CORTICAL & CHROMAFFIN COMPONENTS
- STEM AND DIFFERENTIATED STAGES

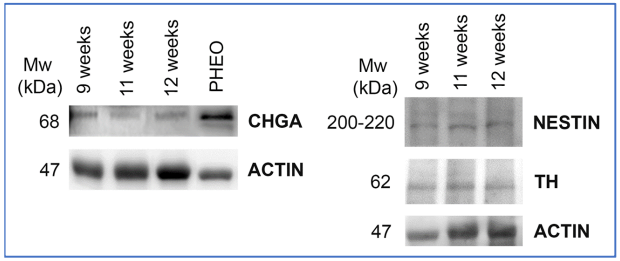
Stem Markers



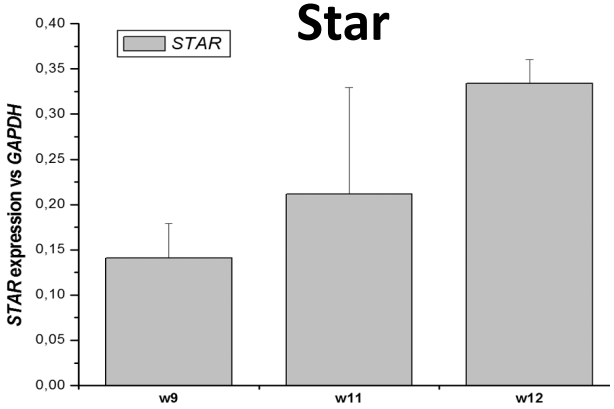
Gestational age

Cortical & Chromaffin Markers



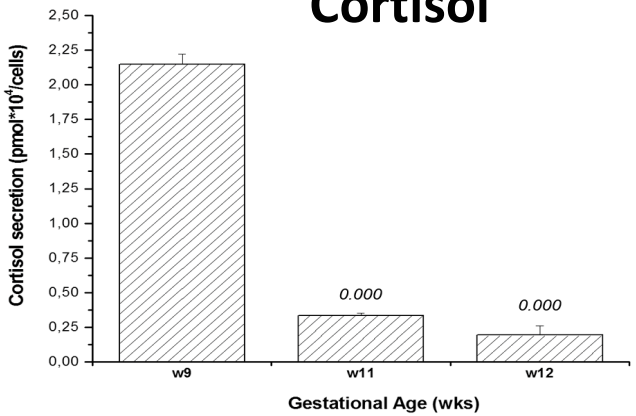


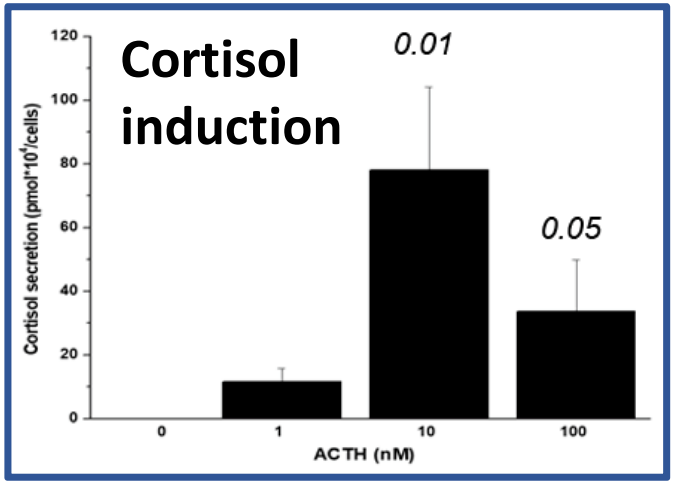
Star



Gestational age

Cortisol



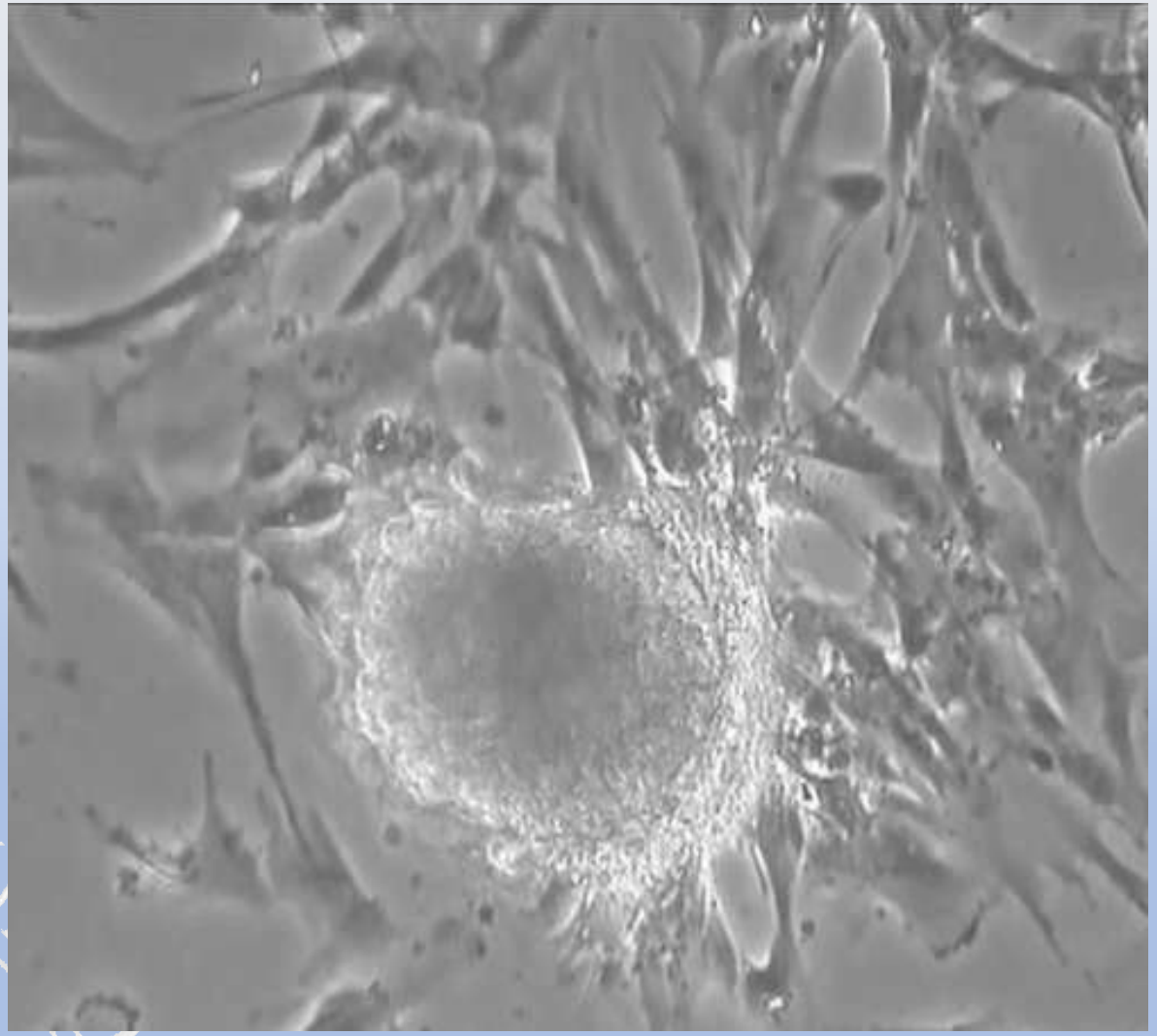


Poli et al, FASEB J 2019

ADRENOID

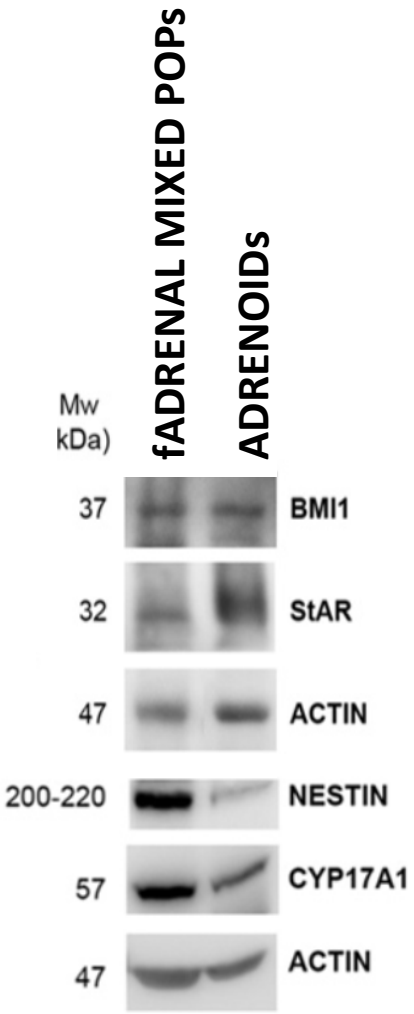
Michaela Luconi

Spontaneous generation of mixed foetal adrenal organoid

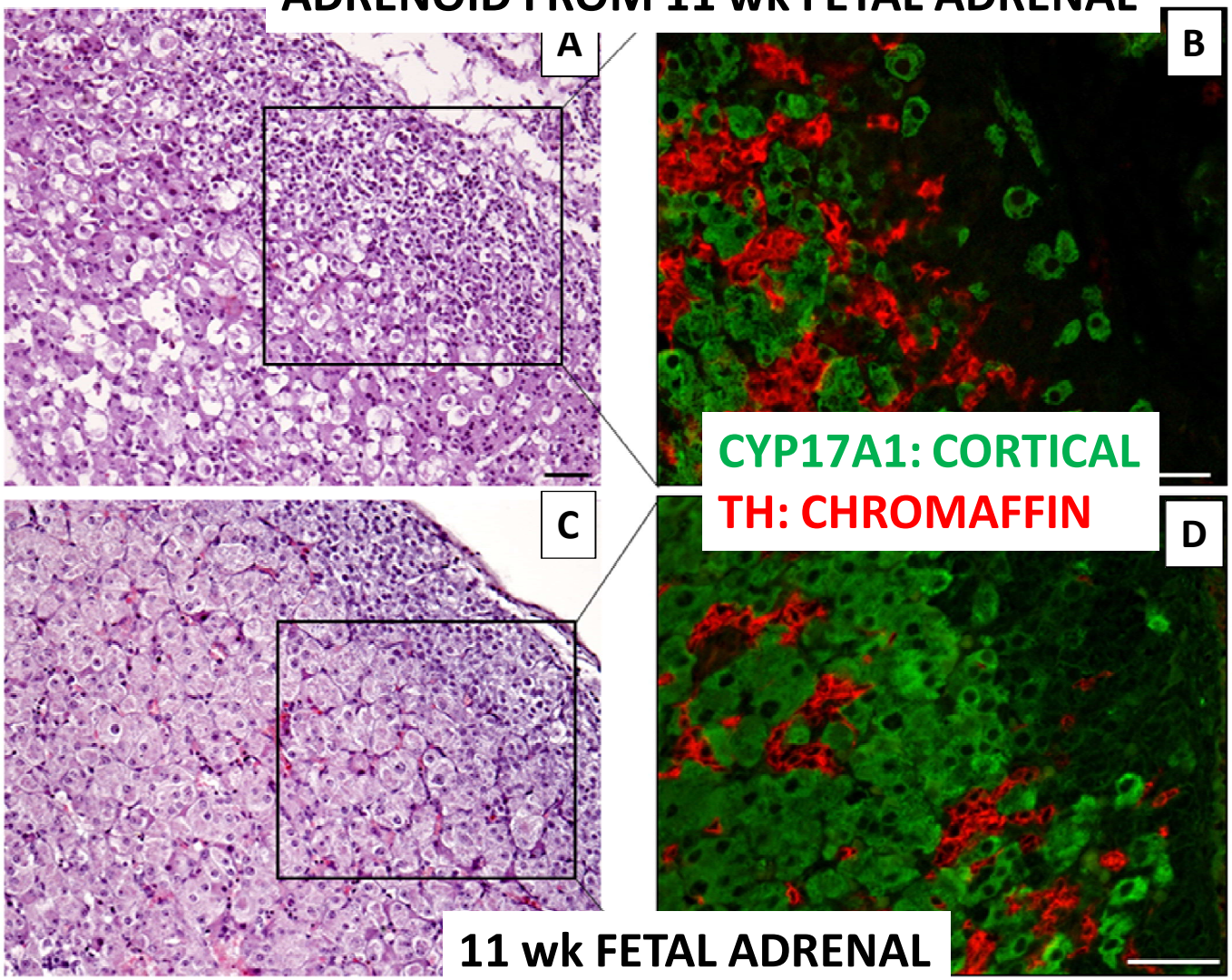


Adrenoid architecture mimics adrenal

Michaela Luconi



ADRENOID FROM 11 wk FETAL ADRENAL



Poli et al, FASEB J 2019

ADRENOIDS FROM CANCER CELLS?

Future prospectives

To improve conditions for long term culturing and polarization

- ❑ Ratio, microfluidic, conditioned media, ECM (scaffolding?)

To generate mixed spheroids with microenvironment cells (adipose cells, fibroblasts, macrophages)

- ❑ To study microevironment effects
- ❑ Screening of TME-target and immune therapies

To generate mixed spheroids with engineered cells

- ❑ Insert mutations in candidate genes of ACC and pheo

TAKE HOME MESSAGE

3D in vitro cell models that reproduce the crosstalk between the steroidogenic cortex and the medullary chromaffin components are required to study

- the tumor development/progression
- the response of the TME to anti-cancer therapies

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Ponte Vecchio....Firenze

Thanks for your attention

Harmonis@tion