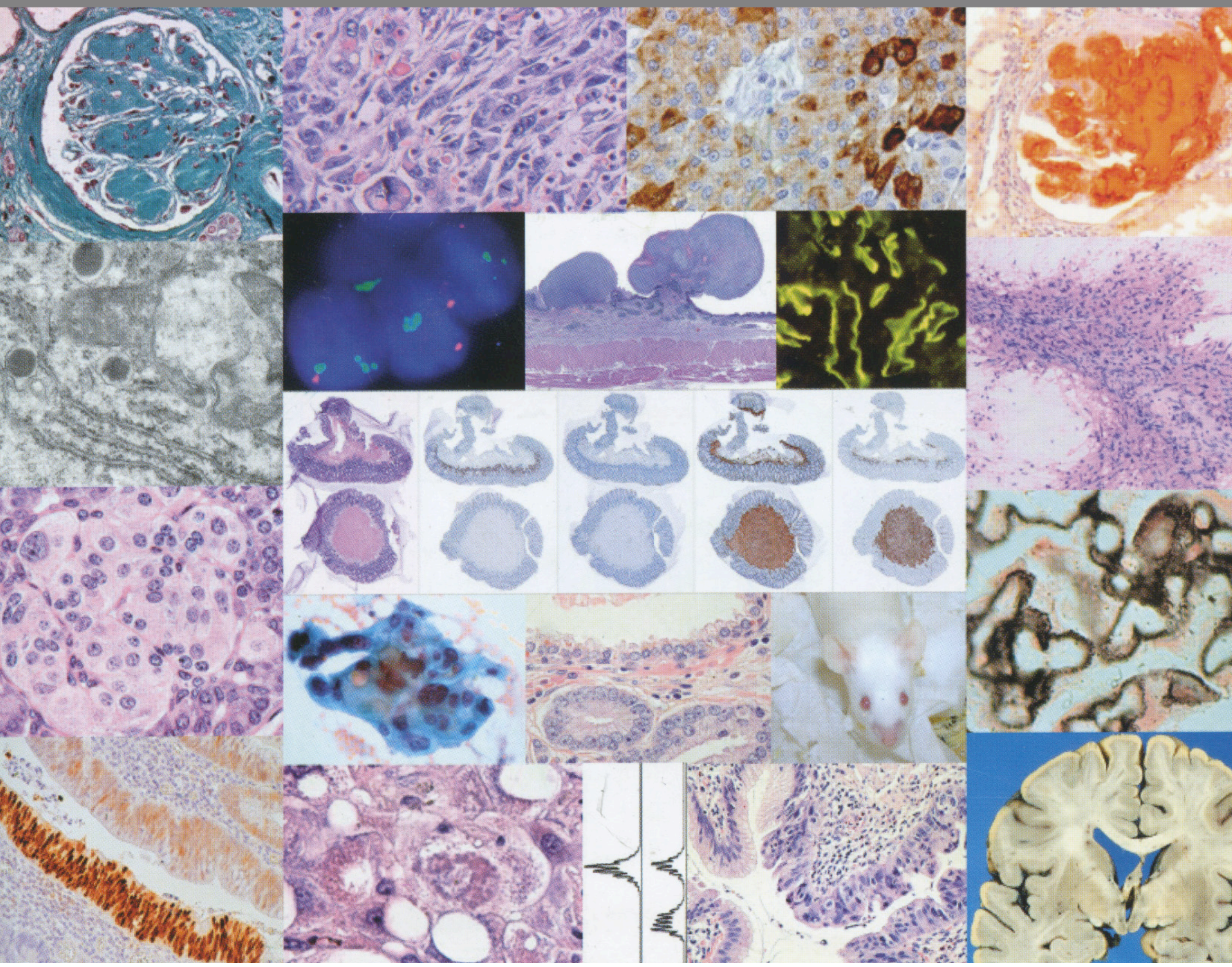
4th BELGIAN WEEK OF PATHOLOGY

in collaboration with

EUROPEAN CANCER PREVENTION

OCTOBER 4 - 5, 2013

HET PAND – GHENT



www.bwpath.be

WELCOME

Dear Colleagues,

The first three editions of the Belgian Week of Pathology (BWP) were a huge success.

To organise the fourth meeting was therefore again a big challenge.

After the success of the previous meetings and starting a real tradition, we have decided to program a fourth edition of the BWP on October 4-5, 2013.

To keep up with the emerging tradition and because of the quality and splendour, the fourth BWP is again hosted in 't Pand in Ghent.

From this year onwards we will organise a BWP yearly as one big unified Society of Pathology.

The scientific committee has been working on a magnificent programme to cover both the state of the art in scientific knowledge and to address other challenges which will influence our profession in the next years.

We would like to thank our partners from the industry on their renewed support!

Some made interesting suggestions that we worked on together.

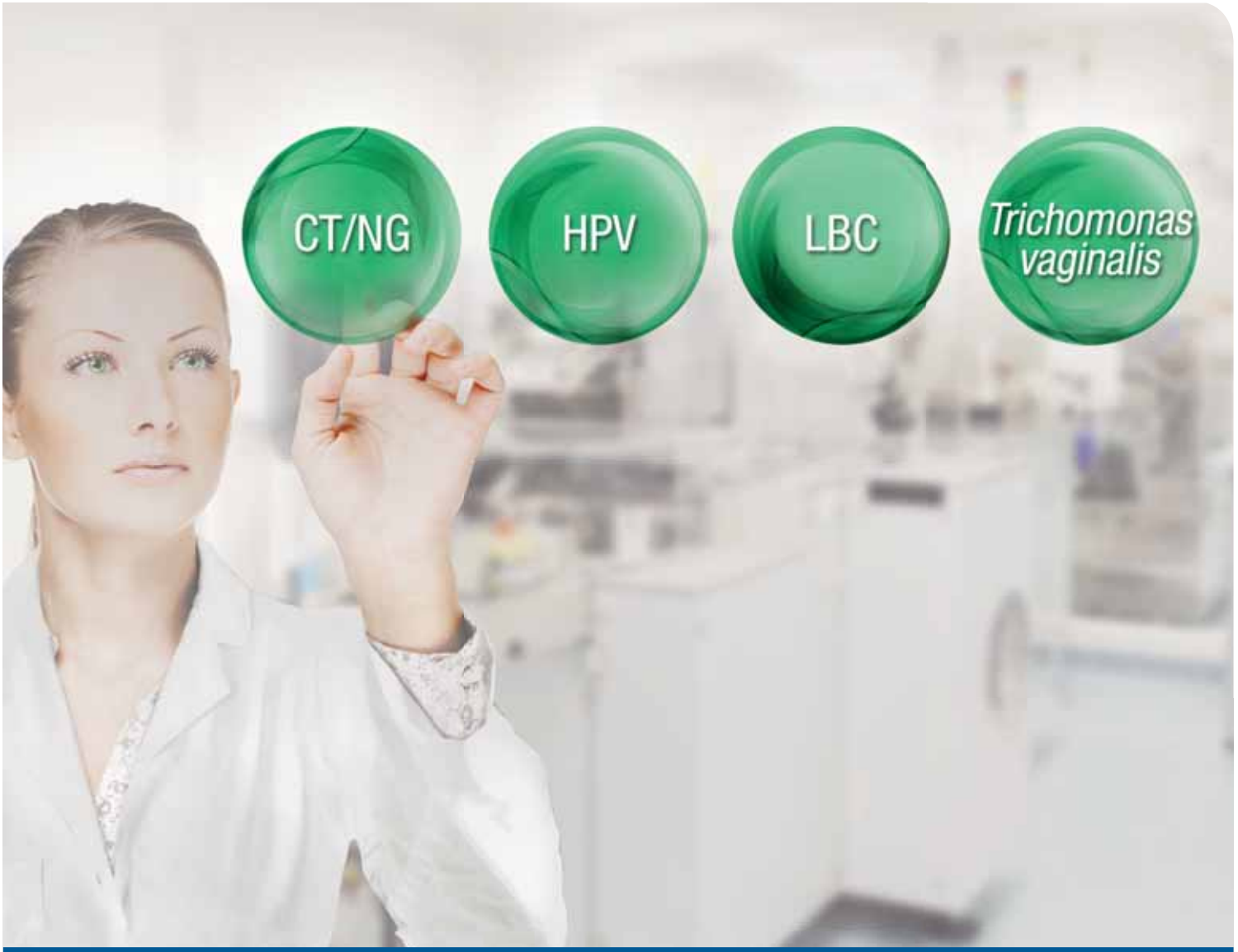
It is also a great pleasure and honour for me and for our society to co-host this meeting together with the European Society of Cancer Prevention (ECP).

Lastly, but most importantly, we are looking forward to welcome you in Ghent in October for the BWP 2013!

Sincerely yours,

J.P Bogers - BWP President

J. Janssens - ECP President



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INDEX




FRIDAY

SATURDAY

GENERAL INFORMATION

Accreditation

Accreditation has been requested for ethics and economy.
From this year on, submission is done on the computers available in the exhibition area. Submission is requested once a day. You will receive a confirmation e-mail after ending the procedure.

Language

The language of the congress is English (British spelling) for abstracts, slides and announcements.

Abstracts

Authors were invited to submit abstracts until August 20, 2013.

All abstracts were accepted as oral presentations and wil be presented during the Free paper Session on Friday from 10:15 to 11:30 and Saturday from 14:00 to 15:50.

The BWP will award the best oral presentation with a price of 500€.

Venue

Het Pand – Onderberg, 1 – 9000 Ghent

Conference rooms are on the first and the second floor. The exhibition, poster area and registration are on the groundfloor.

Parking

Several parkings are located close the congress venue:

- Parking 7 – St Michiels: across the street from the congress venue
- Ramen Parking: in front of the cathedral
- Parking Kouter

Hotels

NH Hotel Ghent Belfort – Tel: +32 (0)9 233 33 31 / Fax: +32 (0)9 233 11 02
Marriott Ghent – Tel: +32 (0)9 233 33 93 / Fax: +32 (0)9 233 33 94

Event Coordinator

Anne-France De Meyer – 102, Av.Carsoel – 1180 Brussels – Belgium
Tel : +32 2 375 36 26 / Fax : +32 2 375 47 84 / E-mail : anne.france.de.meyer@skynet.be

Ghent Tourism Office

Botermarkt, 17A – 9000 Ghent
Tel : +32 9 266 52 32

STEERING COMMITTEE

Belgian Society of Pathology ASBL/VZW, composed of :

- Belgian Society of Clinical Cytology (BVKC-SBCC)
- Belgian Society of Pathology (BVPA-SBAP)
- Belgian Club of Digestive Pathology

And joined by the

- Belgian Association of Pathologists (GBS-VBS)
- Belgian Royal Society of Forensic Medicine (KBGGG-SRMLB)
- Belgian Working Group on Animal Pathology
- Belgian Group of Brain Tumors
- Belgian Group of Neuropathology
- Belgian Club of Dermatopathology
- Belgian Society of Microscopy

European Society of Cancer Prevention

President :	J.P. Bogers
Past President :	I. Salmon
Treasurer :	A. Hoorens
Local Organiser :	C. Cuvelier
Executive Secretary :	P. Demetter

Councillors :

F. Dome
A. Jouret-Mourin
E. Sciot
B. Weynand
F. Willocx

BELGIAN FACULTY

BWP

Aydin J.	Brussels
Bogers John Paul	Antwerp
Bourgain C.	Brussels
Cokelaere K.	Ieper
Colpaert C.	Antwerp
Cosyns J.P.	Brussels
Croes R.	Dendermonde
Cuvelier C.	Ghent
de Jonghe E.	Genk
Delvenne P.	Liège
Demetter Pieter	Brussels
Deschepper S.	Ghent
De Schutter H.	Brussels
Dirix L.	Antwerp
Galant C.	Brussels
Hoorens A.	Brussels
Jouret-Mourin Anne	Brussels
Lambein K.	Ghent
Lelie B.	Terneuzen
Lerut Evelyne	Leuven
Marbaix E.	Brussels
Moens M.	Bonheiden
Nagy Nathalie	Charleroi
Persu A.	Brussels
Sahebali S.	Antwerp
Sieparth P.	Genk
Theunis A.	Brussels
Van de Walle P.	Brussels
Van Eycken Elizabeth	Brussels
Van Huynegem K.	Dendermonde
Vermeulen P.	Antwerp
Weynand B.	Yvoir
Willocx F.	Brussels

Dermatologie:
Sofie De Schepper
Anne Theunis

ECP

Bogers John Paul	Antwerp
Ide P.	Hasselt-Leuven
Janssens J.	Hasselt
Mouallif M.	Liège
Sleurs E.	Ghent

INTERNATIONAL FACULTY

BWP

Algaba F.	Barcelona, Spain
Bartlett J.	Toronto, Canada
Berns E.	Rotterdam, The Netherlands
Dudding N.	Sheffield, U.K.
Hayes M.	Vancouver, Canada
Langner C.	Graz, Austria
Lauwers G.	Massachussetts, USA
Moch H.	Zurich, Switzerland
Reynolds A.	London, UK
Schärer L.	Friedrichshafen, Germany
Van de Vijver K.	Maastricht, The Netherlands
van Kemenade F.	Rotterdam, The Netherlands

ECP

Agorastos T.	Thessaloniki, Greece
Bamias A.	Athens, Greece
Cuzick J.	London, U.K.
Gorostidi M.	San Sebastian, Spain
Jach R.	Krakow, Poland
La Vecchia	Milan, Italy
Murta E.	Abadia, Spain
Paepke D.	München, Germany
Rennert G.	Haifa, Israel
Rodolakis A.	Athens, Greece
Rom J.	Heidelberg, Germany
Schmidt D.	Kiel, Germany
ter Brugge H.	Zwolle, The Netherlands
Namanya V.	Gulu, Uganda

Every door opened could be a discovery made.

Lilly Oncology

No two cancer patients are alike. That's why Lilly Oncology is committed to developing treatment approaches as individual as the people who need them. We've made many contributions toward improved patient outcomes and—with each door we open—we take another step forward. But helping today's cancer patient isn't enough. Even with over 40 drug targets in development, our quest to help you provide tailored therapy is just beginning.

Lilly

Answers That Matter.

PROGRAM OVERVIEW

	REFTER ROOM Ground floor	RECTOR VERMEYLEN First floor - 130pax	INFIRMARY Second floor - 60 pax	RECTOR BLANQUAERT Third floor - 80 pax
08:30-08:35	Exhibition Area	Opening : J.P. Bogers		
08:35-10:00	Exhibition Area	8:35 – 10:00 Digestive Pathology		09:15 – 10:00 Dermatopathology
10:00-10:30	Coffee Break Exhibition Area			
10:30-11:30	Exhibition Area		10:15 – 11:30 Free Paper Session	
11:30-12:30	Exhibition Area	11:30 – 12:30 Keynote Lecture : G. Lauwers (USA)		
12:30-14:00	Lunch in Exhibition Area			
12:50-14:00			12:50 – 14:00 Satellite Symposium ROCHE PHARMA	
13:15-14:00	Exhibition Area			
14:00-16:00	Exhibition Area	14 :00 – 15 :30 Renal and adrenal tumours	14:00 – 15:30 Breast Pathology	14 :00 – 15 :30 Ethics an Economy
15:30-16:00	Coffee Break Exhibition Area			
16:00-17:30	Exhibition Area	16 :00 – 17 :30 Renal and adrenal tumours	16:00 – 17:30 Breast Pathology	16 :00 – 17 :30 Ethics an Economy
17:30-19:00	Exhibition Area Reception Cheese & Wine			
				ECP 2013
9:00–10:30		9:00 – 10:30 Cervical Cytology	9:00 – 10:40 Breast Pathology	9:00 – 10:30 Cervical Cancer: Prevention, early detection and treatment
10:30–11:00	Coffee Break Exhibition Area			
11:00–12:20		11:00 – 12:30 Cervical Cytology	11:00 – 13:00 Breast Pathology	11:00 -12:20 Cervical Cancer: Prevention, early detection and treatment
12:20-14:00	LUNCH Exhibition Area			
13:15-14:00			13 :15 – 14 :00 Satellite Symposium ROCHE DIAGNOSTICS	
14:00-16:00			14 :00 – 15 :50 Free Paper Session	14:00 – 15:20 Cervical Cancer: Prevention, early detection and treatment
15:20–16:30	Coffee Break Exhibition Area			
16:00–17:30				16:00 – 17:30 Cervical Cancer: Prevention, early detection and treatment

FRIDAY

SATURDAY

We take cancer personally

Patients are at the heart of everything we do at Roche.

They motivate and inspire us to search for and develop innovative medicines and therapeutic solutions with the goal of transforming the lives of people with cancer around the globe.

We've come a long way, but there's still a long way to go.

Roche

We Innovate Healthcare

R.E. Dr Christine Lenaerts BR 459 – 07/02/2013

Invitation

Roche Satellite Symposium

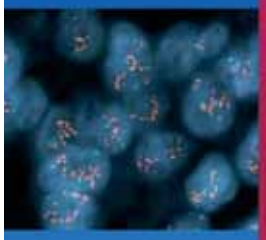
(Lunch is foreseen)

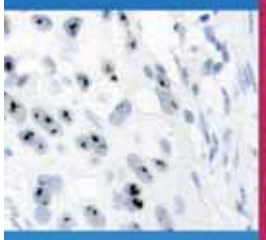
During the Belgian Week of Pathology

- HER2 testing with Prof. Dr. G. Viale
- EGFR/ALK testing with Prof. Dr. P. Pauwels

Roche

Friday October 4th from 12h50 till 14h - Het Pand, Gent





Program | Friday October 4th from 12h50 till 14h.

Chair: Dr. K. Lambein (UZ Gent)

12h50-13h35: Prof. Dr. G. Viale (Milan, Italy)

12h50-13h30 HER2 Diagnosis: A modern Odyssey?

13h30-13h35 Q&A

13h35-14h00: Prof. Dr. P. Pauwels (UZ Antwerpen)

13h35-13h55 Towards Belgian guidelines in Non Small Cell Lung Cancer

13h55-14h00 Q&A

FRIDAY 4 MORNING

Room Rector Vermeylen - 1st floor

08:30-08:35 : **Welcome message** *J.P. Bogers (Antwerp)*

Room Rector Vermeylen - 1st floor

08:35-10:00 : **Digestive pathology**

Chairpersons : *A. Jouret-Mourin (Brussels), A. Hoorens (Brussels)*

08:35-09:15 ***F 01*** ***Dysplasia in inflammatory bowel disease: guidelines 2013*** *C. Langner (Graz-Austria)*

09:15-10:00 ***Slide seminar: Dysplasia and mimics*** *N. Nagy (Charleroi); H. Dano, S. Fonseca, A. Jouret-Mourin (Brussels); E. Meeus (Leuven); A. Driessen (Antwerp)*

Room Rector Blanquaert - 3rd floor

09:15-11:30 : **Dermatopathology**

Chairperson : *A. Theunis (Brussels), S. De Schepper (Ghent)*

09:15-10:00 ***F 02*** ***Autoimmune blistering diseases*** *L. Schärer (Friedrichshafen-Germany)*

10:00-10:30 Coffee break

10:30-11:30 *Slide seminar*

Room Infirmary - 2nd floor

10:00-11:30 : **Free paper session**

Chairpersons: *P. Demetter (Brussels), N. Nagy (Charleroi)*

10:15-10:27 ***O 01*** ***A rare cause of ACTH-independent Cushing syndrome.*** *D. Hastir, C. Maris, B. Corvilain, V. Lucidi, P. Demetter, I. Salmon/Brussels*

10:27-10:39 ***O 02*** ***Immune infiltration in choroidal melanomas.*** *D. Narasimhaiah, R. Remark, E. Brecht, D. Damotte, C. Legrand, P. De Potter, P.G. Coulie, M. Vikkula, C. Godfraind/ Brussels and Paris*

FRIDAY 4 MORNING

10:39-10:51 ***O 03*** ***Endothelial VEGFR1 expression is associated with metastasis-free survival in colorectal cancer.***
N. D'Haene, C. Koopmansch, F. Hulet, J. Allard, C. Decaestecker, I. Salmon/Brussels

10:51-11:03 ***O 04*** ***Liver infiltration by a NK/T cell lymphoma nasal-type mimicking fulminant hepatitis: a case report.***
I. Ferreira, J. Lelotte, C. Sempoux, S. Aydin, G. Schmit, F. Bonbled, I.Theate/Brussels and Gosselies

11:03-11:15 ***O 05*** ***ADAM-17/FHL2 colocalisation suggests interaction and role of these proteins in colorectal cancer.***
L. Verset, S. Sauvage, J. Tommelein, X. Moles Lopez, C. Decaestecker, M. Mareel, M. Bracke, I. Salmon, O. De Wever, P. Demetter/ Brussels, Gosselies and Gent

11:12-11:24 ***O 06*** ***Acquired cystic kidney disease-associated renal cell carcinoma revealed by spontaneous retroperitoneal haemorrhage.***
D. Milowich, B. Henriet, T. Roumeguère, J. Nortier, E. Coppens, S. Rorive/Brussels

Room Rector Vermeylen - 1st floor

11:30-12:30: **Keynote lecture**
Chairperson: *C. Cuvelier (Ghent)*

F 03 ***Uncommon types of dysplasia in the digestive tract***
G. Lauwers (Massachusetts-USA)

LUNCH

FRIDAY 4 AFTERNOON

Room Rector Vermeylen - 1st floor

14:00-17:30: **Renal and adrenal tumours**

Chairpersons: *: J.-P. Cosyns (Brussels), E. Lerut (Leuven)*

14:00-14:45 ***F 04*** ***Epidemiology of renal and adrenal cancer: physically very close, but two completely different entities***
E. Van Eycken (Brussels)

14:45-15:30 ***F 05*** ***Classification of the renal tumours of the adult: histological and immunohistological criteria for the pathological diagnosis***
F. Algaba (Barcelona-Spain)

15:30-16:00 Coffee break

16:00-16:45 ***F 06*** ***Pathology and genetics of renal cell cancer***
H. Moch (Zurich-Switzerland)

16:45-17:30 ***F 07*** ***Pheochromocytoma and paraganglioma: genetical aspects and upgrade*** *A. Persu and S. Aydin (Brussels)*

Room Rector Blanquaert - 3rd floor

14:00-17:30: **Ethics and economy**

Chairpersons: *E. Marbaix (Brussels), R. Croes (Dendermonde)*

14:00-14:45 ***F 08*** ***The Commission for Pathology: who, what and how?***
K. Cokeleare (Ieper)

14:45-15:30 ***F 09*** ***The Scientific Institute for Public Health: who, what and how?***
P. Van de Walle (Brussels)

15:30-16:00 Coffee break

16:00-16:45 ***F 10*** ***Guideline for practice of the Commission for Pathology***
B. Lelie (Terneuzen)

16:45-17:30 ***F 11*** ***Items on the agenda of the working group legislation of the Commission for Pathology.***
H. Van Dijck (Duffel)

FRIDAY 4 AFTERNOON

Room Infirmary - 2nd floor

14:00-17:30: **Breast pathology research session**

Chairperson: *P. Vermeulen (Antwerp)*

14:00-14:45 ***F 12*** ***Non-angiogenic growth of breast cancer metastasis: implications for pathologists and for the treatment breast cancer***
A. Reynolds (London-U.K.)

14:45-15:30 ***F 13*** ***HER-2*** *J. Bartlett (Toronto-Canada)*

15:30-16:00 Coffee break

16:00-16:45 ***F 14*** ***Explanation of the inflammatory breast cancer phenotype***
L. Dirix (Antwerp)

16:45-17:30 ***F 15*** ***Mechanisms of resistance in breast cancer patients***
E. Berns (Rotterdam-The Netherlands)

17:30 **Reception with cheese and wine**

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Invitation

Roche Satellite & Lunch Symposium

During the Belgian Week of Pathology

- Results of the CINtec PLUS test in the PALMS cervical cancer screening and triage trial with Dr. Christine Bergeron
- Discussion on the screening algorithm in Belgium with Professor Dr. Claire Bourgain

Saturday October 5th from 13h15 till 14h -
Het Pand, Room Infirmary, 2nd floor Gent

CINtec® PLUS

Program | Saturday October 5th from 13h15 till 14h

Chair: Professor Dr. Claire Bourgain (Imelda Ziekenhuis, Bonheiden)

13h15-14h00: Dr. Christine Bergeron (Laboratoire Pasteur Cerba, France)

13h15-13h55	«Clinical utility of the CINtec PLUS test in the context of PAP cytology and HPV based primary screening»
13h55-14h00	Q&A

SATURDAY 5 MORNING

Room Infirmary - 2nd floor

09:00-13:00 **Breast pathology**
Chairpersons: *C. Colpaert (Antwerp), C. Galant (Brussels)*

09:00-09:30 ***F 16*** ***ER/PR guidelines.*** *M. Hayes (Vancouver-Canada)*
09:30-10:00 ***F 17*** ***HER-2 guidelines.*** *J. Bartlett (Toronto-Canada)*
10:00-10:20 ***F 18*** ***Ki-67 guidelines.*** *K. Lambein (Ghent)*
10:20-10:40 ***Round table discussion, questions and answers***

10:40-11:00 Coffee break

Chairpersons: K. Lambein (Ghent), C. Galant (Brussels)

11:00-11:20 ***F 19*** ***Belgian Cancer Registry: retrospective analysis of newly diagnosed breast cancer patients in 2008.***
H. De Schutter (Brussels)
11:20-11:40 ***F 20*** ***Synoptic breast cancer reporting.*** *C. Colpaert (Antwerp)*
11:40-12:00 ***F 21*** ***Clinical importance of pathology reviews in breast cancer.***
M. Hayes (Vancouver-Canada)
12:00-13:00 ***F 22*** ***Slide seminar: breast cytopathology with histological correlation.***
K. Van de Vijver (Maastricht-The Netherlands)

Room Rector Vermeylen - 1st floor

09:00-12:30: **Cervical cytology**
Chairpersons: B. Weynand (Yvoir), C. Bourgain (Bonheiden)

09:00-09:30 ***F 23*** ***HPV - The wolf in sheeps clothing.*** *N. Dudding (Sheffield-U.K.)*
09:30-10:00 ***F 24*** ***HPV and other biomarkers for cervical (pre)neoplasti lesions.***
P. Delvenne (Liège)
10:00-10:30 ***F 25*** ***HPV negative HSIL.*** *S. Sahebali (Antwerp)*

10:30-11:00 Coffee break

11:00-11:30 ***F 26*** ***HSIL as red herring.***
F. van Kemenade (Rotterdam-The Netherlands)
11:30-12:00 ***F 27*** ***Rationale for recent decision on 3-years interval for cervical screening.***
M. Moens (Bonheiden)
12:00-12:30 ***Panel discussion: value of cytology in the HPV testing era.***
C. Cuvelier (Ghent), J.P.Bogers (Antwerp), M. Moens (Bonheiden)

LUNCH

SATURDAY 5 AFTERNOON

Room Infirmary - 2nd floor

14:00-16:00 **Free paper session**

Chairpersons: P. Demetter (Brussels), J.P. Bogers (Antwerp)

14:00-14:12 ***O 07*** ***Stromal features are potential prognostic markers for ipsilateral locoregional recurrence in DCIS.***
M. Van Bockstal, K. Lambein, O. Gevaert, O. De Wever, M. Praet, V. Cocquyt, R. Van den Broecke, G. Braems, H. Denys, L. Libbrecht/Gent and Leuven

14:12-14:24 ***O 08*** ***Cytokine synthesis in spleen cells of mice with breast tumours interaction with physical activity.***
D.R. Abdalla, A.A.R. Aleixo, S. Thys, C. Simoens, I. Pintelon, J. Bogers, E.F.C. Murta, M.A. Michelin/ Uberaba and Antwerp

14:24-14:36 ***O 09*** ***Solitary extramedullary plasmocytoma of the thyroid: a case report.***
M. Mertens de Wilmar, L. Knopp, C. Sempoux, X. Geets, A. Camboni/Brussels

14:36-14:48 ***O 10*** ***Next generation sequencing is feasible on FNA specimens and improves diagnosis of thyroid nodules.***
M. Le Mercier, N. D'Haene, N. De Nève, C. Degand, S. Rorive, I. Salmon/Brussels and Gosselies

14:48-15:00 ***O 11*** ***Identification of putative precursor lesion of papillary thyroid carcinoma by cyclin D1 overexpression and p38 MAPK phosphorylation.***
M. Lamba Saini, B. Weynand, J. Rahier, M. Mourad, M. Hamoir, E. Marbaix/ Brussels and Yvoir

15:00-15:12 ***O 12*** ***Immune reconstitution inflammatory syndrome in progressive multifocal leukoencephalopathy after highly active antiretroviral therapy.***
C. Maris, D. Hastir, I. Salmon/ Brussels

15:12-15:24 ***O 13*** ***Familial presentation of a granular cell astrocytoma and an oligodendroglioma.***
D. Narasimhaiah, M. Amyere, N. Janin, C. Raftopoulos, M. Vikkula, C. Godfraind/ Brussels

15:24-15:36 ***O 14*** ***Are there specific biomarkers of glioma stem cells?***
A.-L. Trépant, C. Bouchart, A. Verrellen, C. Nicaise, S. Sauvage, S. Rorive, C. Decaestecker, I. Salmon/ Brussels and Gosselies

15:36-15:48 ***O 15*** ***Knowledge, attitudes and acceptability of human papilloma vaccination amongst primary school girls (9 years and above) in Minakulu sub-county Oyam district.***
V. Namanya, R. Opio, D. Niwasasira, A. Onyege, M. Naisanga/Gulu, Uganda

SATURDAY 5 MORNING

Room Rector Blanquaert - 3rd floor

09:00-12:20: **Cervical cancer: prevention, early detection and treatment**
Chairpersons: *E. de Jonge (Genk), E. Murta (Abadia-Brazil)*

09:00-09:10	***Introduction*** *(J. Janssens, Hasselt)*
09:10-09:40	***Oral contraceptive use, HPV and cervical cancer.*** *C. La Vecchia (Milan-Italy)*
09:40-10:00	***Replacement of cytology by an HPV-based screening method: which is the best option?*** *T. Agorastos (Thessaloniki-Greece)*
10:00-10:30	***Diagnostics and surgical treatment of premalignant lesions.*** *H. ter Brugge (Zwolle-The Netherlands)*
10:30-11:00	Coffee break
11:00-11:20	***The importance of stromal factors.*** *J.P. Bogers (Antwerp)*
11:20-11:50	***Vaccination: Who needs the cervical cancer vaccine? How many doses? Can boys be vaccinated, too? What about side effects?*** *J. Cuzick (London-U.K.)*
11:50-12:05	***Awareness of cervical cancer and human papillomavirus (HPV) and acceptability of the HPV vaccine in Morocco.*** *M. Mouallif (Liège)*
12:05-12:20	***Knowledge, attitudes and acceptability of HPV vaccination among primary school girls in Uganda.*** *V. Namanya (Gulu-Uganda)*

LUNCH

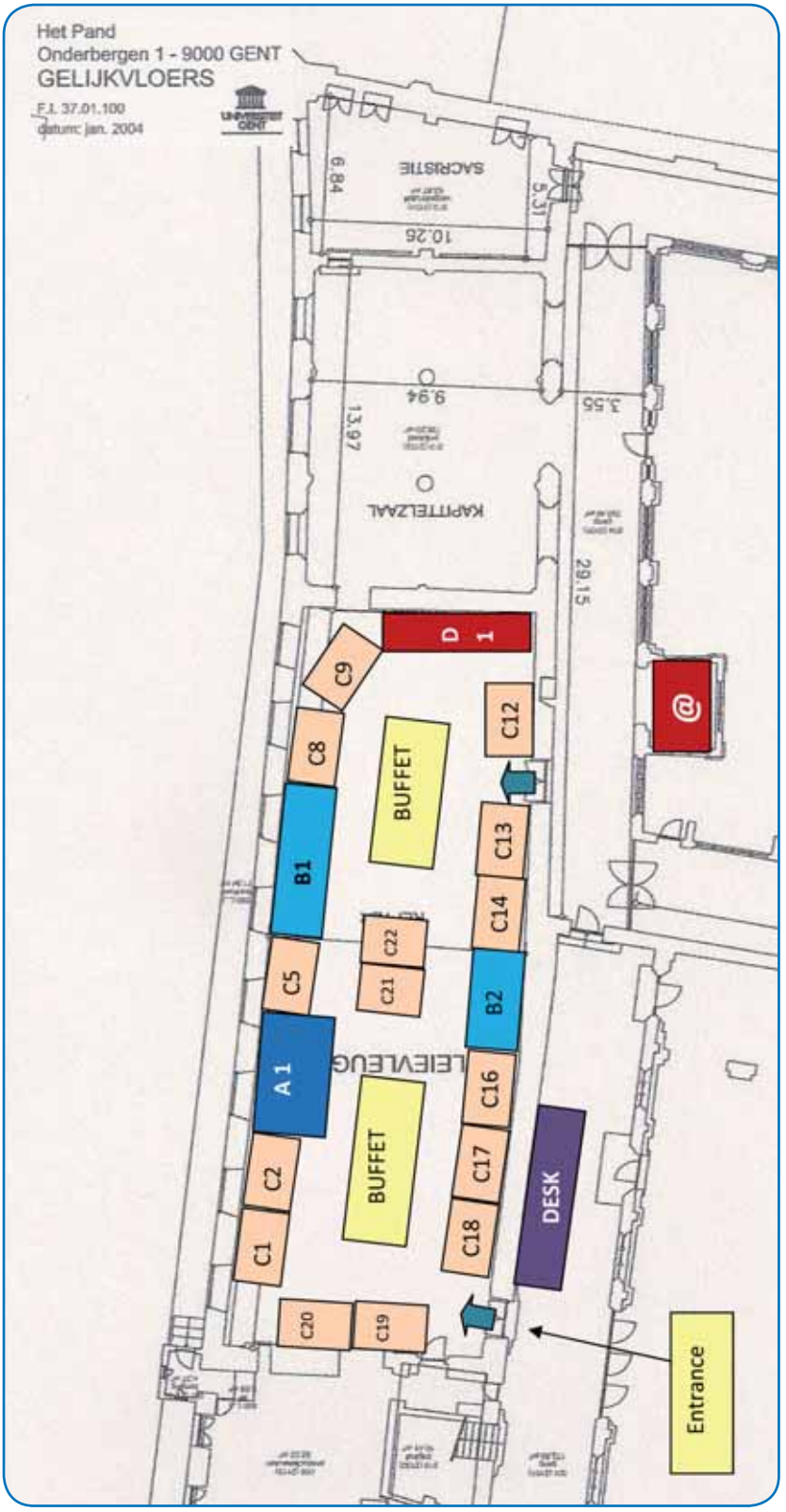
SATURDAY 5 AFTERNOON

Room Rector Blanquaert - 3rd floor

14:00-17:30: **Cervical cancer: prevention, early detection and treatment**
Chairpersons: *D. Schmidt (Kiel-Germany), P. Sieprath (Genk)*

14:00-14:20	***European screening strategies.***
	E. Sleurs (Ghent)
14:20-14:40	***Clinical-molecular scoring system to predict CIN outcome.***
	R. Jach (Krakow-Poland)
14:40-15:00	***Colposcopic findings in "in situ" adenocarcinoma.***
	P. Ide (Hasselt-Leuven)
15:00-15:20	***Trachelectomy: a conservative approach.***
	A. Rodolakis (Athens-Greece)
15:20-16:00	Coffee break
16:00-16:20	***Surgery for invasive lesions.***
	J. Rom (Heidelberg-Germany)
16:20-16:40	***Staging of advanced cervical cancer: laparoscopy or robotics? Advantages and Disadvantages.***
	M. Gorostidi (San Sebastian-Spain)
16:40-17:00	***Systemic therapy in advanced-inoperable disease.***
	A.Bamias (Athens-Greece)
17:00-17:30	***Integrative Oncology: an example from the gynecological department of the Technical University of München.***
	D. Paepke (München-Germany)
17:30	***End of the session***

EXHIBITION FLOOR



EXHIBITIORS

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A 1	ROCHE PHARMA ROCHE DIAGNOSTICS
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D1	ACCO

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INVITED LECTURES

F 01
Dysplasia in Inflammatory Bowel Disease: Guidelines 2013

Dr. Cord Langner
Institute of Pathology, Medical University, Graz, Austria

Patients with inflammatory bowel disease are well known to carry an increased risk of colorectal cancer. In ulcerative colitis, the most important risk factors are young age at onset, long disease duration and extensive large bowel involvement, indicating a cumulative effect of intestinal inflammation (dysplasia-carcinoma sequence). Additional risk factors include primary sclerosing cholangitis, severity of inflammation, presence of pseudopolyps, and a family history of colorectal cancer. Endoscopy with biopsy is used for secondary prevention and the detection of dysplasia in ulcerative colitis and may similarly be used in patients with Crohn's disease depending on the extent of colon involvement. With respect to small bowel cancer, the relative risk is particularly high. Owing to the overall rarity of the disease, however, the cumulative risk is still low and surveillance is not recommended. Within the small bowel, neoplastic lesions most commonly affect the distal jejunum and ileum.

According to WHO guidelines, dysplasia is defined as "histologically unequivocal neoplastic epithelium without evidence of tissue invasion". It is the best and most reliable marker of increased risk of malignancy in patients with inflammatory bowel disease. For diagnostic reasons, dysplasia is separated into four distinct categories: negative for dysplasia (regenerating epithelium), indefinite for dysplasia ("questionable" dysplasia), and positive for low-grade dysplasia and high-grade dysplasia. Interobserver agreement is poor for low-grade and indefinite dysplasia. Confirmation of dysplasia by an independent expert gastrointestinal pathologist is recommended by the ECCO / ESP Consensus panel (based upon similar recommendations for Barrett's oesophagus).

Dysplasia related to inflammatory bowel disease develops only in areas with chronic inflammation and may occur in any part of the colorectum, often multifocal. The microscopic features used for diagnosis are analogous to those characterizing neoplastic growth in general, including both architectural and cytological abnormalities.

There are two gross patterns of dysplasia in patients with IBD: flat and elevated lesions. According to the AGA technical review on the diagnosis and management of colorectal neoplasia in IBD, the term flat dysplasia refers to lesions that are endoscopically undetectable, whereas elevated dysplasia refers to endoscopically detectable lesions. It should be noted, however, that sometimes the term "flat dysplasia" has been used to describe endoscopically detectable, but only slightly

raised lesions. Hence, flat dysplasia is generally detected in random biopsies from unremarkable mucosa. It carries a high risk for cancer: At the time flat high-grade dysplasia is diagnosed adenocarcinoma may already be present in 42-67% of cases. Elevated or raised dysplastic lesions are a heterogeneous group including adenoma-like lesions and non adenoma-like lesions. Adenoma-like lesions, i.e. sporadic adenomas, occur independently of the inflammatory bowel disease, within or outside the inflamed areas of the large bowel. In contrast, non adenoma-like lesions, i.e. dysplasia associated lesions or masses (DALMs), are causatively related to the inflammatory process ("colitis-associated dysplasia") and do only occur in areas of inflammation. Macroscopically, non adenoma-like lesions can either appear as large velvety patches, irregular plaques, irregular bumps and nodules, wart-like lesions, large sessile polypoid lesions with a broad base or even as localized strictures. Adenoma-like lesions are usually well-circumscribed small lesions, with sometimes a sessile configuration similar to those of sporadic adenomas unrelated to inflammatory bowel disease.

Several clinical and microscopic features have been identified which may help to differentiate colitis-associated dysplasia from adenoma-like lesions (**Table 1**). The distinction is of eminent importance as the clinical management differs significantly, ranging from local therapy (polypectomy) to proctocolectomy. According to a recent large retrospective analysis of patients undergoing proctocolectomy for dysplasia, patients with high-grade dysplasia had cancer in 29%, compared to 3% in low-grade dysplasia. In addition, patients with preoperative elevated non-adenoma-like dysplasia had cancer in 25%, compared to 8% in flat dysplasia. Hence, risk of cancer for patients with diagnosis of high-grade dysplasia or elevated non-adenoma-like dysplasia is substantial, and the threshold for surgery should be low given the high likelihood of finding cancer in the corresponding resection specimen.

Recent studies have focused on ancillary methods to improve interobserver variability in detecting dysplasia. The p53 tumour suppressor gene is a key factor in the initial steps of IBD-associated carcinogenesis. P53 is overexpressed in 33-67% of patients with dysplasia and in 83-95% of patients with IBD-associated carcinomas. However, occasional positivity of regenerating, non-dysplastic epithelium limits the diagnostic value of this marker.

As already indicated by the title, the talk will inform about the guidelines on the histopathology of inflammatory bowel disease, produced on behalf of the European Crohn's and Colitis Organization (ECCO) and the European Society of Pathology (ESP), which have just been published online. It will focus on criteria for diagnosis and differential diagnosis, including ancillary techniques, such as

immunohistochemistry and molecular pathology.

Table 1 Microscopic and Clinical Features used for the Differential Diagnosis of Neoplastic Lesions in Inflammatory Bowel Disease (from Magro et al. 2013)

	Colitis-associated dysplasia	**Adenoma-like lesion (sporadic adenoma)**
Age	< 50 years	> 60 years
Extent of disease	Usually total	Usually subtotal
Activity of disease	Usually active	Usually inactive
Disease duration	Long (>10 years)	Short (<10 years)
Associated flat dysplasia	Common (no sharp delineation)	Absent (sharp delineation)
Histology of lesion	Irregular neoplastic glands (varying configuration, size and diameter) with varying amounts of stroma	Regular neoplastic glands (similar configuration, size and diameter) with low amounts of stroma
Increased (mononuclear) lamina propria inflammation	Usually present	Usually absent
Mixture of benign / dysplastic crypts at surface	Usually present	Usually absent

INVITED LECTURES

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F 04

Epidemiology of renal and adrenal cancer: physically very close, but two completely different entities.

Pieter Vande Putte, Kris Henau, Liesbet Van Eycken
Belgian Cancer Registry

A/ Renal Cancer

Cancer of the kidney amounts to 3% of the global cancer burden in Europe, with approximately 73.200 new cases diagnosed each year (Globocan 2008). These tumours occur in all world regions, with a preference for developed and industrialized countries. Renal cell cancer (RCC) represents on average over 90% of all malignancies of the kidney that occur in adults in both sexes.

In 2010, a total of 1.532 kidney cancers were diagnosed in Belgium. The 981 registered tumours in men account for 3% of all tumours. Renal cancer is the 7th most frequent malignancy in males. In women, kidney cancer is the 13th most frequent tumour and represents 2% (n=551) of all tumours. Renal cancer has a male predominance and is diagnosed at a mean age of 64 years in males and 66 years in females. Mortality from this cancer type is limited, representing the 12th most frequent cause of cancer death in males and the 13th most frequent cause of cancer death in females.

For the period 2004-2010, more than 90% of the kidney tumours in Belgium are renal cell cancers. About 55% are classified as renal cell cancers without any further specification, about 20% are clear cell adenocarcinoma and about 6% (5.9% in males and 7.3% in females) are chromofobe RCC. Papillary RCC is twice as frequent in males (11.5%) than in females (5.2%). Sarcomatoid RCC, Bellini Duct, cyst associated and granular cell carcinoma are rare histological subtypes, the distribution is comparable between men and women.

Age specific incidence for kidney cancer for the period 2004-2010 is shown in **figure 1**. A first and small peak can be observed from birth to the fifth year of life, due to nephroblastoma (Wilms tumour). Almost all nephroblastoma cases (approximately 15 new cases a year) occurred in this age category. A gradual increase in incidence is seen from the age of 35 years old, with a second peak at the age of 75 years old for men and 70 years old for women.

The incidence data are equivalent to an average annual crude incidence of 18,5 new cases per 100.000 person years in men and 10 new cases per 100.000 in women. Age standardised incidence according to the World Standard Population is 10/100.000 in men and 5/100.000 in women.

Trends in incidence and mortality (estimated annual percentage of change, EAPC) were only calculated for the Flemish Region (2001-2010) because of the availability of a longer registration period and stable methodology for the cancer registration activities when compared to the Brussels and the Walloon Region.

Kidney cancer incidence and especially stage I tumours are increasing both in males and females between 2001 and 2010. Mortality at the contrary, is significantly decreasing for both sexes (**figure 2**) and for the same period. These are known trends, most probably due to the increased use of more (sophisticated) imaging procedures. The detection of pre-symptomatic tumours could explain this phenomenon: more patients are diagnosed at an earlier, curable stage, with a better prognosis and a higher survival rate. Increase in incidence between 2001 and 2010 is observed in all the age categories in males (age group 30-44 years, 45-64 years and 65+) while in females it is only noted for the age group 65 years and older.

For the incidence years 2004-2008, kidney cancer in Belgium reaches a 5-year relative survival rate of 71% in both males and females. Survival is inversely related to the age of the patient. The best outcome is noticed for the youngest age group (5-year relative survival of 79.6% in males and 86.9% in females) followed by the 45-64 years age group (74.4% in males and 76.9% in females) and the 65+ years age group (67.0% in males and 65.4% in females). Five-year relative survival for kidney cancer is highly dependent on the extent of the disease, ranging from 94.5% (stage I) to 17.7% (stage IV) in males, and from 91.2% (stage I) to 15.0% (stage IV) in females. More than half of the patients is diagnosed with stage I or II (60.1% of cases with known stage in males and 63.6% in females) and have a 5-year relative survival rate of more than 80%.

The relative 5 year survival by histology is comparable between men and women for RCC (72%), clear cell adenocarcinoom (77%) and chromofobe RCC (83%). For papillary RCC, sarcomatoid RCC and Bellini duct carcinoma, the prognosis is worse for men. Papillary RCC has the best relative 5 year survival (90.4% in women, 82.1% in men), sarcomatoid RCC the worst (27.4% in women, 19.2% in man). Nephroblastomas in Children have a good prognosis (5 year survival of 94%).

Etiology

Tobacco smoking is a major cause of kidney cancer as well as hypertension, which is a known risk factor for renal diseases in general and more specific for renal cancer. Exposure to carcinogenic arsenic compounds in industrial processes or through drinking water increases the risk of renal cancer by 30%. A history of radiotherapy, women for cervical cancer and men for testicular cancer, is associated with an increased risk by almost 2 to 3 times after 30 years. Renal cancer appears also to be more common in patients with obesity (high BMI), end-stage renal failure and acquired renal cystic disease. Phenacetin containing analgetics are known to increase the risk of kidney cancer. There are also inherited conditions that increase the risk for renal cancer, they include: Von Hippel-Lindau syndrome (VHL), Tuberous sclerosis, Birt-Hogg-Dube syndrome and hereditary non-VHL clear cell and papillary renal cell cancer.

Since the widespread use of abdominal imaging (ultrasonography, computed tomography and magnetic resonance imaging), it is estimated that more than 50% of the renal cancers are detected incidentally.

In childhood, nephroblastoma (Wilms Tumor) is the most frequent occurring renal cancer. It arises from mesodermal precursors of the renal parenchyma (metanephros) and occasionally is found to arise in the extrarenal retroperitoneum. Two loci on chromosome 11 (locus 11p13 known as the WT1 gene and locus 11p15 known as the WT2 gene) have been implicated in the genesis of a minority of Wilms tumors. However, the genetics appear to be multifactorial and abnormalities at other sides are also recognized.

B/ Adrenal cancer
Background

The three main oncological disorders that may arise from the adrenal gland are the adrenocortical carcinoma (ACC) from the adrenal cortex and the neuroblastoma and malignant pheochromocytoma from the adrenal medulla.

The reported annual incidence of *adrenocortical carcinoma* (ACC) is 0.5–2.0 cases per million individuals. Updated population-based studies on incidence are scarce. The age specific incidence for ACC follows a bimodal age distribution with a peak in childhood and in the fourth to fifth decade of life. The disease is more frequent in females than in males. The majority of ACC's are sporadic and little is known about their etiology. However, sometimes these malignancies are part of hereditary syndromes such as the Li-Fraumeni syndrome, Beckwith-Wiedeman

syndrome, multiple endocrine neoplasia (MEN) 1, congenital adrenal hyperplasia, familial polyposis coli and B-cantenin mutations.

Neuroblastomas (NB) are neuroendocrine tumours, arising from any neural crest element of the sympathetic nervous system. Most frequently it originates in one of the adrenal glands, but it can also develop in nerve tissues in the neck, chest, abdomen or pelvis. The etiology is not well understood. Several risk factors have been proposed but the results of research have been inconclusive. About 1%-2% of the cases run in families and have been linked to specific gene mutations. Germline mutations in anaplastic lymphoma kinase (ALK), PHOX2A and KIF1B gene have been implicated in familial NB. NB is also a feature of neurofibromatosis type I and Beckwith-Wiedeman syndrome. N-myc oncogene amplification within the tumor is also a common finding in NB.

Pheochromocytomas are catecholamine-producing neuroendocrine tumors arising from chromaffin cells (of the adrenal medulla or extra-adrenal paraganglia). The incidence is higher in patients with hypertensive symptoms. Up to 30% of pheochromocytomas are associated with a variety of inherited conditions, including MEN2, Von Hippel-Lindau disease, neurofibromatosis type 1 and heredity paraganglioma syndromes. Only a few (10%-17%) pheochromocytomas are malignant. Although the likelihood of malignancy varies among different genetic backgrounds, it is below 10% for most sporadic pheochromocytomas, except in patients with mutations in the succinate dehydrogenase B gen and/or extra-adrenal locations, among whom more than 30%-50% may develop a malignant tumor.

Adrenal cancer, Belgium, 2004-2010

Adrenal cancer is a very rare cancer. In 2010, only 58 cases were registered in males (n=29) and in females (n=29).

Between 2004 and 2010, a total of 282 new cases of adrenal cancer were diagnosed in Belgium. In this group, a total of 90 new cases of neuroblastomas were diagnosed and all but one occurred in the youngest age group of 0-14 years. In this youngest age group, a total of 91 new cases of adrenal cancer were diagnosed and all but two were neuroblastomas. ACC was the most common diagnosed adrenal cancer, with 118 new cases, representing 42% of all newly diagnosed adrenal cancers. In 65% of the cases, patients were 50 years or older. Phaeochromocytoma was diagnosed in 48 patients, in 75% of the cases, people were 50 years or older.

Age specific incidence for the period 2004-2010 is shown for both sexes in **figure 3.** Neuroblastoma has the highest incidence in the youngest age group, with almost no cases after the age of 10 years. From the age of 30 years onwards, the incidence of phaeochromocytoma and ACC steadily increases by age with a peak incidence at the age of 70 years.

The best 5-year relative survival is observed for phaeochromocytoma (more than 80%). The worst survival is seen for the ACC, with a relative 5-year survival of about 40%. For neuroblastoma this is about 75%. Foetal neuroblastomas very often differentiate into benign neoplasms. In infants and young children, the number of patients needing therapy for an aggressive variant increases. In older children, neuroblastomas are very frequently aggressive malignancies. Age, dichotomized at 18 months, is one important parameter in determining prognosis. In the Flemish Region, 10-year survival rates are very high for children up to 18 months of age (92%). For patients of 18 months of age and older, prognosis is much worse (50% 10-year survival rates).

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Figure 1: Kidney cancer: Age specific incidence by sex, Belgium, 2004-2010

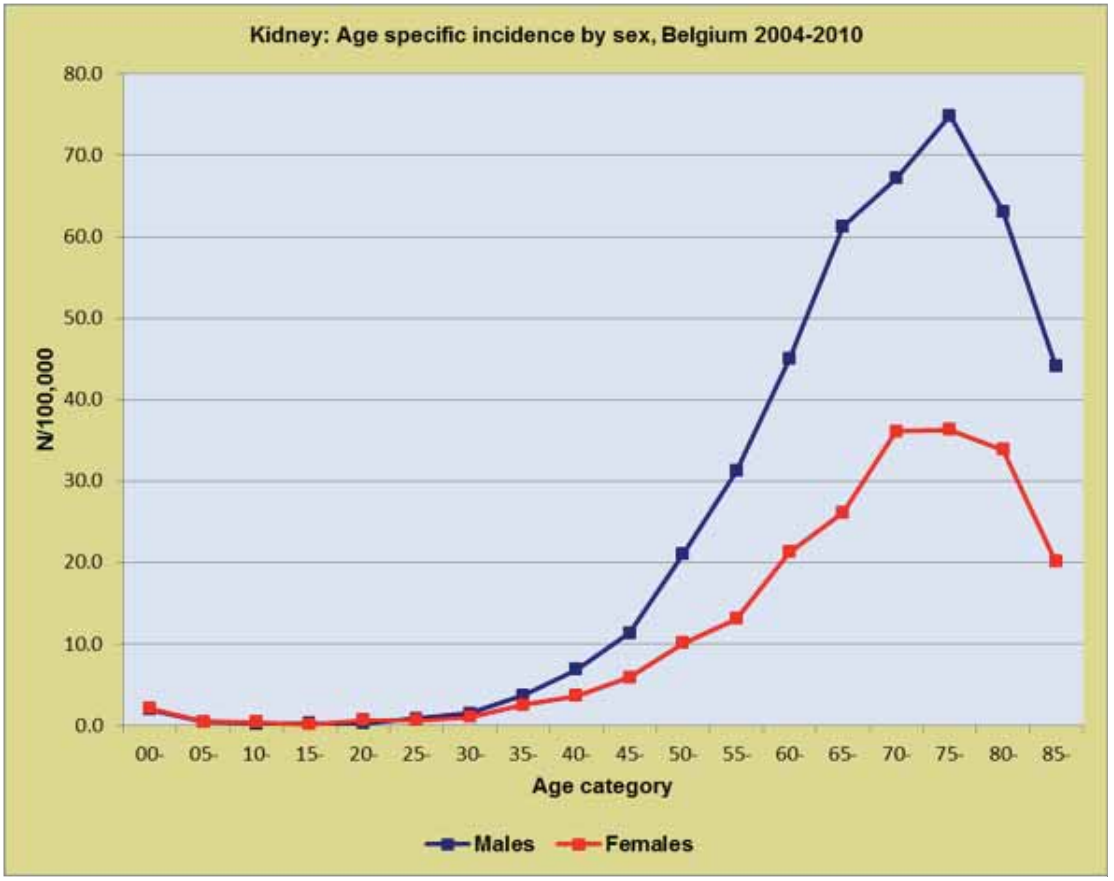


Figure 2 Kidney cancer: incidence and mortality by sex, Flemish region, 2001-2010

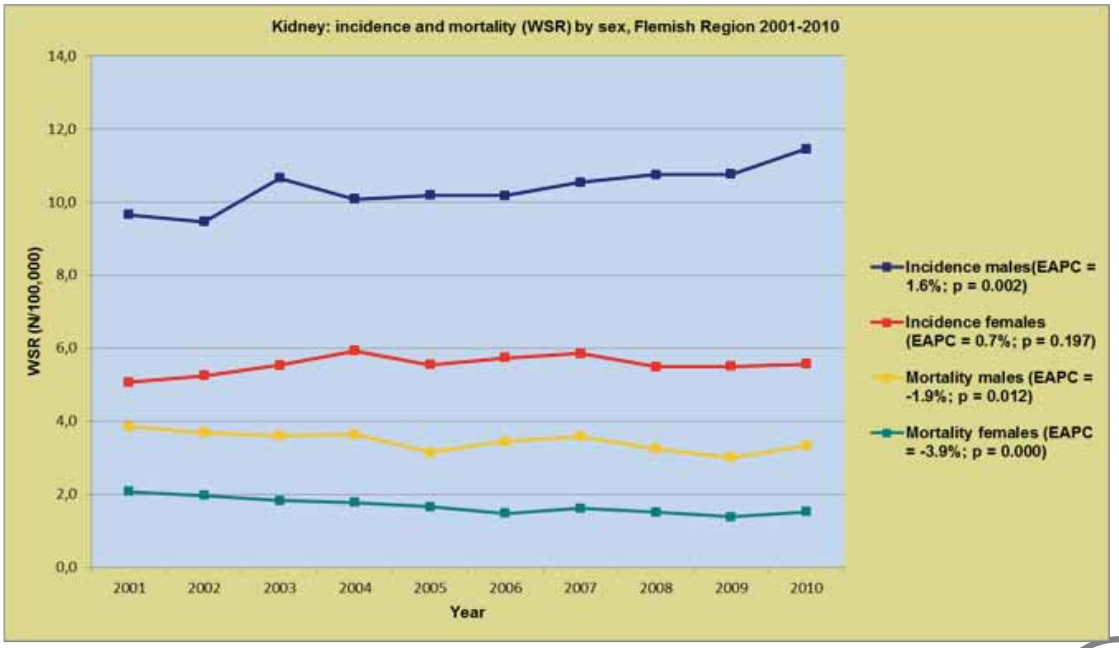
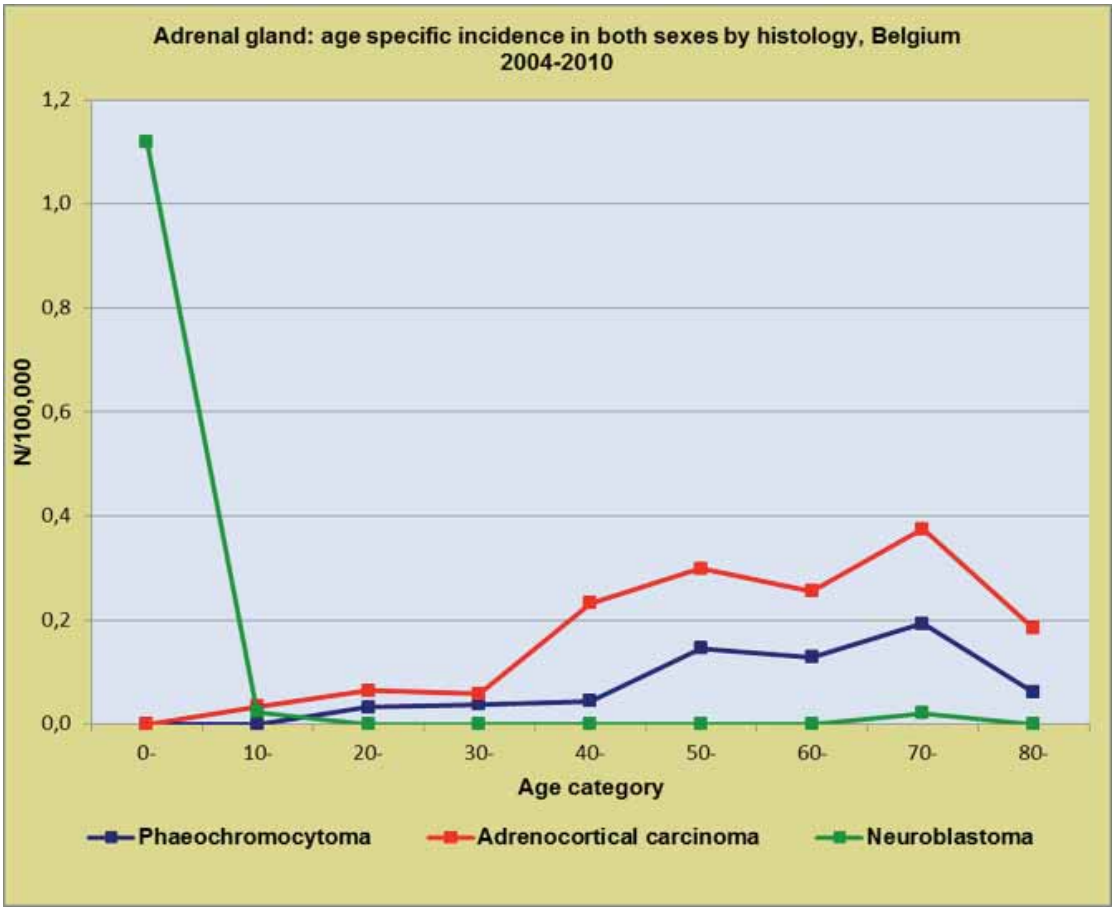


Figure 3 Adrenal cancer: age specific incidence by histology, Belgium, 2004-2010



F 05

Classification of the renal tumours of the adult: histological and immunohistological criteria for the pathological diagnosis.

F. Algaba.
Pathology Department. Fundació Puigvert. Universitat Autónoma de Barcelona- Spain

The identification of the specific chromosomal and genetic alterations of the familiar and hereditary syndromes of the distinct histological subtypes of renal cell carcinoma (RCC) has been able to check that a big percentage of the sporadic forms of these subtypes have the same genetic changes, by what can interpret that the described morphological subtypes are expression of specific genetic changes. By this reason when developing target therapies against different genetic pathways the precise identification of the histologic subtype can determine the election of the drug and by this reason the WHO 2004 RCC classification follows being of utility to its correlation with the molecular bases of the RCC.

CLEAR CELL RENAL CELL CARCINOMA (RCC WITH LOH 3p)

Is the more frequent subtype of sporadic RCC in the adult (of 70% to 85%) (1). The proximal nephron is its origin.

The typical histological feature is the clear aspect of the cells by the big quantity of glycogen and lipids in their cytoplasm. They are distributed in tubular and solid areas with a very prominent capillary stroma.

Three genes have been located on the short arm of chromosome 3 that are probably involved in RCC. The suppressor gene in 3p25-26 (VHL) which coincides with von Hippel-Lindau disease but is expressed in 34-56% of sporadic carcinomas (2), and those located on 3p14.2 and on 3p.12, whose deletion is more frequent that of the former.

Carbonic anhydrase IX is a trans-membrane protein and it is expressed in 84-100% of clear cell RCC. The membranous pattern of CD10 is also typical.

The multilocular cystic RCC, composed entirely of numerous cysts lined by clear cells, probably is a variant of low aggressivity of this subtype (3).

PAPILLARY RCC (RCC WITH TRISOMIES)

Around 7% to 15% of all RCC in the adult are papillary (4). The proximal nephron is also its origin.

Its name is for the distribution of the malignant cells around capillary cores (papillae) in a 50% to 70% of the tumor (4).

In 73% of cases the cells have scarce cytoplasm (type 1), and in a 42% the cytoplasm is eosinophilic (type 2) (5, 6). This subtyping correlates with less or more biological aggressivity, respectively (5).

Trisomy or tetrasomy 7, trisomy 17 and loss of the chromosome Y, are the most frequent anomalies (7). The type 1 is related with the proto-onco-gene c-MET (7q34) activation, that codes HGFr. The type 2 is in relation with Fumarate hydratase gene. A strong expression of alpha-metylacil-CoA racemase (AMACR) is a typical feature, but not exclusive.

CHROMOPHOBE RENAL CELL CARCINOMA (RCC WITH MULTIPLE MONOSOMIES)
Its incidence is 5% to 10% of adult RCC (8). The intercalated cells of distal cortical nephron are the origin (9).

The typical cells are polygonal with a good delimitation of the cytoplasmic membrane (that gives them the appearance of a plant cell). The pale reticulated cytoplasm (chromophobe) is due to the presence of abundant cytoplasmic invaginated 150-300nm diameter vesicles. These cells may have a clear cytoplasm (clear cell subtype) due to loose glycogen deposits (but not as clear as that of the clear cell renal cell carcinoma), or a more eosinophilic one (eosinophilic variant) depending on the number of mitochondria.
An extensive chromosomal loss in 1, 2, 6, 10, 13, 17 and 21 is present (10). LOH 17 associates this tumour with the Birt-Hogg-Dubé syndrome (11). A strong expression of c-kit is a typical feature.

COLLECTING DUCT RCC
Less than 1% of RCC are from the medullary distal nephron or Bellini ducts.
The mandatory morphology of the cells is a high nuclear grade, eosinophilic cytoplasm, predominant tubular arrangement, desmoplasia and expression of high molecular weight cytokeratins (12).
Losses in 1q, 6p, 13q, 14, 15, 21q and 22 are described (13).
Expression has been observed of high molecular weight cytokeratins (34 E12 and CK19) and of *Ulex europaeus* lectin in almost all cases which justifies them to be major criteria within the WHO classification. CD10 and Vimentin are variably positive
The medullary RCC is considered as an undifferentiated collecting duct (14).

TRANSLOCATION RCC
The translocation of Xp11.2, with the gene TFE3, the most frequent is t(X;1)(p11,2;q21) (15) and the translocación t(6;11)(p21;q12) and fusion TFEB (16) are the definitions of these infrequent RCC with a peculiar morphology with clear cell and eosinophilic cells mixture in solid and papillary growth and calcifications.

Cathepsin K is a good markers of these tumors in addition f the TFE3 and TFEB nuclear expression. AMACR is frequently positive.

Other les frequent morphological subtypes are:
MUCINOUS TUBULAR AND SPINDLE CELL CARCINOMA is a low-grade carcinoma composed of tightly packed tubules separated by pale mucinous stroma and a spindle cell component. It seems to derive from the distal nephron but some authors believe it could be a variant of papillary RCC with proximal tubule origin. Express AMACR and the typical chromosome anomalies are monosomies on 1, 4, 6, 8, 9, 13, 14, 15 and 22 (17).

RCC ASSOCIATED WITH NEUROBLASTOMA.
UNCLASSIFIED RCC In surgical series, it represents 4-7% of renal tumors, it seems to be a heterogeneous group of tumors whose prognosis seems to be related to histologic features known to be relevant in common forms of renal cell carcinoma

After the 2004 WHO Classification some other histological subtypes are proposed, among them the most accepted (but not yet included in a official classification) are:
TUBULOCYSTIC RCC composed by packed tubules and cysts lined by cuboidal or hobnail cells with abundant eosinophilic cytoplasm and large nuclei showing prominent nucleoli with gains of chromosomes 7 and 17 (18). CD10 and AMACR are positive in greater than 90% of tumors. It may represent a subset of papillary RCC.
CLEAR CELL PAPILLARY RCC some cases are associated with end renal disease. It show in 50% of cases a prominent cystic component; solid, tubular and microcystic with papillae areas are also present. The tumor cells show a clear cytoplasm and a low-grade nuclear pleomorphism with nuclei situated towards the surface of the papillary tufts. This RCC not show neither LOH 3p nor trisomies of chromosomes 7 and 17 (19).
ACQUIRED CYSTIC DISEASE ASSOCIATED RCC are composed by eosinophilic cells with a rounded nucleus and large nucleolus arranged in variety of architectural patterns and characterized by the presence of oxalate crystal. Multiple gains of chromosomes including 1, 2 , 6, 10, 3, 7, 17 are described (20).

These Morphological-Genetic RCC subtypes can correlate with:
The hypoxia-inducible pathway (Clear cell RCC, Papillary RCC type II through Fumarate gene) (21)
The mTOR (mammalian target of rapamycin) signalling pathway (Clear cell RCC and Papillary RCC type II). (22)
The c.Met –RAF-MEK-ERK pathway (Papillary RCC type I and Translocation RCC) (23).
The c-kit-RAF-MEK-ERK pathway (Chromophobe RCC)
All of these pathways can represent a potential targets for treatment.

INVITED LECTURES

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INVITED LECTURES

F 06
Pathology and genetics of renal cell cancer.

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Zurich, Switzerland

The von Hippel-Lindau (*VHL*) tumor suppressor gene alterations dominate the genetic landscape of clear cell renal cell carcinoma (ccRCC) (Gnarra *et al*, 1994; Latif et al, 1993) and formed the basis of the current WHO classification with a clear genotype/phenotype correlation (Moch, 2013). Such alterations control cellular oxygen sensing via HIF (Shen & Kaelin, 2013). Recent studies have reported new ccRCC genes, including *SETD2, KDM6A, KDM5C, BAP1, and PBRM1 (Varela et al, 2011)*. Strikingly, all these genes fall into a category of histone/chromatin regulators. Polybromo-1 (*PBRM1*) is the second most frequently mutated gene after VHL (*Kapur et al, 2013; Pawlowski et al, 2013; Pena-Llopis et al, 2012; Varela et al, 2011*). In a previous study, additional mutated genes have been identified. The PI(3)K/AKT was recurrently mutated, suggesting this pathway as a potential therapeutic target (2013). Widespread DNA hypomethylation was associated with mutation of SETD2. Further, aggressive cancers demonstrated evidence of a metabolic shift with downregulation of genes involved in the TCA cycle, decreased PTEN levels and other alterations including miR-21 promoter methylation. Remodelling cellular metabolism constitutes a recurrent pattern in clear cell renal cancer (Gerlinger *et al*, 2012b; Linehan & Ricketts, 2013). The understanding of the metabolic basis of kidney cancer will provide the potential for development of new treatment options (Linehan & Ricketts, 2013). Future studies will show the relevance of intratumoral heterogeneity in these pathway alterations (Gerlinger *et al*, 2012a; Xu *et al*, 2012).

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INVITED LECTURES

F 07
Pheochromocytoma and paraganglioma from clinics to genetics through pathology

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Summary

During the last decade, several new genes have been found to be involved in the pathogenesis of pheochromocytoma and paraganglioma, particularly genes encoding the subunits of succinate dehydrogenase (SDHx genes). Currently, 10 predisposing genes have been identified and the proportion of patients with pheochromocytoma or paraganglioma harbouring a mutation in one of these genes is about 20 to 30%. The identification of mutations in these predisposing genes is of interest, as well for the family (genetic counselling) as for the patient him/herself (for instance, SDHB mutations are associated with an increased risk of recurrence and malignity). Genetic screening is clearly indicated in case of early diagnosis (before 45 years old), positive family history, suspicion of a syndromic form, or extra-adrenal, multifocal or malignant presentation. Due to the loose genotype-phenotype correlation and, on the other hand, the prognostic implication of the identification of a mutation in one of the known predisposing genes, many experts recommend performing genetic screening in every patient diagnosed with pheochromocytoma. In the near future, immunohistochemistry of SDHx genes, search for somatic mutations within the tumour and identification of specific gene expression profiles (microarrays) are likely to play an increasing role in the diagnosis, management and follow-up of pheochromocytoma.

When to look for a familial pheochromocytoma

A familial pheochromocytoma should be considered in presence of at least another case of pheochromocytoma and/or paraganglioma in the family or in presence of manifestations suggesting the existence of a syndromic form (Multiple Endocrine Neoplasia type 2, von Hippel-Lindau syndrome or Neurofibromatosis type 1). However, mutations in the VHL gene as well as in newly identified predisposing genes (see lower) can also be found in apparently sporadic cases without

syndromic manifestations, because of the occurrence of a de novo mutation or, more frequently, due to an incomplete penetrance. Finally, inheritance of SDHD-related tumours is autosomal dominant with maternal imprinting (subjects who inherited the mutation from their mother have a 50% risk to transmit the trait but will not develop the phenotype). In this case, the disease can skip a generation or be found in single subject, especially in small nuclear families frequently encountered nowadays.

How frequent are familial forms of pheochromocytoma?

Until recently, pheochromocytoma was known as the « tumour of 10% » : 10% bilateral, 10% extra-adrenal, 10% malignant and 10% familial. However, this description definitely needs to be updated, particularly the frequency of inherited pheochromocytoma. Indeed, following the identification of new predisposing genes, the prevalence of mutations in large recent cohorts of pheochromocytomas/paragangliomas is about 20-30%.

Which genes are involved?

Until the end of the year nineties, known predisposing genes were limited to RET, VHL and NF1, respectively associated with Multiple Endocrine Neoplasia type 2 (MEN2), von Hippel-Lindau syndrome and Neurofibromatosis type 1.

Since the early 2000s, mutations in SDHD, SDHB and less often SDHC and SDHA genes coding for the 4 subunits of succinate dehydrogenase, - a mitochondrial enzyme involved in oxidative phosphorylations and the Krebs cycle - were also described in 5-20% patients.

Finally, these last five years, several additional predisposing genes have been identified in a small proportion of cases, in particular SDHAF2, coding for an enzyme involved in the flavination of succinate dehydrogenase and the tumour-suppressor genes TMEM127 and MAX (MYC-associated Factor X). Overall, 10 different genes have been involved in the pathogenesis of pheochromocytoma and paraganglioma, and the list may still increase!

Interest of genetic screening in patients with pheochromocytoma

Identification of a mutation in one of the known predisposing genes may have a diagnostic and prognostic importance, both for the patient and his family. In particular, mutations in SDHB were consistently associated with an increased

risk of recurrence and malignancy. With extra-adrenal location, SDHB mutations are the most powerful predictive factor of malignant behaviour in patients with pheochromocytoma. In view of the poor specificity of current histological markers of malignancy, this finding is highly relevant for clinical practice.

Genetic screening: in which patients?

Genetic screening is clearly indicated in case of early diagnosis (before 45 years old), positive family history, suspicion of a syndromic form, or extra-adrenal, multifocal or malignant presentation. Due to the loose genotype-phenotype correlation and, on the other hand, the prognostic implication of the identification of a mutation in one of the known predisposing genes, many experts recommend performing genetic screening in every patient diagnosed with pheochromocytoma.

Which genes should be tested in priority?

While several screening algorithms have been proposed, none gives entire satisfaction. Irrespective of the algorithm, several simple guidelines can be proposed:

- In case of presentation suggestive of MEN2 or von Hippel-Lindau syndrome, the corresponding genes- RET and VHL, respectively - must be screened in priority. The NF1 gene is very large and clinical criteria are sufficient for the diagnosis of neurofibromatosis.
- In case of familial head and neck paraganglioma, the first candidate gene to be tested is SDHD.
- In case of extra-adrenal abdominal and/or thoracic pheochromocytoma, malignant or paediatric tumour, and in rare cases associated with kidney tumours, SDHB screening should be prioritized.
- Finally, in case of bilateral pheochromocytoma, the first gene to be screened is VHL.

In daily practice, in the absence of manifestations suggesting a syndromic form, it is more straightforward to test VHL, SDHD, SDHB and possibly SDHC genes in parallel. Besides screening for mutations in coding regions and/or in intron-exon boundaries, a comprehensive analysis should include a search for large rearrangements (~ 10% of mutations in SDHx genes are deletions). The latter is usually performed using Multiplex Ligation-dependent Probe Amplification (MLPA). Nowadays, the search for mutations in SDHA, SDHAF2, TMEM127 and MAX is usually restricted to a research context.

Immunohistochemistry: an alternative to conventional genetic screening?

Several publications from the Erasmus Medical Centre in Rotterdam and the Hôpital Européen Georges Pompidou in Paris demonstrated the interest of immunohistochemistry using antibodies directed against the SDHB and SDHA subunits of succinate dehydrogenase for the genetic characterization of the pheochromocytomas and paragangliomas. Based on these preliminary works, the existence of a mutation in SDHB, SDHD or SDHC is associated with a destabilization of the enzyme, leading to abolition or substantial decrease in the staining intensity for SDHB, without alteration of staining for the SDHA subunit. In case of rare SDHA mutations, staining for both SDHA and SDHB subunits is abolished.

Immunohistochemistry appears to have an almost 100% sensitivity for the detection of SDHx mutations and is less expensive, less time-consuming and faster than classical genetic analysis. Accordingly, it may become the first-line screening test for the identification of mutations in SDHx genes. In this hypothesis, genetic analysis of germline DNA would be carried out only in the presence of abolished staining for SDHB, in order to identify the responsible SDH subunit. Our centre currently takes part to a multicentric research program aiming to validate on a broader scale the sensitivity and specificity of immunohistochemistry for the detection of mutations in SDHx genes.

New research directions

Recent works based on RNA microarrays showed the existence of two different patterns of gene activation reflecting distinct pathophysiological mechanisms. The first - called cluster 1 - is associated with activation of hypoxia genes in the absence of hypoxia (*pseudo-hypoxic pattern*) and is found in VHL and SDHx-related tumours. The second pattern - cluster 2 -, characterized by activation of PI3 kinase/AKT, RAS/RAF/ERK and mTORC1/p70S6K pathways, is found in case of mutations in RET, TMEM 127 and MAX genes.

The use of microarray profiling opens new perspectives: identification of new predisposing genes (this approach contributed to the identification of TMEM127 and MAX mutations in a subset of pheochromocytomas), better understanding of the pathophysiology of the disease and development of individualized therapies.

Finally, somatic mutations of NF1 or copy number variations (CNV) in at least one of the known predisposing genes have been identified in a high proportion of pheochromocytoma without germline mutations. In the near future, these

abnormalities may provide prognostic information and influence the follow-up. If these findings are confirmed on a larger scale, most pheochromocytomas may prove to be associated with genetic abnormalities, whether constitutional or acquired.

In the next decade, diagnosis, prognosis and individualized management of pheochromocytoma will largely benefit from a tight collaboration between the clinician, the pathologist and the geneticist.

Adapted from:
Aydin S., Evenepoel L., Severino F., Mendola A., Vikkula M., Persu A. Et si c'était un phéochromocytome familial ? De la clinique à la génétique. Louvain Médical 2013; 132 ; 121-125.

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F 15
Mechanisms of resistance in breast cancer patients.

Dr. Els M.J.J. Berns
On behalf of D. Ramirez, E.A. Reijm and Dr. M.P.H.M. Jansen.
Department of Medical Oncology, Erasmus MC – Cancer Institute, Rotterdam, The Netherlands

Annually one million women are diagnosed with estrogen receptor-positive breast cancer. These breast tumors are of the luminal A and B subtype and tumor cell proliferation is thought to depend on activity of the estrogen receptor, i.e. ERα.
For over three decades both primary and metastatic breast cancer patients with estrogen receptor-expressing tumors greatly benefit from **endocrine therapies** like tamoxifen or state of the art aromatase inhibitors. This means that inhibition of ER by endocrine therapy is a major treatment modality. However, its highly heterogeneous character leads to differences in response to therapy, with approximately 30% non-responders and eventually, in all initial responders, development of progressive disease. Consequently, there is a high need for markers to identify patients likely to benefit from endocrine therapy in order to obtain a more personalized treatment approach.

To date three molecular markers (**ER, PR and CYP2D6**) have been used in the clinical setting to predict the benefit of the anti-estrogen tamoxifen therapy. The hepatic drug-metabolizing enzyme cytochrome P450 2D6 (CYP2D6) is involved in the conversion of tamoxifen to its abundant metabolite: endoxifen. Depending on the genotype and/or co-administration of other drugs the levels of endoxifen in patients differs.

Central in endocrine therapy resistance, however, is the function of ER . The estrogen receptor, when activated by estrogens, acts as a nuclear transcription factor and needs to interact with DNA/chromatin at specific loci to result in expression of specific genes. Many different **ER-related mechanisms** have been suggested to explain tamoxifen resistance and it is evident that multiple factors are involved.
We will discuss several mechanisms to account for resistance to endocrine therapy including decreased or loss of expression of ER, altered phosphorylation, amplification or truncated receptors. Increased activity of AP1, activation of MAPK or PIK3 pathways also influence ER function. As known, overexpression of the HER2 receptor or hyperactivation of the phosphatidylinositol 3-kinase (PI3K)

pathway already led to the development of new targeted therapies.

ERα rarely binds promotors and most estrogens receptor/chromatin interactions occur at distal enhancers which are involved in chromatin loop structures to regulate gene expression. Thus slowly it is becoming apparent that **epigenetics** plays a key role in breast cancer as well. Epigenetics refers to the function of the chromatin that does not depend upon the coding sequence of the DNA itself but on modification to the DNA or to histone proteins attached to the DNA. This means that in contrast to non-reversible genetic alterations, epigenetic alterations are reversible and epigenetic compounds are now in development or tested in clinical trials, for example a target being EZH2.

EZH2 is the catalytic enzyme that trimethylates histone 3 lysine 27. Histone 3 trimethylation of lysine 27 (H3K27me3) results in closed DNA/chromatin, thus inaccessible for transcription factors, which leads to gene expression silencing.
We have demonstrated an association between high EZH2 protein expression and poor outcome to first-line tamoxifen treatment in MBC patients. Moreover, we have also shown that silencing of EZH2 in the ER-positive cell line MCF7 leads to a higher expression of ER and increased sensitivity to tamoxifen. This is overcoming or postponing the emergence of endocrine therapy resistance. Therefore overexpression of Enhancer of Zeste in tamoxifen resistant breast tumor samples underscores the need for EZH2 inhibitors.

Cancer is a disease of the **DNA** and in breast cancer targeted therapies based on DNA copy number changes (e.g Herceptin in the context of HER2 amplification) have provided clear clinical benefit for breast cancer patients. Recent studies using whole genome sequencing analyses on breast tumor samples revealed a set of 18 genes to be mutated in breast cancer patients that correlate with differential survival upon **aromatase** inhibitor treatment, including PI3K, TP53, MAP3K1 and GATA3.

The aim is to validate **new biomarker candidates**. Different platforms are used for these studies including high throughput qRT-PCR, microarrays, TMA, SNPs. Tumor tissues, cell lines and animal models are queried. We will present studies on **metagenes** that are ongoing. For example, in clinical samples we and others revealed discriminatory gene-signatures with resistance to first-line tamoxifen and aromatase treatment. Functional annotation of the signature genes showed that tamoxifen resistance is not only the result of alterations in the tumor itself (as is the case for HER2/neu amplifications), but that the tumor microenvironment also plays a key role as well.

INVITED LECTURES

What is the **future**?
Tumor clonality: Is the molecular evolution of cancer genetic constitution of the tumor changing over time and during treatment pressure?
Development of state of the art methodology: ultra deep sequencing, cell free circulating tumor DNA and circulating tumor cells studied in patients with solid cancers, a liquid biopsy?
In silico analyses: Share and integrate data: an example is the TGCA platform.

F 16
Hormone Receptor Guidelines in Breast Cancer

Dr. Malcolm Hayes
BC Cancer Agency

Why the ASCO Guidelines?

- Results from multi-centre quality assurance programs showed that many laboratories had sub-optimal ER assays

UKNEQAS ER results 2002

- 290 participating laboratories
- 66% passed on the test slide
- 87% passed on their internal control slide
- 60% of 139 using DAKO ID5 antibody passed
- 79% of 112 using Novocastro 6F11 antibody passed.
- 9 different antibodies were used
- 8 different antigen retrieval methods were used
- 15 different detection systems were used
- 12 different chromogens were used
- 10 different automation systems were used.

- The BCCA lab failed the test slide
  - Switched from ID5 to 6F11 antibody
  - Changed antigen retrieval system

Assessment of ER

- Each participant is provided with TWO unstained slides that have FFPE sections of 3 different breast cancers each with different levels of ER expression. They are asked to stain both slides are return the best together with their **"in house"** control.

- "in house" slides are included so that there is an element within the module that the participants are routinely familiar with

- UK NEQAS now request that the participants use multiblock controls that include cases with different levels of receptor expression

- Participants are also asked to provide the protocol employed via the on-line system

- All slides are coded so as to protect anonymity

ER from Run 90 – 2010 High Expressor

UK NEQAS ICC & ISH

Assessment Report Images - RUN 90

Selected Images from the BREAST STEROID HORMONE RECEPTOR Module showing Optimal and Sub-optimal

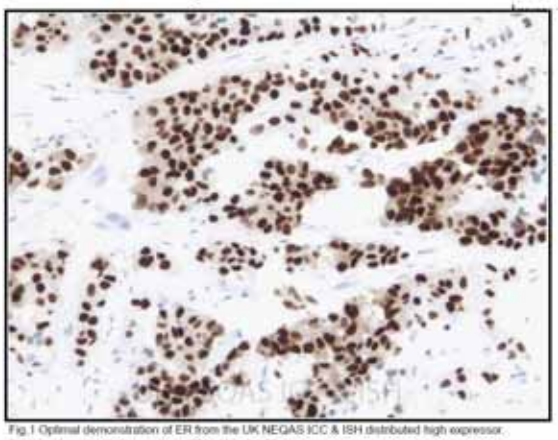


Fig.1 Optimal demonstration of ER from the UK NEQAS ICC & ISH distributed high expressor. Section stained with Neomarkers SP1, diluted 1:50.

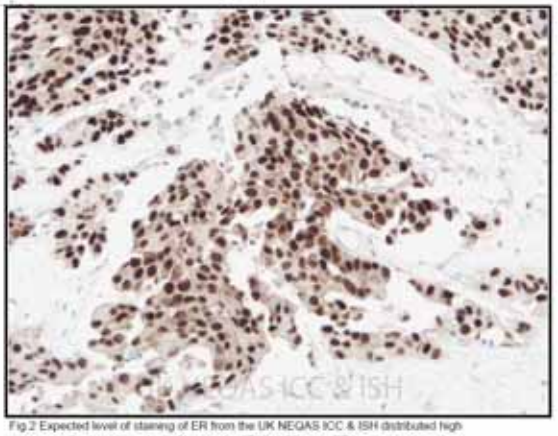


Fig.2 Expected level of staining of ER from the UK NEQAS ICC & ISH distributed high expressor. Section stained with Novocastra 6F11, 1:20 using ER1 antigen retrieval.

ER from Run 90 – 2010 Mid-Expressor

Stained Well

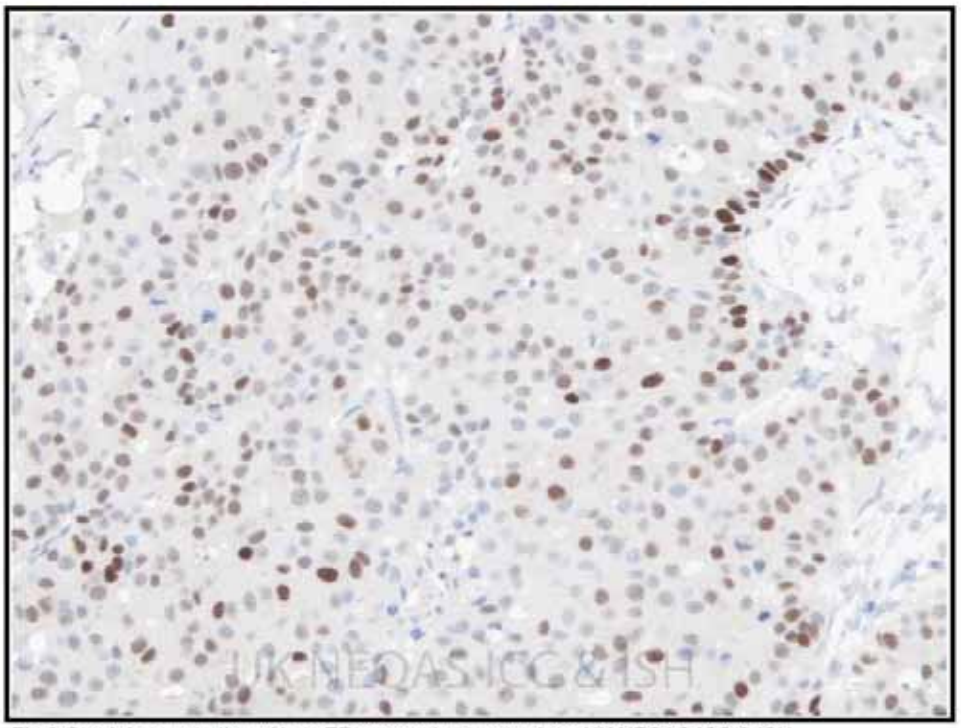


Fig.3 Good demonstration of Low ER-expressing tumour from UK NEQAS ICC & ISH distributed section. Section stained with Novocastra 6F11, 1:20 using ER1 antigen retrieval.

Stained Badly

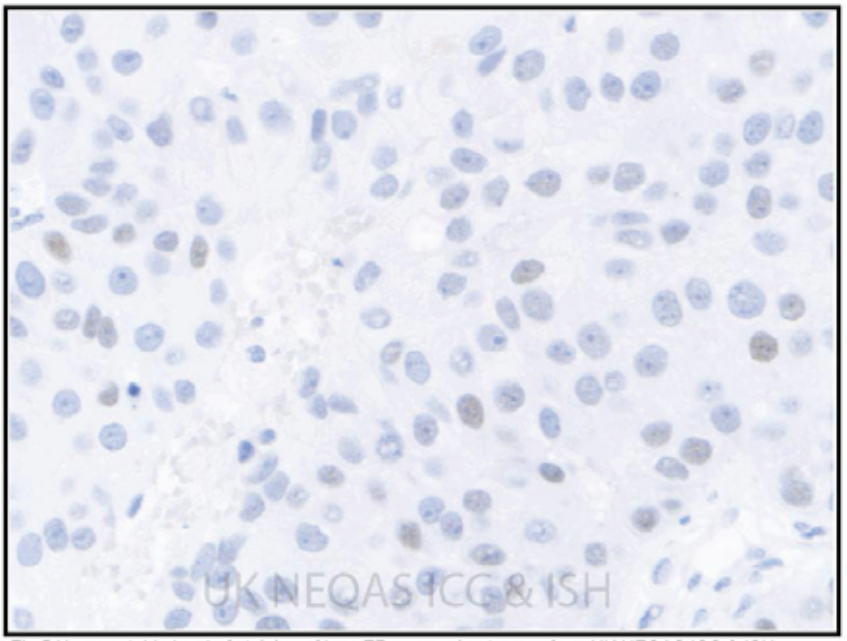


Fig.5 Unacceptable level of staining of Low ER-expressing tumour from UK NEQAS ICC & ISH distributed section. The majority of cells are negative with only a few cells showing a hint of nuclear ER-positivity. Section stained with Vector 6F11, 1:600 using a Dako PT link, with Flex target retrieval solution.

ER from Run 90 – 2010 negative tumour

Stained appropriately

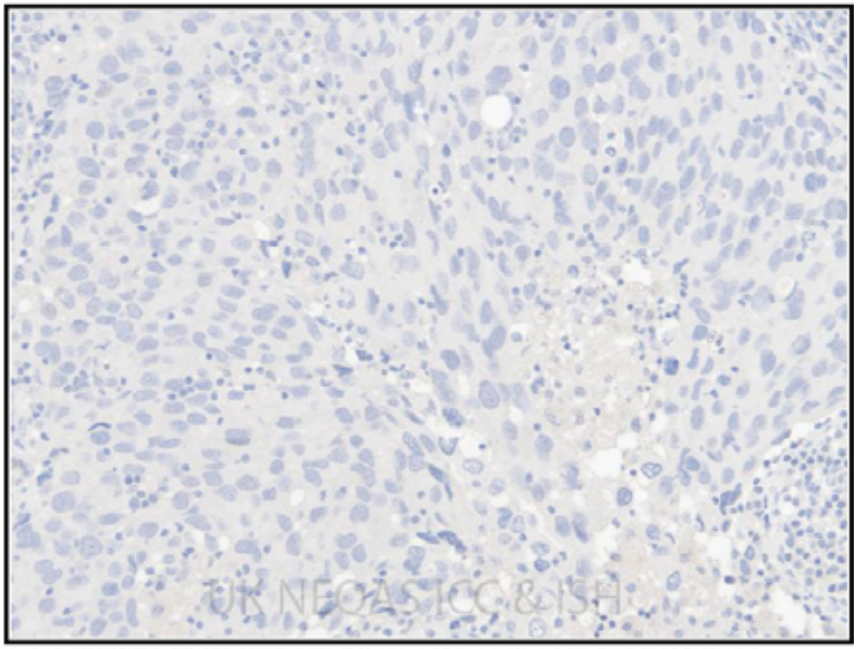


Fig.4 Oestrogen negative tumour from the UK NEQAS ICC & ISH distributed slide. Section stained with Novocastra 6F11, 1:20 using ER1 antigen retrieval.

Stained badly

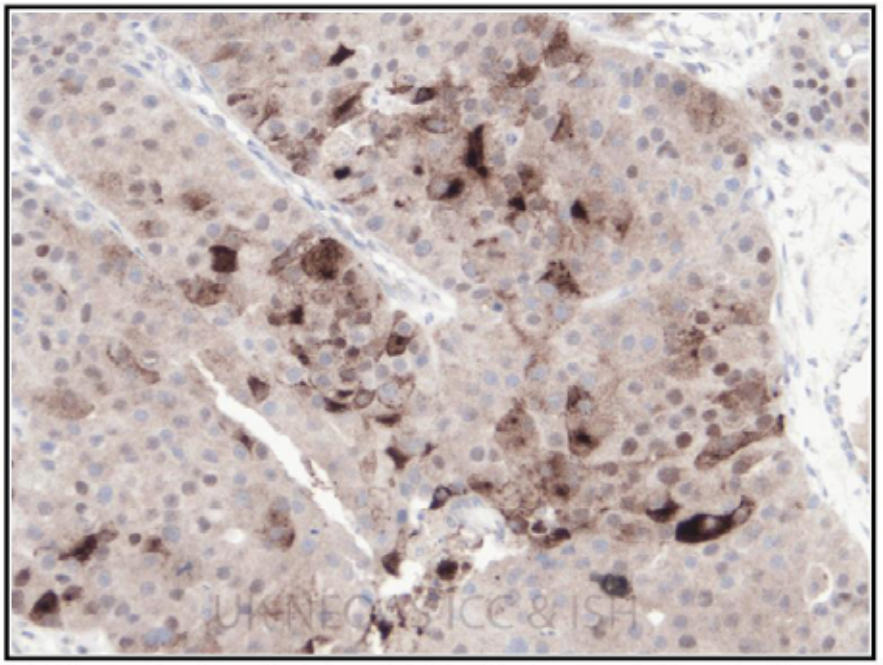


Fig.6 Non-specific and excessive background staining of Low ER-expressing tumour from UK NEQAS ICC & ISH distributed section. Stained using the Dako 1D5 antibody on a Leica Bond Max using ER1 antigen retrieval.

UK NEQAS assessment system

Slides are assessed by 4 independent assessors, each with a laptop computer, using a multihead microscope.

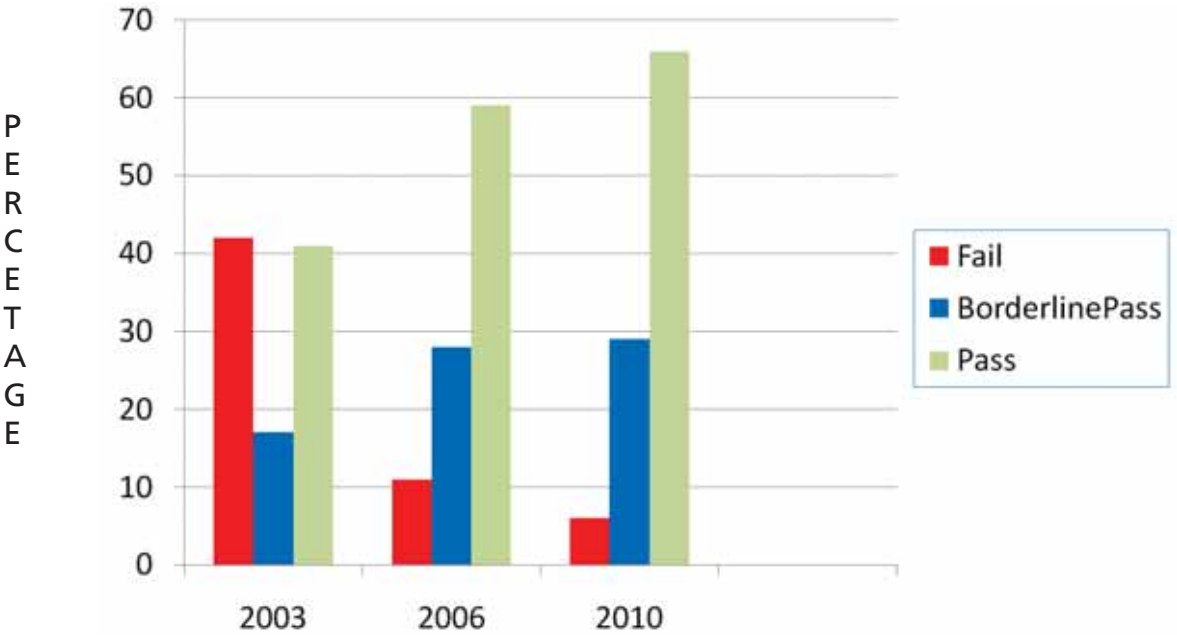
- Scores and comments are entered into the laptops and are totalled.
- **>12/20 is given for immunostaining that ranges from good to excellent.**
- 10-12/20 is a borderline score. This indicates that whilst some improvement is required, the staining still provides a reliable guide to the hormonal status of the cancer in question.
- **<12/20 the staining is poor and a significant improvement is required.**

UK NEQAS

2003	**2013**
• Number of participants 325 • Scoring >12/20 40% • Borderline 10 - 12/20 19%	• Number of Participants 319 • Scoring >12/20 59% • Borderline 10 -12/20 20% • Fail <10/20 score 21%

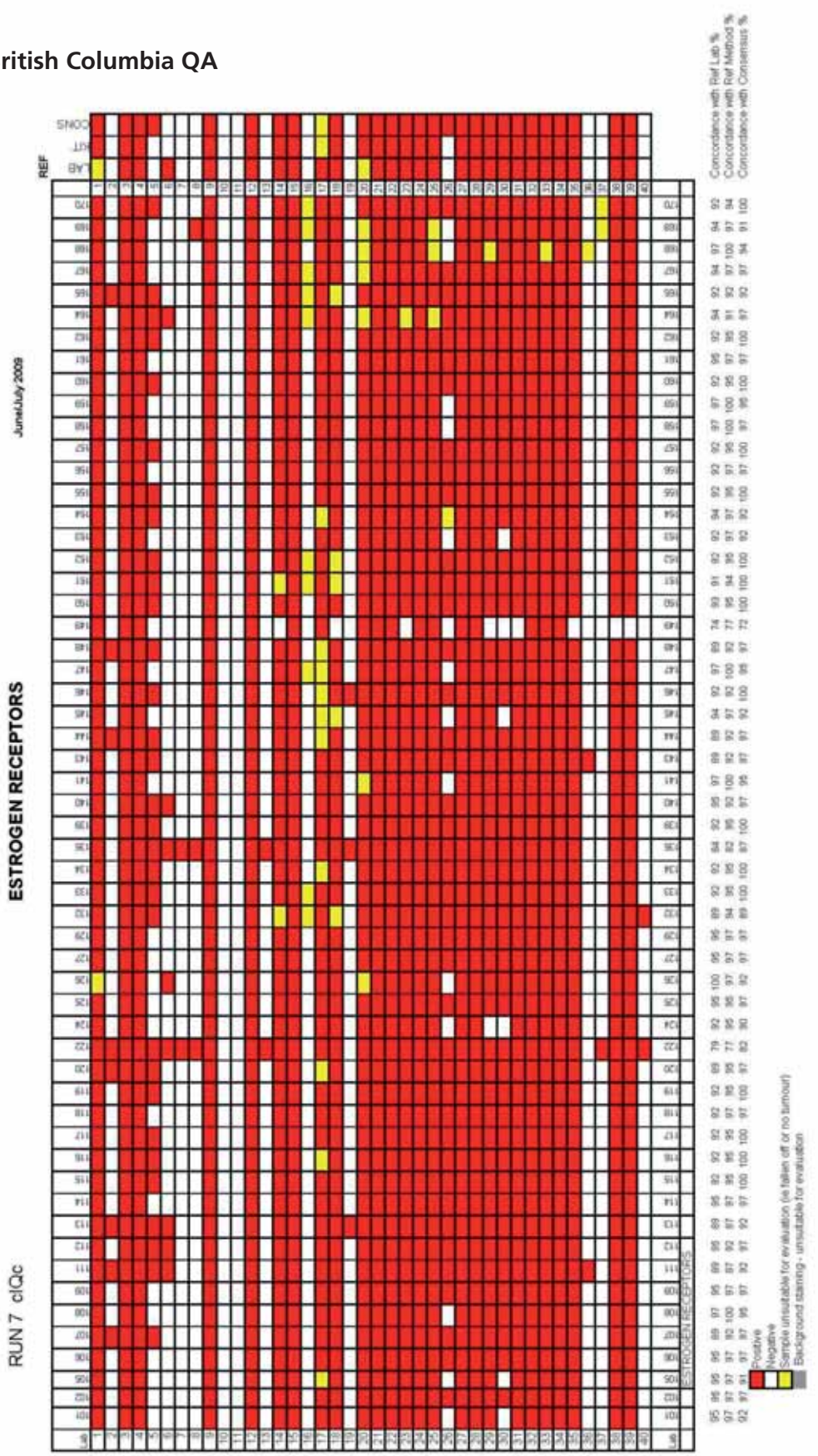
NB. A borderline score is given to suboptimal immunostaining whereby an improvement is desirable. For example, the background staining needs to be reduced. Otherwise the ER stain is clinically acceptable.

Effective Feedback From UK NEQAS Does Improve Quality of IHC ER pass rates 2003-2010

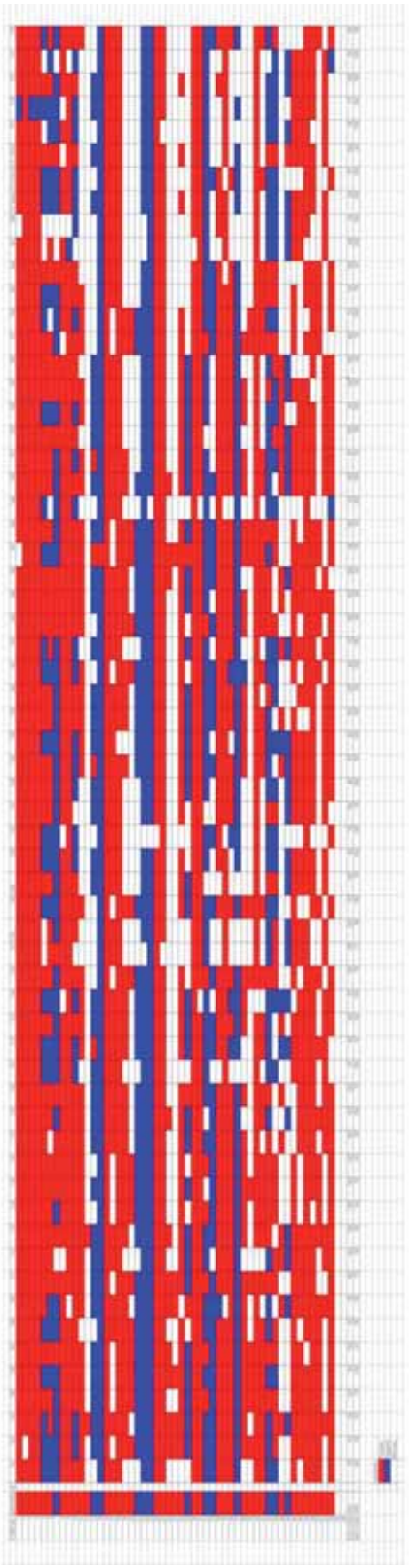


2010 data is for UK labs only

ER British Columbia QA



ER Canadian QA test



INVITED LECTURES

What aspects were covered by ASCO Guidelines

• **Pre-analytic**
- Type of specimen
- Fixation time
- Fixation solution
- Processing methodology

• **Analytic**
- Antigen retrieval
- Antibody selection
- Staining methods
- Validation

• Post-analytic
- Interpretation – Cut-off points
- Controls – internal and external
- Report structure

• Quality Assurance
- Internal
- External

Issues to be addressed
• Ischaemic time must be recorded
- Biopsies
- Resections – time thin slices put in formalin

• Fixation time must be recorded

• Alternative methodology
- Formalin-free fixatives
- Xylene-free processors
- Microwave fixation
- Rapid processors

• Alternative assay methods – RTPCR

• New antibodies

INVITED LECTURES

- New staining amplification methods

- Validation of new testing methods against clinical response

- What is the value of running percentage figures?

- What is an acceptable performance by a laboratory?
  Accreditation requirements?

- What steps are taken when a laboratory fails and who will enforce this?

Validation and Verification

- Verify a new test in the laboratory if you use an approved commercial method and adhere to the accepted protocol.

- Verify a validated test if minor technical change introduced (eg. Ab. dilution)

- Validate a test if you modify an approved commercial test or use an in-house test, fixation, or different processing or testing machine/protocol.

Samples for validation

- ER status of the sample known and validated previously by another method – ligand binding assay – own lab or commercially available.

- Samples from an established proficiency testing QA program – best to get microarrays enriched for low expressors

Verification/Validation procedures and Standards

- Check at least 20 positive and 20 negative cases for verification.

- At least 5 low-ER expressors

- Split between at least 2 runs.

- Double the above required for validation.

- Standards:

- ER+ves 90% concordance
- ER-ves 95% concordance.

• Documentation of validation required.

Benchmarks for ER in USA

• Overall ER-ve rate <30%

• Age 65+ ER-ve rate <20%

• Low-grade breast carcinoma >95% ER-positive rate

• PR +ve rates 10-15% lower than ER

• PR+ve/ER-ve profile 1-3%

• Note: Some population groups are enriched for ER-ve breast cancers.

Pathologist interpretation

• Hormone receptor immunostains should be part of the regular workload of pathologists reporting ER/PR.

• Every reporter should be seeing an adequate volume of cases.

• Performance should be monitored and documented on a regular basis for each reporting pathologist – best done by Allred scoring of microarrays +/- digital images.

Pathologist reporting standards

• 6 monthly testing

• 40 sample test set

• 95% concordance

• Demonstrate adherence to reporting requirements

• Should be documented

Pathologist interpretation

• If ER/PR negative:

- Check external controls - if OK
- Check internal controls - if OK

- Check ischaemic time
- Check fixation time
- Check grade of tumour
- Check tumour type - consider metastasis
    - DCIS, WS keratin, history, etc.
- Recommend repeat testing on excision specimen

BC Cancer Agency
Department of Pathology
600 West 10th Avenue
Vancouver, BC
V5Z 4E6
Tel: 604.877.6000 Fax: 604.877.6178

Biomarkers.

Name:	[redacted]			Accession #:	**T13-1426**
PHN:	9062101792	Agency ID:	**BCC1313099**	Taken:	13/05/2013
DOB:	31/10/1934	Client:	PHSA	Received:	13/05/2013
Gender:	F	Location:	BC Cancer Agency	Reported:	15/05/2013
Ord. Phys.:	PHYSICIAN - NO PRINT	Service:	Anatomic Pathology		
Addl. Phys.:	JOHN ALEXANDER CARR	PHSA ID:	9062101792		
		Copy To:	None Given		

Specimen(s) Received:
NS13-8314 NRGH Breast, NOS

Date Ordered: 13/05/2013
Status: Signed Out

Interpretation
TEST: Breast Biomarkers

Specimen Received: **Hospital:** Nanaimo Regional General Hospital; **Accession #:** NS13-8314; **Block #:** C3.

Specimen Type: **Site:** Breast; **Side:** Left; **Procedure:** Mastectomy;
Outside Diagnosis reported as: Infiltrating carcinoma.

Estrogen Receptor (SP1 antibody): **Positive** (Intensity: Strong, Percent: 100%), Allred Score: 8/8.

Progesterone Receptor (PGR 1294 antibody): **Positive** (Intensity: Strong, Percent: 100%), Allred Score: 8/8.

Her-2/neu Protein (4B5 antibody): **Negative** (Numeric Score 0/3).

For optimal results, the time from tumor removal to fixation (Ischemic Time) should be kept to less than 1 hour, and fixation in 10% formalin (Fixation Time) should be no less than 6 hours and no more than 72 hours.

Ischemic Time: Unknown.
Fixation Time: Unknown.

dk/15/05/2013

Electronically Signed Out
Gregory Naus, MD Pathologist

Other components of Breast marker report often provided

- Grade of the cancer – of use in subset analysis of ER+/-%
- Antibody used – available from laboratory documents on request (only 1 component of the method).
- Note on validation of the laboratory test.
- Note on participation of the laboratory in EQA and proficiency testing program.

What is an "Equivocal" result?

• <1% staining with moderate or strong intensity (Allred scores 3 or 4).

• This population has never been studied.

• Recommendation :
  - Check the staining nuclei are malignant – not entrapped normal cells.
  - Treat as for ER+ve and review the response to endocrine therapy through the BOCU.

Responsibility of the Clinician

•Incongruous results should be questioned
  - ER-negative low-grade cancer
    • Is the ER wrong?
    • Is it not a breast cancer?
  - ER-negative lobular cancer
  - ER+ve metaplastic carcinoma
  - Discrepancy between IHC and gene expression tests.

Biomarkers on Core versus Resection

1. Core tissue is better fixed than resected tumour.
2. Cores are a sample of a larger tumour.
3. There is a small discrepancy between marker results when comparing core with excision.
4. Markers results are available more quickly from the core to allow treatment planning.
5. Locally advanced cancers will be treated prior to excision.
6. All tumours contain different cell clones some differing in marker status.
7. ASCO guidelines for Her2-neu require >30% of tumour cells to be 3+ and >10% to be 2+ but do not differentiate between core and whole section.

Recommendations

• Stain the core biopsy.
• If ER-ve repeat on the excision.
• If core ER+ and excision ER- act on the core result.
• If core ER- and excision ER+ act on the excision result.
• If Her2-neu is discrepant by immuno do FISH. If still discrepant make a decision based on patient's clinical status and risk.

Testing of Multifocal and Multicentric breast cancer Recommendations:

- If practical, breast markers should be performed on all separate invasive foci >10mm in size if Grade 1 or 2 and all foci if nuclear Grade 3.
- If multiple cancers are present and there are nodal metastases, the markers should also be performed on the metastatic cancer.

Testing of recurrent breast cancer

Issues:

1) "Recurrent" breast cancer can represent residual original tumour or a new cancer clone.
2) Different cancer clones may have a different breast marker profile.
3) The marker profile of a breast cancer can evolve over time – most often ER+ turning to ER-ve
4) The relationship between ER+ve status of cancer recurrent after previous endocrine therapy and future response to further endocrine therapy is largely unknown/unstudied.

Testing of Metastatic breast cancer

Issues:

1. The marker status of metastatic breast cancer may differ from the original primary in up to 30% of cases.
2. Metastatic breast cancer is often in inaccessible sites more amenable to sampling by FNA than tissue biopsy.
3. Assay of ER in FNA material is not properly validated and is disallowed by the ASCO guidelines.
4. Metastases in different sites may have different breast marker profiles. How many if any should be tested?
5. Most patients with distant metastatic breast cancer are incurable.

Recommendations

1. Think in a practical clinical way – will the test results really alter your therapy choices and alter prognosis in a meaningful way?
2. Validate assays on FNA material.
3. Consider alternative methodology – RTPCR – which has other issues!.

F 21
Breast cancer case review at the British Columbia Cancer Agency

Dr. Malcolm Hayes
BC Cancer Agency
Vancouver

BCCA breast pathology review process

- From its inception, the BCCA reviewed the pathology slides of all patients with a diagnosis of breast carcinoma.
- SMPBC reviewed all pathology detected by screening until the local hospital was deemed to be "up to standard".
- Later, review was confined to selected cases – particularly node-negative carcinomas and ADH which increased in number due to SMPBC.

Breast pathology review process

- Move towards core biopsy, partial mastectomy, and often, re-excisions - associated with increased number of slides per case (40-100) which made the review process more onerous.
- The current BCCA standard is that node-negative breast cases are reviewed.
- Figures show that many cases are not reviewed.
- Some oncologists are satisfied with their "own" local pathology and want mandatory review to be discontinued.
- This appeal is stated to be "because they do not want to be held legally responsible should one of their patients be reviewed later resulting in a change in the information upon which their original treatment was based"

BREAST REVIEWS

	2007	2008	2009	2010	2011	2012	2013 June
BRNOS	1247	1006	1000	805	683	688	332
BRNNR			329	608	797	806	411
TOTAL	1247	1006	1329	1413	1480	1494	743

What are the advantages of pathology review?

- To check that the pathologist in the institution treating the patient concurs with the pathology assessment made by the outside pathologist when the case is re-examined in the context of the clinical and imaging findings.
- To supply additional information to oncologists that will tailor the therapy to that individual patient.
- To obtain better uniformity of grading, typing of cancer, and other parameters.
- Ensure better of uniformity in information entered into the Breast Outcomes Unit Database for future research on treatment efficacy.
- Provide constant feedback to outside pathologists with regards to diagnosis, grading, and information required.
- Allows pathologists at the BCCA to acquire additional expertise through seeing a large volume of cancer cases. This enables them to act as consultants to assist pathologists in small hospitals with diagnosis of problem cases.
- Promotes better integration of pathology in the multidisciplinary management of patients by the cancer care team (medical oncologists, radiation oncologists, surgical oncologists, radiologists, pathologists, and others).
- Medicolegal buffer should the diagnosis and/or management be questioned in the future.
- Results of pathology review can provide outside hospitals with excellent EQA, performance statistics, and CME material . Our figures have shown that this system has resulted in improved quality of reports.
- The knowledge that a case is liable to be re-examined by an independent reviewer results in more care being taken at the time of primary diagnosis and more intradepartmental consultations about any controversial points

Pathology review (general AP).

Author	Major error	Minor error
Manion 2008	2.3%	9.0%
Tsung 2004	6%	
Abt 1995	6.0%	9.1%
Frable 2006	1.5-5.7%	
Chan 1999	6.5%	12.5% (gyn)
Khalifa 2003	8.3%	19.9% (gyn)
Coblentz 2001	18% (bladder)	
Epstein 1996	1.3% prostate benign	
Kronz 2005	5-7% (head & neck)	
Westra 2002	7% (head & neck)	

Other studies on Breast-specific cancer review discrepancy rate

Staradub VL	Chicago	7.8% major
Price MM, et al	Toronto	4.1%
Tsung JSH	Taiwan	6.3%
Chang JH	Pennsylvania	4% (major)
NewmanEA	Michigan	9% (major)

Staradub, VL et al. Ann Surg Oncol 2002;9:982-987 Chicago Breast Cancer Study

- Studied 340 patients who presented for a second treatment opinion 1997-2001.
- Routine review of the pathology slides.
- 80% change in pathology or prognostic factors.
- **7.8% major changes resulting in altered surgical therapy.**
- 40% change in prognostic factors only
- Changes more common with DCIS than Invasive cancers.

Price JA, et al. Inter-institutional pathology consultations for breast cancer: impact on clinical oncology therapy recommendations.
Current Oncology 2010;17:25-32.

- Retrospective analysis of 100 random breast cancer cases 2004.
- 93 cases were evaluable by review pathologist
- Any discrepancies evaluated by 2 radiation oncologists and 2 medical oncologists to determine clinical impact.
- 10 cases deemed disagreement with high or medium clinical impact.
  - DCIS with microinvasion missed
  - Invasive lobular ca upstage from T1a to T1c
  - **Invasive ductal ca changed from ER+ to ER-**
  - **Invasive ductal ca changed from ER- to ER+**
  - Nodal stage changed from N1mi to N0i+
  - Invasive ductal ca stage change from T1c to T2
  - **Margin status changed from 5mm to 1mm**
  - **Margin status change from 6mm to 1mm**
  - **LVI change from absent to present**

- **Grade change from G2 to G1**
- **Grade change from G2 to G3**
- **LVI not stated to positive**
- **LVI changed from uncertain to positive**
- **Size change from T1b to T1c**
- **Margin change from <1mm to >2mm**

Impact of review:
- Further surgery 2
- Altered hormone therapy 3
- Altered chemotherapy 3
- Altered radiotherapy 3

Vestjens JH, et al. Ann Oncol 2012;23:2561-6. Holland
Studied 2842 pN0, pN0(i+), or pN0mi breast cancer patients

- 1082 pN0 pts – 22% were up-staged
- 623 pN0(i+) pts – 26% upstaged, 1% downstaged
- 1137 pNmi pts – 11% upstaged, 15% downstaged.

Conclusion: Review changed N-classification in 24%

Major Cancer Centres

- Mayo Clinic
- Memorial Sloan Kettering (Rosen PP 2002)
- MD Anderson

All demand mandatory pathology review prior to treatment
Assoc. of Directors of Anatomical Pathology recommend path review of all outside cases.
Gupta 2000: 110/126 US hospitals surveyed encouraged or required pathology review.

Tsung JS. Institutional pathology consultation. Am J Surg Pathol 2004;28:399-402
- "as the Association of Directors of Anatomic and Surgical Pathology recommended, second pathology review should be standard practice"

Kennecke HF et al. J Clin Oncol 2012;30:2227-31. BCCA Node Negative Breast Review Study

- 906 Node-negative cancers were studied
- 405 had pathology review
- 81 had change of data of potential clinical significance (20%).
  - Grade 40%
  - LVI 26%
  - Nodal status 15%
  - Margin status 12%
- 25 patients had treatment changes (6%)
- Only 2 changes were related to tumour biology in patients with ER+ve,N0 breast cancer.

Diagnosis discrepancy in cases sent to the BCCA for breast marker analysis.

- 2186 cases studied.
- 19 discrepant diagnoses (0.9%)
- 8 cases reported as invasive ductal carcinoma changed to in-situ carcinoma or a benign lesion.
  - DCIS
  - Atypical apocrine adenosis
  - Sclerosing papilloma/complex sclerosing lesion
  - Sclerosing adenosis
- 3 invasive ductal ca. cases changed to another malignancy
  - Pleomorphic lobular ca
  - DLBCL
  - Myeloma

Diagnosis discrepancy in cases sent to the BCCA for breast marker analysis.

- 7 DCIS discrepancies
  - 3 changed to invasive carcinoma
  - 4 changed to DIN1A(FEA), ADH, atypical papilloma, benign breast parenchyma.
- 1 case called benign was shown to contain invasive carcinoma.

- **Conclusion**: Centralization of biomarkers provides
  - Accredited standardised staining protocols
  - More consistent interpretation
  - Detects some diagnostic errors pre-treatment.

Conclusions to be drawn

- The BCCA pathologists appear to be no worse and probably better than most elsewhere in the world with regards to quality and accuracy of reporting breast cancers - most likely because of the efficacy of the established BCCA review process.
- However, a significant number of patients have changes effecting treatment.
- **Many of such changes (margin and nodal status)would not be covered by molecular testing procedures.**

Bleiweiss IJ. "Look again: The importance of second opinions in Breast Pathology" JCO 2012;30:2175-6.

- Breast cancer pathology is complex and often yields differences of opinion.
- High volume and pathology labs in small towns precludes breast cases being restricted to specialised breast pathologists.
- Second opinions may become critical for a significant number of patients. (60 per year in BC).
- Molecular tests will not cover many of the significant changes (margins, nodal status, LVI, DCIS vs. Invasive).
- Cost effectiveness is complex and impossible to assess.
- Breast cancer physicians must determine for which patients a second pathologic opinion is needed.

Why stop reviews?

- The pathologists in the community are well trained and are better than those at the BCCA.

  Maybe – BUT …….everyone makes mistakes!
- Disagreements create professional and interpersonal conflict and problems – Sure, but we learn from our mistakes!

Pathology review for breast cancer is a luxury that the Canadian/Belgian public health system cannot afford.

Maybe – BUT …Can we afford another Newfoundland debacle?

Maybe in the future only Lady Prime Minister or Lady Lawyer will have her breast pathology reviewed!

SURE "some pigs are more equal than others" (Napoleon the pig)

Health Economics Questions

- What is the proven cost benefit of an oncologist taking a clinical history of a patient with breast cancer?
- What is the proven cost benefit of an oncologist performing a clinical examination on such a patient?
- What is the cost benefit of performing a review of the imaging studies on such a patient?
- What is the cost of a pathology review?
- What is the cost of sub-standard treatment based on incorrect pathology information?

Pathologist Review

Quality, Assurance, Diagnosis, Treatment, and Patient Care. Patient Safety and Quality Healthcare
March / April 2006 Julia Dahl, MD

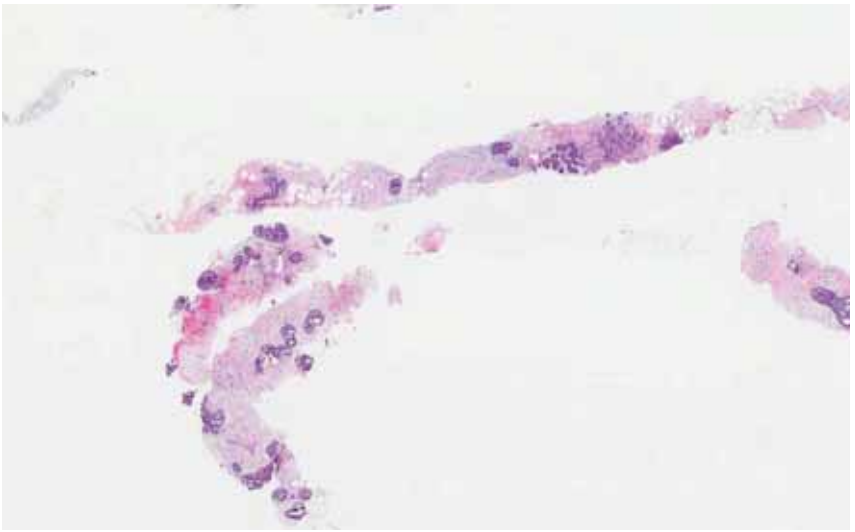
- Consultative review of pathology materials (second opinions) is an essential component of total quality assurance programs in diagnostic surgical pathology and cytopathology. This is likely to be most accurate and cost-effective when a program combines:
  - prospective intradepartmental review of cases
  - retrospective intradepartmental review of diagnoses rendered,
  - selected utilization of inter-institutional second opinions referred to pathologists with subspecialty expertise within specific organ systems and disease categories
  - **mandatory review of pathology materials in which the diagnosis was reported at external institutions when patients are referred for definitive therapy within the «home» institution (Tomaszewski, et al., 2000; Sarewitz, n.d.).**
- While these components of quality assurance programs may appear «self-evident» or universally accepted, self-reporting surveys of academic and community hospitals demonstrate that each of these measures is performed consistently within the pathology departments of only 30% to 65% of institutions (Gupta & Layfield, 2000).

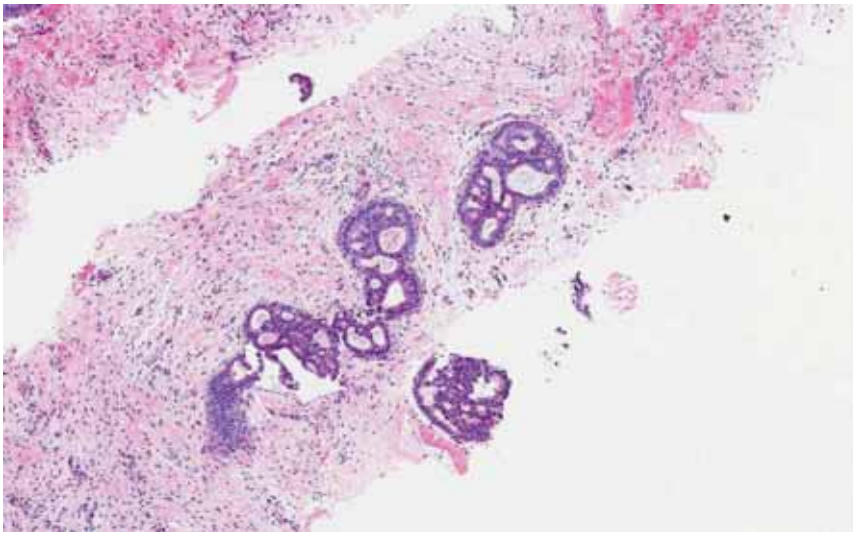
Allen TC. Second Opinions:

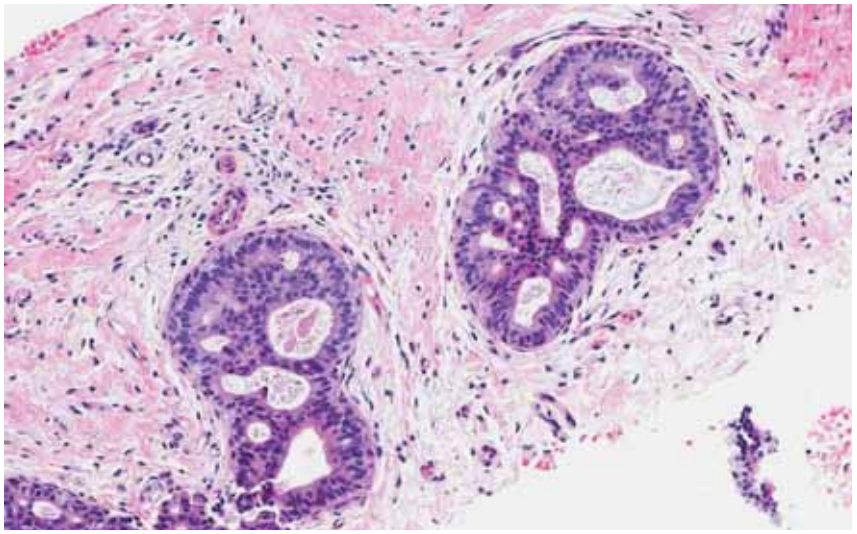
Pathologists' Preventive Medicine. Arch Pathol Lab Med 2013;137:310-11

- "2 decades of studies consistently have shown that second opinions are cost-effective preventative medicine, preventing unnecessary surgeries, chemotherapies, and radiotherapies, as well as the potential litigation costs of misdiagnosis therapeutically acted upon."

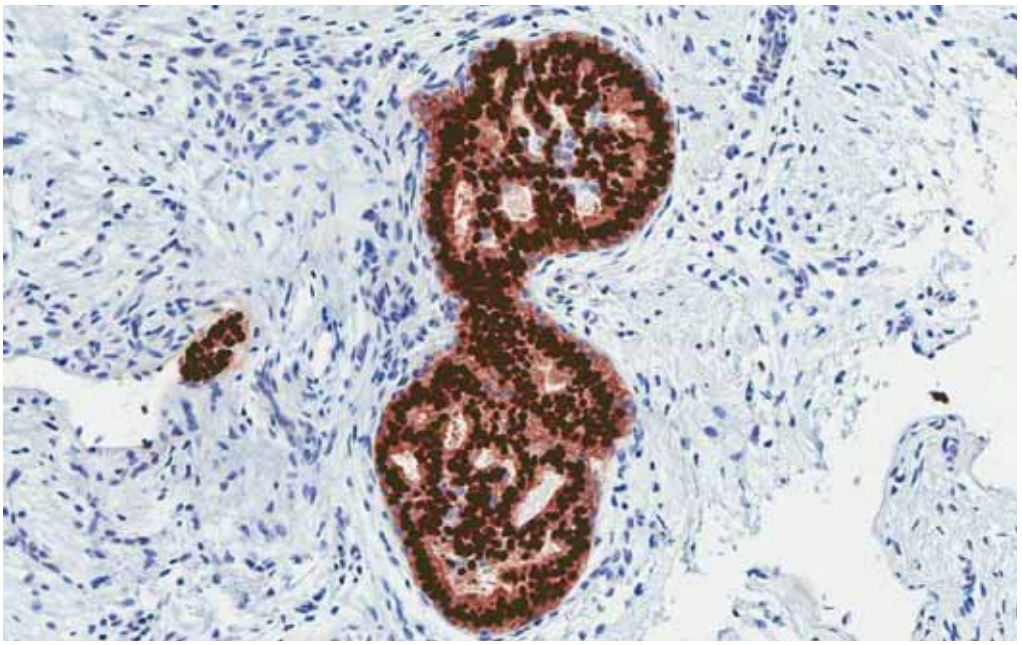
57F Left breast 13mm mass



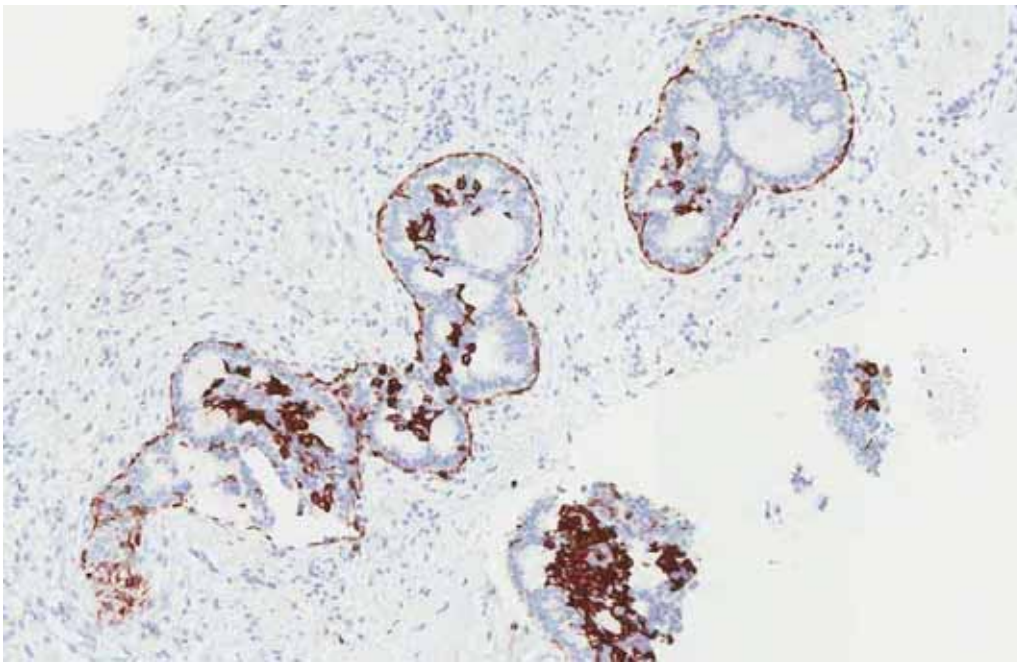




ER



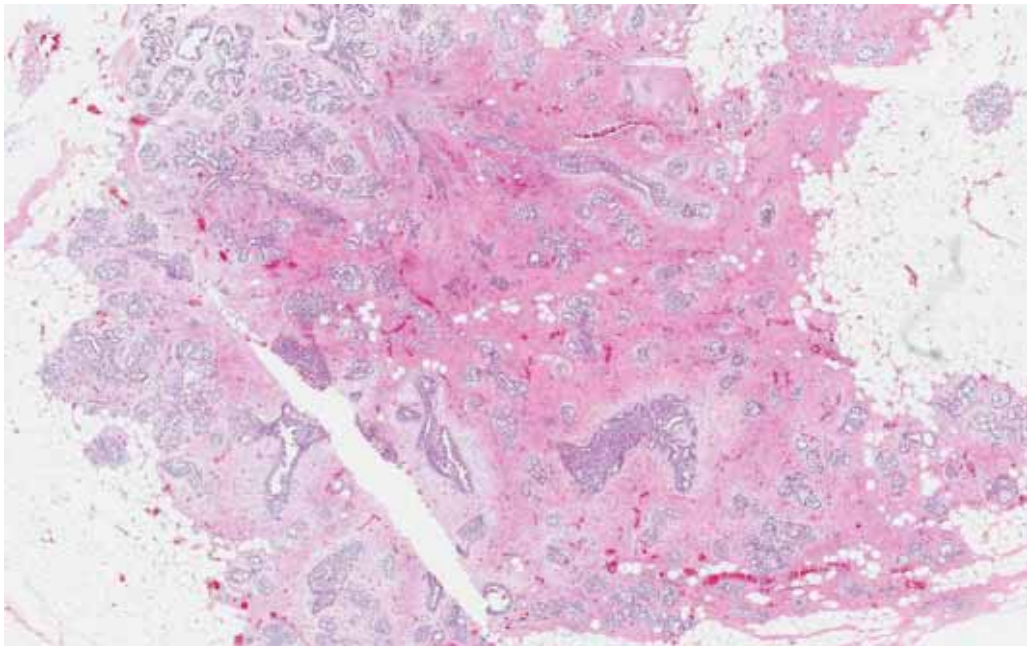
CK5/6

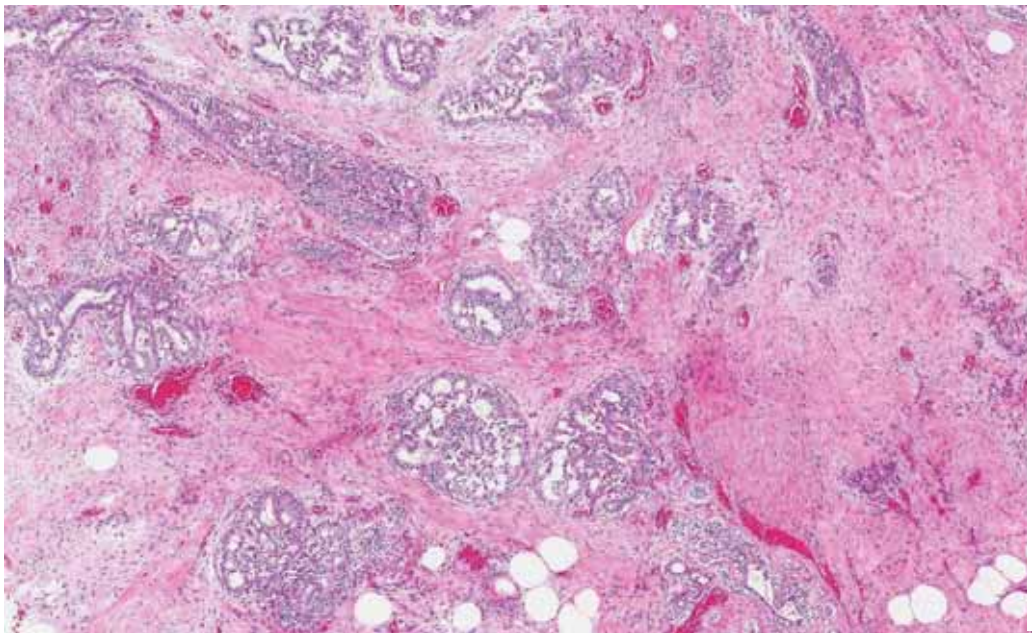


Original Outside diagnosis

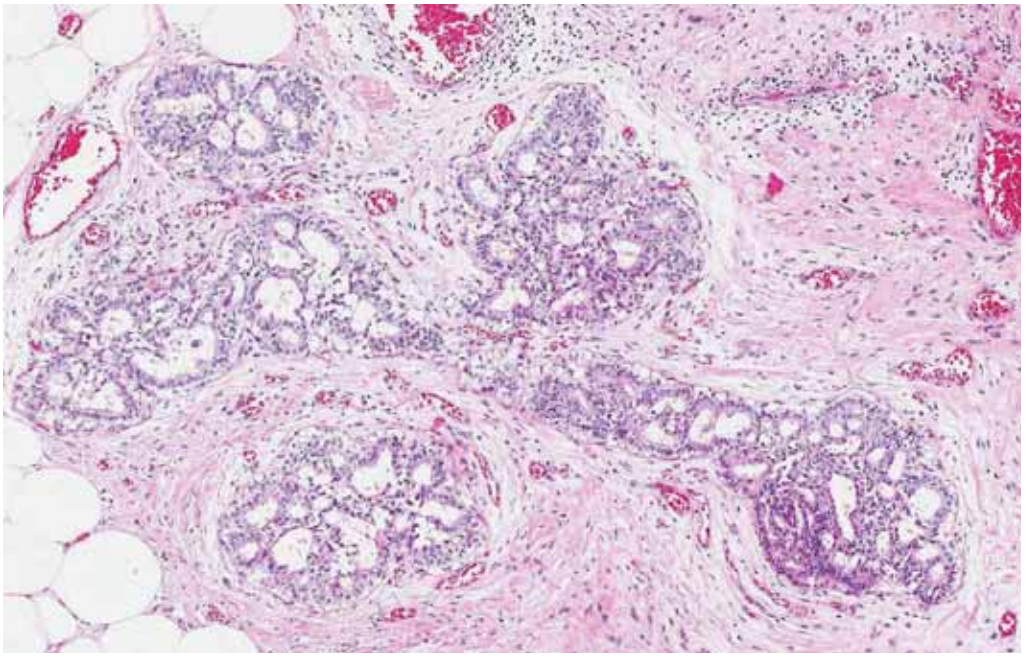
- DCIS intermediate grade
- Patient referred to another hospital
- No second opinion or review of pathology slides performed at hospital B.
- Bilateral total mastectomies and left sided sentinel lymph node biopsies performed.
- Referred to BCCA for radiotherapy opinion.

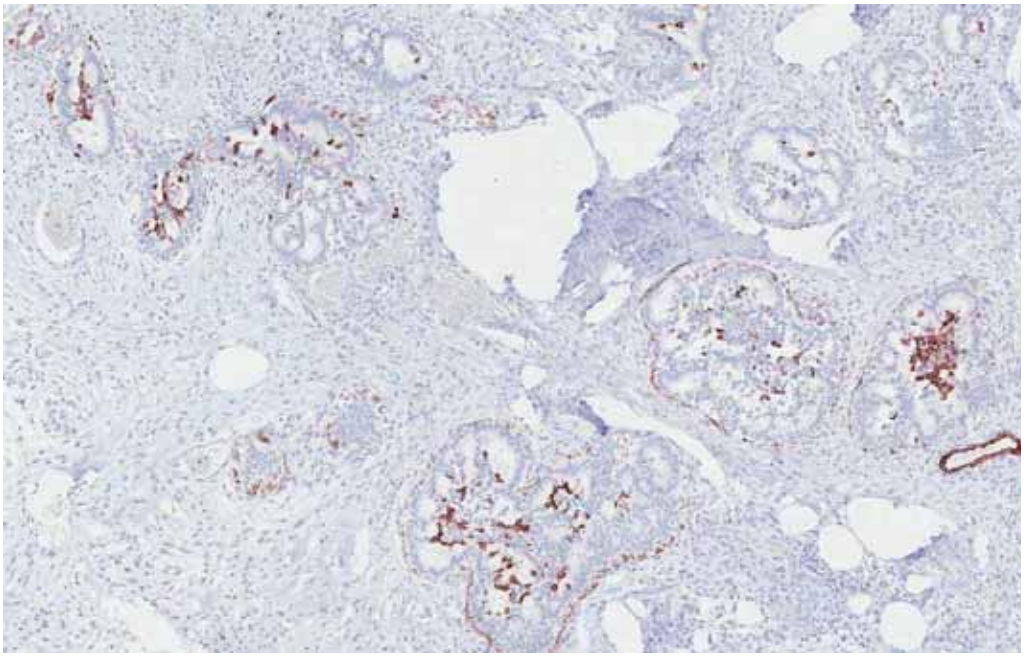
Mastectomy showed 13mm nodule





INVITED LECTURES

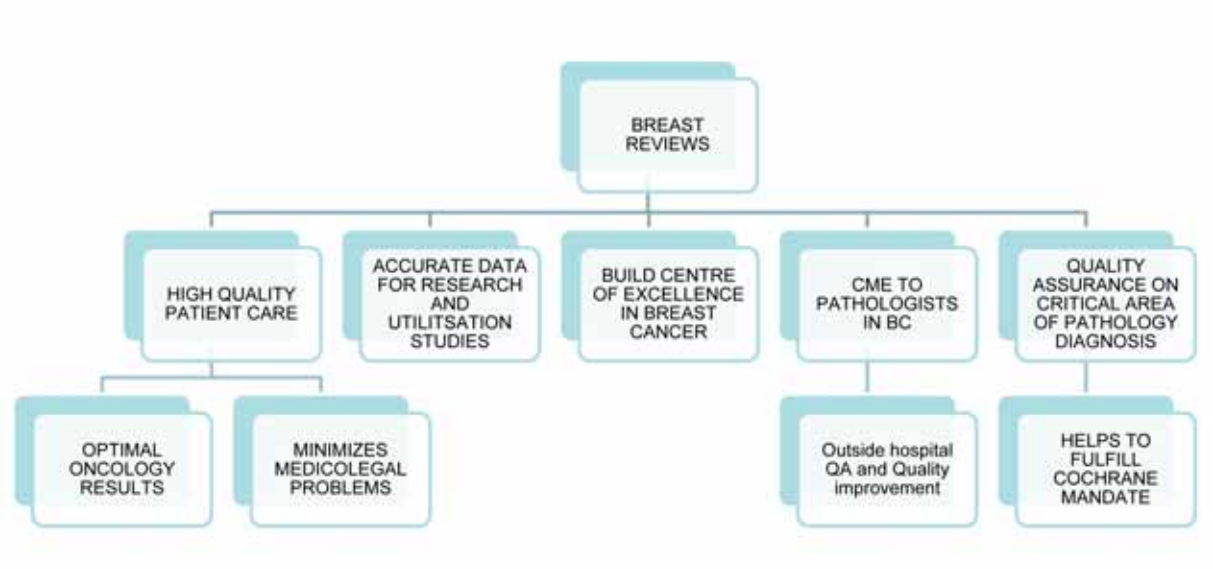




Pathology Review

- BCCA:
  - Negative for malignancy
  - UDH and ADH in gynecomastia-like hyperplasia
- Other expert opinions
  - 2mm Low grade DCIS in fibroadenoma (Yale)
  - ADH in fibroepithelial lesion (Schnitt Boston)
- CONCLUSION: BILATERAL MASTECTOMY AND SENTINEL NODE BIOPSY NOT INDICATED.

Advantages of Routine Breast pathology review



The Gold! – why accept second best ?

Kyra van Graan

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- http://www.youtube.com/watch?v=t1gTbZDmGEM

INVITED LECTURES

Vanden Bussche CL, et al. Borderline atypical ductal hyperplasia/Low-grade Ductal carcinoma in situ on breast needle core biopsy should be managed conservatively. Am J Surg Pathol 2013;37:913-923.

- 74 patients with above diagnosis had excision biopsy.
  - Group A: 38 patients
    - 27% Benign or LIN.
    - 24% ADH
  - Group B: 36 patients
    - 45% DCIS
    - 4% DCIS + Invasive carcinoma

Group A Follow-up: No further surgery or XRT
- 37 No recurrence (All > 3yrs, mean 54 months)
- 1 Recurrence/ residual DIN1B/C

Group B: Further treatment
- 10 Observation alone
- 4 Hormone therapy
- 5 Hormone therapy + XRT
- 9 Mastectomy – 6DCIS – 3 limited to 1 quadrant
- 1 Unknown

- Group B: Further follow-up – All > 3yrs, mean 70 months
  - 1 Pagets after 2 yrs – Mastectomy
  - 1 Invasive ca @ site after 1 yr – Mastectomy
  - 1 DCIS adjacent to site after 1yr – Mastectomy
  - 1 DCIS contralateral breast after 8 yrs
- No cancer deaths.

- **Conclusion**: Mastectomy and XRT can be avoided in most patients with borderline ADH/DCIS on core biopsy.

F 23
HPV. The wolf in sheeps

Dudding N.

HPV. The Wolf in Sheep's Clothing....

Nick Dudding
Sheffield Teaching Hospitals
East Pennine Cytology
Training Centre

www.cytologytraining.co.uk

Nick Dudding

- I am a Consultant Biomedical Scientist at Sheffield Teaching Hospitals. UK
- We process around 100,000 samples per annum
- Used Surepath LBC since 2003
- HPV triage and test of cure since 2009
- Started HPV primary Screening in 1st May 2013

HPV Testing

- Triage of low grade abnormalities
- Test of " cure"
- In USA Co-testing
- **Primary Screening**

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Triage of low grade abnormalities

- Used by all English laboratories
- Very successful....

www.cytologytraining.co.uk

Triage of low grade abnormalities

- In Sheffield has **NOT** improved outcomes
- **BUT** has improved patient pathway
  - Has Speeded up referral
  - Avoided referral for those that don't need it
  - Reduced number of re-tests
  - Returned women to normal recall earlier
- Is cost saving

9/18/2013 EPCTC: L:\East England\BNC Hg - HPV Day

Test of Cure

- Also used by all English laboratories
- Women treated for CIN
- Previously had annual cytology for 10 yrs
- If negative for HPV at 6 months return to normal recall

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Test of Cure

- Improves patient pathway
  - Reduces number of re-tests
  - Returns women to normal recall earlier
- Highly cost saving

However

- PPV for residual disease not good
  - 3.3% for CIN2+

The Real Debate

- The real debate is around the potential role of HPV as the primary screening tool
- This is based on the idea that HPV testing is **"better"** than cytology in this role

HPV testing is "better"

- What do "they" mean – **"Better"?**

HPV testing is "better"

- We often hear this
- Is it more sensitive ?
- Is it more specific
- Or does it save money...?

More sensitive ?

- Most of you would say that testing with HPV is more sensitive than with cytology
- In most countries this will be true
- **BUT**
- What if you provide high quality cytology screening?

ARTISTIC Trial

- A Randomised Trial In Screening To Improve Cytology – ARTISTIC
- Used HC2
- 25,000 women (20 – 65) in Manchester. UK
- Results NOT quite what expected
- ***Lancet Oncol 2009; 10: 672–82***

ARTISTIC

- Results demonstrated that liquid based cytology plus HPV was **NOT** more sensitive over 2 screening rounds than cytology alone
- **Sensitivity of cytology > 90%**

ARTISTIC

- High grade dyskaryosis rates over study period
- Revealed arm (effect of HPV + Cyto)
  - 1.9% - 0.37%
- Concealed arm (effect of LBC only)
  - 1.6% - 0.33%
- **In other words – where you do cytology well it can match the sensitivity of HPV**

Is it More Specific ?

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HPV PPV / Specificity

- **Not even close**
- ARTISTIC trial
- 15% of women positive for high risk HPV types had high grade disease (HC2)
- Sheffield to date – 860 samples HPV positive
- 11% high grade disease (Roche Cobas)

HPV PPV / Specificity

- **In our Triage population in Sheffield**
  - Only 14% of women with ascus / HPV positivity have CIN2+
- **Test of cure Sheffield (with Roche)**
  - 39 women with negative cytology referred to detect 1 case of CIN 2+
  - A PPV of 3.4%

HPV Primary Screening

- Despite this the UK is introducing HPV primary screening
- First laboratories including my own started May 1st
- Why ?

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HPV Primary Screening

- Despite this the UK is introducing HPV primary screening
- Why ?
- It has a better negative predictive value
- In other **words – it is CHEAPER**

ARTISTIC 2

Kitchener et al; Eur J Cancer Feb 2011

- After 3 rounds (6 years) cumulative rate for CIN 2+
- 1.41% following a negative cytology
- 0.87% following a negative HPV test.
- The conclusion was that;

ARTISTIC 2

- " A negative HPV test was significantly more protective than cytology over three screening rounds."
- "Screening intervals could be extended to 6 years if HPV testing replaced cytology as the primary screening test"
- In other words – **it is CHEAPER**

HPV Primary Screening

- Ok. What is my problem ?
- Cost is not the best reason to choose a test
- It might be OK for UK, but the rest of you take care
- Because as well as the PPV & specificity issues HPV is **not as safe** as many of the speakers at this meeting would have you believe.

HPV Primary Screening

- If we look at CIN there are one or two problems, but if we look at cancer.....

HPV Risks ?

- HC2
- UK ARTISTIC Trial - using HC2
- 10.9% of CIN IIs and 4.3% of CIN IIIs in revealed arm were **HPV negative**

(Lancet oncology;10:672-82)

HPV Risks ?

- PCR Methods
- Naucler et al – NEJM Oct 2008
- Rjkaart *et al.* in the POBOSCAM study
  - Only 86% of CIN II + cases HPV positive !
- ATHENA Trial - Cobas 4800
  - Sensitivity of 90% for CIN II+
- **In other words false negative rates for CIN II+ of between 10 and 15%**

HPV Test Results in Women Histological CIN2/3 Dx: Magee

Time from HPV test to histologic diagnosis	Patients Tested	Patients Positive	% HPV+
Immediately before Biopsy	807	786	97%
HPV test prior 3 years to bx (not including above)	454	377	83%

HPV Test Results and Histopathologic **CGN** Diagnoses

Time from HPV test to Histologic Diagnosis	Patients Tested	Patients Positive	% HPV+
Simultaneous HPV and Biopsy testing	72	70	97%
HPV test ≤3 Years Before Bx	29	25	86%

Australian Registry Data
Acta Cytologica 2011; 55: 307-312

Table 1. Percentage of negative HPV tests and the interval by which the HPV tests precede high-grade histology

	HPV tests, n	HPV negative
Concurrent	709	11%
<6 months	745	9%
6–12 months	203	14%
12–24 months	265	22%
24–30 months	80	29%
All	2,002	13%

HPV Risks

- Now this makes me NERVOUS BUT.....
- GETS MUCH WORSE IF WE PONDER CARCINOMA.....

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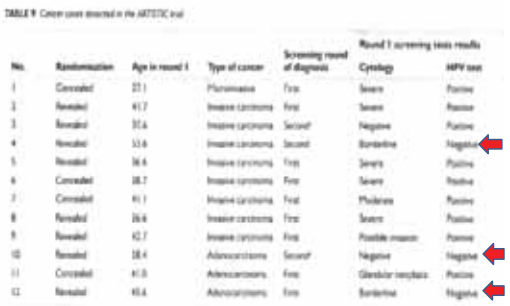
UK ARTISTIC TRIAL

Health Technology Assessment 2009; Vol. 13: No. 51



INVITED LECTURES

Prior HPV Test Results in Patients with Invasive Cervical Cancers

CAP Pre-Survey 2012 (14 hospitals)

Period	Patient#	HPV#	Positive#	Positive Rate
<1y	41	42	38	90.5%
1-<3y	17	21	16	76.2%
3-5y	7	8	6	75.0%
Total	54	71	60	84.5%

Impact of Pap and HPV Co-testing 2003-2005

(Over 300,000 Screened Women 30 and older)
Kaiser Permanente, Northern California
Lancet Oncology 2011; 12: 662-672

	#	CIN3/ AIS	SqCa	AdCa	CaCx
Total at baseline	331,818	817	49	27	87
HPV-	95%	17%	18 (37%)	6 (22%)	27 (31%)
HPV+	5%	83%	31 (63%)	21 (78%)	60 (69%)

The Expanded Use of HPV Testing in Gynecologic Practice per ASCCP-Guided Management Requires the Use of Well-Validated Assays

Mark H. Stoler, MD, Philip E. Castle, PhD, MPH, Diane Solomon, MD, and Mark Schiffman, MD, MPH

Am J Clin Pathol 2007;127:335-337
DOI: 10.1309/MW3C0LAADQCLAP

2. To meet currently achievable standards, the test should have a clinical sensitivity to detect at least 92% ± 3% of CIN 3+ such that the negative predictive value of the test

Does the data I have presented suggest we might even be missing this figure ??

The Big Question ?

- If "virtually all" cervical cancers are caused by persistent high risk HPV infections……
  why do cancer patients have baseline negative high risk HPV results?

HPV Type

- Could it be HPV types not tested for?
  - HC2 only tests for 13 types
  - PCR based tests 14 types
- Unlikely in most cases
- All the IARC group 1 types are tested for

L1 deletions ?

- Possible deletions in the L1 region that the DNA probes are targeted against
- HC2 uses large DNA probes so doesn't really explain the HC2 results.

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HPV infections are episodic

- reactivation of endogenous infection
- low levels of infection fluctuating around the threshold of detection
- inadequate sampling
- new exposure.
- **The fact that viral load varies during the course of an HPV-positive episode has implications for a single point in time test.**

Woodman et al. Lancet. 2001 Jun 9;357(9271):1831-6

Low Viral Loads

- **Prospective study of HPV16 viral load and risk of in situ and invasive squamous cervical cancer**

- Karin Sundstrom, Alexander Ploner, Lisen Arnheim Dahlström, et al
- *Cancer Epidemiol Biomarkers Prev Published OnlineFirst November 15, 2012*

Sundstrom et al

Showed that;
viral loads were unexpectedly low early in the invasive disease process

Sundstrom et al

"Low viral loads early in the SCC disease process may have implications
While the first priority of cervical screening programs is the detection of precancerous lesions, a secondary goal is early detection and down-staging of invasive disease. Our data indicate that less sensitive HPV- testing than our PCR could have failed to detect low-grade HPV infections that still correlated with invasive disease in the long-term perspective"

Sundstrom et al

"highly sensitive thresholds may therefore be of importance in the long term. However any gain in sensitivity should be weighed against a subsequent loss in specificity in the larger population of women – an issue of import in large-scale HPV-based screening"

The Next Debate is Which Test ?

- We also need to give much more thought as to which system we use
  - HCII
  - Cervista
  - Abbott
  - Roche
  - Aptima
- **Most of these kits are approved for use in the UK, but we have only limited information on the clinical performance of these various kits**

INVITED LECTURES

Which Test ?

- Early results from other UK labs suggesting different rates with Roche than with Hybrid Capture
- **Since changing from HC2 to Riche Cobas 4800 in Sheffield**
  - **HPV triage rates have fallen from 76% - 69%**
  - **ToC "fail" rates increased from 12% to 29%**
- **Increased number of women referred to colposcopy do NOT have disease**

Which Test ?

- Latest data from Denmark (Horizon study) also showing significant variations in
  - Positivity
  - Cross reactivity
  - Reproducability

HORIZON DATA – Denmark

Citation: Preisler S, Rebolj M, Untermann A, Ejegod DM, Lynge E, et al. (2013) Prevalence of Human Papillomavirus in 5,072 Consecutive Cervical SurePath Samples Evaluated with the Roche Cobas HPV Real-Time PCR Assay. PLoS ONE 8(3): e59765. doi:10.1371/journal.pone.0059765

- **Roche cobas PCR assay using SurePath samples**
- 26.8% of women tested positive on cobas,
- 20.4% on HC2
- "In Copenhagen cytology was replaced by cobas in women above age 30 years, an extra 11% of women would be expected to have a positive cobas test without an underlying CIN 3 or worse.

HORIZON DATA – Denmark

28th International Papillomavirus Conference – Puerto Rico Presenter: Ditte Ejegod

- Reproducibility
  Samples tested twice in succession but in different batch runs - Surepath medium
- Positive reproducibility
  - HC2 was 99.3%
  - cobas 90.7%
  - APTIMA 84.8%

Conclusion

- **Many countries are looking at replacing cytology with HPV Testing**
- **I am NOT against its introduction**
- **It will be necessary in the vaccinated era**
- **BUT....**

Conclusion

- **Need to be more thoughtful....**
- Will it even prevent more cancers ?
- Does it even meet agreed criteria ?
- Which is the best HPV test ??
- What are the most appropriate algorithms
- Are we using the right collection media ?

INVITED LECTURES

Conclusion

- **CIN2+ data has been used to distract everyone's attention from the really significant subset- women developing cervical cancer**
- **We need to be careful we do not over sell it !**

Conclusion


Smear test to end all doubts

Conclusion

- **If we do where will we be medico legally ??**
- Will plaintiff's attorneys start watching for the missed cervical cancer patients so that they can then sue the program organizers for ignoring data that HPV tests being relied on were known in advance not to perform at extended screening intervals up to international standards!

Thank You for Your Attention

Any Questions ?

My email address is:
nick.dudding@sth.nhs.uk

F 25
HPV negative HSIL

Shaira Sahebali

Background & set-up

Most screening guidelines for cervical cancer now include some form of HPV testing. A high-grade lesion is supposed to contain HR-HPV.

In our laboratory cervical cytology screening is performed on LBC samples. All cytology is robotically processed and automatically prescreened according to a specific software algorithm. All samples are tested with MY09/11 consensus PCR and typed with type specific quantitative real-time PCR for HR-HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68. Viral load is determined as well. All abnormal cytology is seen by at least one cytotechnician and one cytopathologist. All ASC-H/ HSIL cases which are HPV negative are reviewed by another cytotechnician as well as another cytopathologist. HPV PCR results are reviewed and retested as well. Still, in many of these cases the diagnosis remains unchanged. This raises the important issue of which advice to give to the gynecologist on further management of such patients. At this time there are no clear guidelines.

From a time interval of 1st of January 2007 up to 31st of December 2009 a study-set of 90 slides was compiled, consisting of 70 cytological samples diagnosed either ASC-H or HSIL, HPV negative. Both cytological diagnosis and PCR results have been reviewed as part of QC as descibed above. The set has been enriched with 10 double negative cases and 10 HSIL/HPV+ cases. This study-set was submitted to external review by an international panel of experts (dr Longatto, Brazil; dr Cibas, USA; dr Tampouret; USA). Each expert diagnosed the cases in the set independently and blinded to both the first diagnosis as well as the diagnoses given by his/her fellows.

The primary goals are to:

- Identify mistakes and pitfalls in cases where the diagnosis is not upheld.
- Identify ways to avoid these mistakes in the future.
- Formulate an advice on how to manage those patients in which the diagnosis is upheld.

Cytology & histology

For cytology diagnoses Bethesda terminology was used. Other terms refer to histology.

Biopsy results were extracted from our follow up database, all in-house results were reviewed. We had 9 in-house confirmations of CIN2+ and 11 other histological confirmations made elsewhere after referral.

Out of 70 cases 1 ASC-H was upheld bij all panellists. 9 cases of HSIL were unanimously upheld. All these cases were confirmed to be high-grade lesions on biopsy. Another 20 cases were not unanimously upheld (4 out of 5), but many did show a high-grade lesion on biopsy. One of these was a case, which was called unsatisfactory by 1 panellist.

Unfortunately we did not have follow up of 9 cases.

In total there are 38 cases in which we have at least 1 strong indicator that a high-grade diagnosis is not overcalling but may indicate the possibility of a false negative HPV test.

In non-confirmed cases there are a few LSILs which seem to have been overcalled and in 2 cases an endocervical polyp was removed afterwards.

HPV re-testing

HPV testing and typing was performed on all cases. We collaborated with dr. Markus Schmitt from the German Cancer Research Center (DKFZ) in Heidelberg. He worked with a different PCR primer system (GP5+/6+) and had a lot of primers for more exotic HPV types.

For 2 cases there was not enough DNA left for re-testing. 14 cases were HPV negative. Of these cases 4 were histology confirmed CIN2+ and 1 CIN1. All other cases contain 1 or more HPV types. A few of these cases contained 1 of the 14 HR-HPV and were missed in our lab. However, there are also a lot of the more exotic types, which are often not tested for. So these cases would almost always turn out false-negative.

It is important to remember that HPV testing is a major support for screening, but good morphology still is vital for the end result!

F 26
HSIL as red herring.

Folkert J van Kemenade

Screening-, case finding-, diagnostic- and test of cure cytomorpology can all pose problems to microscopists. Differences in brushes for obtaining samples, differences in sample preparation, timing of the cervical smear as well as influences of chemotherapeutics, radiation therapy of invasive tumorgrowth from adjacent areas. To complicate matters, the cycle change of hormonal influence, the gradual disappearance of estrogen drive, atrophic or inflammatory states, presence intrauterine devices, unusual neoplasms and metastases can all complicate recognition of non-neoplastic boundaries, or insinuate presence of neoplastic cells of the uterine cervix. Supply of clinical information can vary notoriously.
All these situations can lead the microscopist astray and lead to overdiagnosis of a putative leasion or an erroneous diagnosis with respect to the location or origing of the leasion. In a short overview, an array of these dialy and not so daily difficult lesions will be shown.

ORAL PRESENTATIONS

ORAL PRESENTATIONS

ORAL PRESENTATIONS

O 12	*Monika Lamba Saini, Birgit Weynand , Jacques Rahier, Michel Mourad, Marc Hamoir, Etienne Marbaix*	Identification of putative precursor lesion of papillary thyroid carcinoma by cyclin D1 overexpression and p38 MAPK phosphorylation.	118
O 13	*Maris C., Hastir D., Salmon I*	Immune reconstitution inflammatory syndrome in progressive multifocal leukoencephalopathy after highly active antiretroviral therapy	120
O 14	*Narasimhaiah D., Amyere M., Janin N., Raftopoulos C., Vikkula M. & Godfraind C.*	Familial presentation of a Granular cell astrocytoma and an Oligodendroglioma	121
O 15	*Anne-Laure Trépant, Christelle Bouchart, Audrey Verrellen, Charles Nicaise, Sébastien Sauvage, Sandrine Rorive, Christine Decaestecker, Isabelle Salmon*	Are there specific biomarkers of glioma stem cells?	123
O 16	*Namanya Vian K. (PI), Opio Ronald1, Niwasasira Dickson, Onyege Alex1, Naisanga Molly*	Knowledge, attitudes and acceptability of human papilloma vaccination amongst primary school girls (9 years and above) in minakulu sub-county oyam district	125

O 01
A rare cause of ACTH-independent Cushing syndrome

D. Hastir[(1)], C. Maris[(1)], B. Corvilain[(2)], V. Lucidi[(3)], P. Demetter[(1)], I. Salmon[(1)]/
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A 25-year-old woman with one year history of adrenocorticotropic hormone (ACTH) independent Cushing syndrome was admitted to our hospital for complete endocrinological work-out. Computed tomography scan and nuclear magnetic resonance showed normal-sized adrenal glands without any nodules. Iodocholesterol scan revealed incorporation in both glands. Undetectable plasma ACTH confirmed ACTH independent Cushing syndrome.

Since primary pigmented nodular adrenocortical disease (PPNAD), whether or not associated to Carney complex, was suspected, cardiac echography, dermatological clinical examination and genetic testing were performed. Cardiac echography did not show any abnormalities. Dermatologists detected lentiginous lesions and a blue naevus. Genetic analysis revealed a mutation in the *PRKAR1a* gene.

The patient underwent left laparoscopic adrenalectomy and histology revealed adrenal hyperplasia with small pigmented cortical nodules confirming the diagnosis of PPNAD with incomplete Carney complex.

Carney complex was first described in 1985 as the association of myxoma, spotty pigmentation, and endocrine overactivity. It is an autosomal dominantly inherited multisystem tumour syndrome in which the tumours are multicentric (heart and skin) in affected organs and bilateral in paired organs (adrenal, breast, and testis). The most frequent endocrine manifestation of Carney complex is adrenocorticotropic hormone independent Cushing syndrome caused by PPNAD.

At the histological level, PPNAD is characterised by pigmented adrenocortical nodules ranging in size from sub-microscopic to 10mm in diameter.

The cortical nodules are unencapsulated and appear black brown containing large, globular cells with pigmented-laden eosinophilic cytoplasm, whereas the inter-nodular cortex is usually normal or atrophic.

PPNAD is the result of genetic processes associated with defects of the cAMP signaling pathway. PPNAD tissue expresses consistently high levels of phosphodiesterase 11A (PDE11A); this finding has led to the identification of PDE11A mutations as a low-penetrance predisposing factor to PPNAD. Both *PRKAR1A* and *PDE11A* gene products control the cAMP signaling pathway, which can be altered at various levels in endocrine tumours.

Although in some PPNAD cases unilateral adrenalectomy resulted in remission of hypercortisolism, bilateral adrenalectomy is considered treatment of choice for Cushing syndrome due to confirmed PPNAD. In our case, right adrenalectomy will be performed if follow-up reveals increased cortisol secretion.

O 02
Immune infiltration in Choroidal Melanomas.

Narasimhaiah D.[1, 2], Remark R. [3], Becht E.[3], Damotte D.[3], Legrand C.[4], De Potter P.[5], Coulie P.G.[6], Vikkula M.[1], Godfraind C.[2]/

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Introduction

Choroidal melanomas are the most common primary, intra-ocular malignant tumors in adults. In these tumors, the presence of CD8+ T-lymphocytes is associated with bad prognosis. In contrast, in most other solid tumors, the CD8+ T-cell infiltrate is related to good prognosis. The objective of this study was to better understand the immune infiltration in choroidal melanomas.

Materials and Methods

The primary untreated choroidal melanomas were analyzed for gene expression using Affymetrix U133 plus 2.0 array (n=15) and by immunohistochemistry (n=86).

Results

Gene expression profile analysis led to the identification of a gene signature consisting of 34 upregulated genes in a subset of choroidal melanomas. These genes were associated with: antigen processing and presentation, cell adhesion molecules, interferon-gamma and chemokine signaling pathways. On immunohistochemistry, signature positive tumors displayed a dense intra-tumoral infiltrate of HLA-DRA+CD163+ macrophages and CD3+CD8+ T-cells. It was mild to moderate in tumors lacking the signature.

On this basis, additional tumors were analyzed by immunohistochemistry. In total, 19 melanomas had high immune infiltrate and 67 low. Kaplan-Meier plots demonstrated that tumors with high immune infiltrate had shorter disease-free survival (log-rank $p < 0.001$).

Conclusions

We identified in choroidal melanomas an interferon-gamma induced gene signature associated with infiltration of macrophages and CD8+ T-lymphocytes. The presence of tumor-infiltrating immune cells correlated with higher risk of occurrence of metastases.

O 04

Endothelial VEGFR1 expression is associated with metastasis-free survival in colorectal cancer.

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Introduction

Research on tumour angiogenesis has mainly focused on vascular endothelial growth factor (VEGF) family and methods to block its actions. The fact that VEGFR1 could promote angiogenesis in pathological conditions makes it an attractive potential target. However, reports on the VEGFR1 expression in tumor-associated endothelial cells (EC) are limited. We decided to investigate the prognostic value of VEGFR1 expression in EC of colorectal cancer (CRC).

Material and Methods:

Immunohistochemistry for VEGFR1 was performed on a retrospective series of 269 patients diagnosed with CRC without occurrence of metastatic processes at the time of surgery. EC VEGFR1 expression was submitted to quantitative (computer-assisted microscopy) evaluation, and results were related to clinical variables.

Results:

The data show that in normal tissue, expression of VEGFR1 was low. A great heterogeneity was observed for CRC samples. There was no association between EC VEGFR1 expression and overall survival. However, for pT1-4N0-2M0-staged CRC, we identified female gender ($p=0.03$), N Stage ($p<10-6$) and higher EC VEGFR1 expression ($p=0.03$) being independent negative prognostic factors in terms of metachronous metastasis. Moreover in the subgroup of N2-staged CRC (n=31), EC VEGFR1 expression was significantly associated with the development of metachronous metastases ($p=0.02$), although gender was no longer an independent prognostic factor.

Conclusion:
This work illustrates the importance of studying target distribution. Interestingly, higher EC VEGFR1 expression appears as being involved in CRC progression, suggesting that targeting EC VEGFR1 could offer novel opportunities for CRC treatment.

O 05

Liver infiltration by a NK/T cell lymphoma nasal-type mimicking fulminant hepatitis: a case report.

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Introduction

We report an unusual case of liver infiltration by an extranodal NK/T cell lymphoma, nasal-type, in a Caucasian man.

Case report :

A 64-year-old man, with past history of hypertension and gout, had an extensive and persistent skin rash, located on the trunk, back, and the base of the lower limbs, present since two and a half months. The patient's general condition deteriorated sharply with significant fatigue, anorexia, apathy and weight loss. Next, appeared a conjunctivitis, a clear runny nose and a dry cough. He was admitted to the emergencies for acute renal failure. Rapidly, he developed liver failure, in a context of fatty liver. US and computed tomography showed steatosis and homogeneous hepatomegaly with a adrenal nodular infiltration. Laboratory analyses confirmed severe alterations in renal parameters and hepatic enzymes. Moreover, they also showed hyperbilirubinemia and macrophagic activation syndrome. Serology (viral and autoimmune) was negative. A liver biopsy was performed (described below). Despite the transfer to intensive care, the patient died two days later in a severe and refractory circulatory shock, multisystem organ failure, DIC and ARDS. Autopsy findings showed massive lymphoid infiltration of the thyroid, heart, liver and adrenals.

Results:

A liver biopsy was performed, showing a massive infiltration of portal spaces, sinusoids and lobules by pleiomorphic atypical lymphoid cells. The cells were of intermediate to large size with frequent prominent nucleoli. Macrovacuolar steatosis, sinusoidal congestion and Councilman bodies were also noted. The tumor cells expressed CD3, CD2, CD8, cytotoxic

granules (TiA1, perforin, granzyme B) and CD56. CD5, CD4, betaF1, TCR-gamma (GM1), CD20, TFH markers, CD30 and ALK were negative. In situ hybridization EBER detected the massive presence of EBV RNA in tumor cells. A monoclonal rearrangement was observed by PCR TCRgamma.

Conclusion:
Infiltration of the liver by T cell proliferations is rare and among them, hepatosplenic T cell lymphoma, secondary T-cell lymphoma NOS and angioimmunoblastic T-cell lymphoma are the most frequent. Our case shows that extranodal NK/T-cell lymphoma nasal type must be considered in this differential diagnosis, even in Caucasians, and that this entity may have a clinical presentation of fulminant hepatitis.

O 06

ADAM-17/FHL2 colocalisation suggests interaction and role of these proteins in colorectal cancer.

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Introduction

ADAM-17, a disintegrin and metalloprotease, is responsible for the ectodomain shedding of transmembrane proteins, including Epidermal Growth Factor Receptor (EGFR) ligands.

Four-and-a-half LIM domains protein 2 (FHL2), a molecule involved in multiple protein-protein interactions, interacts with the cytoplasmic tail of ADAM-17 and regulates its localisation and activity. Previously, we demonstrated that high expression of FHL2 in colorectal cancer is associated with poor prognosis. The present study aims to compare ADAM-17/FHL2 colocalisation in colorectal cancer cells and myofibroblasts versus normal colon epithelium.

Materials and methods:

ADAM-17 and FHL2 expression were studied in several colon cancer cell lines (HT29, HCT8/E11, Caco-2, SW480, SW620, Colo320DM) and in an immortalised myofibroblast cell line (CT5.3-hTERT) by immunocytochemistry. To highlight a possible colocalisation of ADAM-17 and FHL2, we used the Duolink® kit for a proximity ligation assay (PLA), performed on SW480, Colo320DM and CT5.3-hTERT cells.

The PLA was also performed on biopsy specimens of 10 colorectal adenocarcinomas as well as on normal colonic mucosa from the same patients. To quantify ADAM-17/FHL2 colocalisation, numbers of PLA signals were counted. Comparison between matched PLA signal numbers was performed using the non-parametric Wilcoxon signed-rank test.

Results:
ADAM-17 was detected by immunocytochemistry in all cell lines except in Colo320DM. All cell lines exhibited FHL2 positivity. The PLA revealed ADAM-17/FHL2 colocalisation in SW480 and in CT5.3-hTERT, but not in Colo320DM cells.
PLA signals were 2 to 28-fold more frequent in neoplastic colorectal epithelium than in matched control epithelium ($p<0.01$).

Conclusions:
ADAM-17 colocalises with FHL2 in neoplastic epithelium in colorectal cancer. This colocalisation is more obvious in cancerous than in matched control tissue, suggesting a role for ADAM-17/FHL2 mediated ectodomain shedding in colorectal cancer.

O 07

Acquired cystic kidney disease-associated renal cell carcinoma revealed by spontaneous retroperitoneal haemorrhage.

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Patients with end stage renal disease, and acquired cystic kidney disease (ACKD) in particular, are at greater risk of developing renal cell carcinoma compared to the general population. We describe two cases of acquired cystic kidney disease-associated renal cell carcinoma (ACKD-RCC), a histological subtype of renal cell carcinoma occurring exclusively in patients with ACKD. In both cases, the tumours occurred in male patients and were discovered after spontaneous retroperitoneal haemorrhage. Histologically, ACKD-RCCs are characterised by variable architectural patterns, cribriform or microcystic patterns being the most common. ACKD-RCCs are composed of large cells with abundant, eosinophilic cytoplasm, marked nuclear pleomorphism and prominent nucleoli. They show strong and diffuse positivity for CD10 and alpha-methylacyl-CoA-racemase and only focal staining for cytokeratin 7. Due to their recent discovery and infrequency, the natural course of these tumours, as well as the patients prognosis, still remains uncertain.

O 08

Stromal features are potential prognostic markers for ipsilateral locoregional recurrence in DCIS.

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Aims:

Ductal carcinoma in situ (DCIS) is regarded to be a non-obligate pre-invasive precursor of invasive ductal carcinoma. The incidence of DCIS has considerably increased since the widespread introduction of screening mammography. Up to one in three patients will develop local recurrence after breast-conserving surgery (BCS) alone, and approximately half of these recurrences will be invasive. Recurrence prediction is still not completely accurate and could be improved by identifying additional prognostic markers. Periductal stroma actively participates in early breast cancer progression. Therefore we explored the prognostic potential of stromal characteristics in DCIS.

Materials and methods:

Histopathological features and hormone receptor/HER2 protein status were analyzed in a first cohort of 65 DCIS with a median follow-up of 112 months (range 6-230). Patients were treated with either BCS or mastectomy. Cox regression analysis with correction for surgical treatment was used to determine hazard ratios for all variables in relation to time to recurrence. Next, immunohistochemical staining of nine stromal proteins was

performed in a second cohort of 82 DCIS without available follow-up data, and correlated with stromal architecture. Decorin protein was selected and further analyzed in the first cohort with available follow-up data.

Results:
Cox regression analysis revealed that myxoid stromal architecture was significantly associated with increased ipsilateral locoregional recurrence ($p = 0.015$) in cohort I, independent of surgical treatment. Therefore, stromal architecture was applied as a surrogate outcome marker in cohort II. Out of an immunohistochemical screening of nine stromal proteins, reduced stromal decorin expression correlated most strongly with myxoid stroma ($p < 0.001$). This association was confirmed in cohort I ($p < 0.001$), and patients with reduced periductal decorin expression had a significantly higher risk of ipsilateral locoregional recurrence ($p = 0.008$).

Conclusions:
Periductal myxoid stroma and reduced periductal decorin expression seem to be prognostic for ipsilateral locoregional recurrence in DCIS. Additional investigations should validate the prognostic power of these promising biomarkers, and elucidate the underlying pathogenetic mechanisms in early breast cancer biology.

O 09
Cytokine synthesis in spleen cells of mice with breast tumours interaction with physical activity.

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Background and Aims:
Different aspects of health sciences are gaining interest into cancer research to improve treatment and quality of life of cancer patients. Understanding the interaction between the immune system and physical activity is one aspect. This study aims to investigate the synthesis of cytokines by spleen cells in the animals with presence of breast tumours and the interaction with physical activity.

Methodology:
Female Balb/c virgin mice (8 weeks old) were used, divided into four groups: a non- tumour/non-trained control group; a non-tumour/trained group subjected to swimming for 45 min, 5 times per week for 8 weeks; a tumour/non-trained group in which the animals received 7,12-dimethyl-benzanthracene (DMBA; 1 mg/ml weekly for 6 weeks); and a tumour/trained group in which animals were subjected to the aforementioned DMBA tumour induction and swim training protocols. The physical activity started 16 weeks after finalizing the DMBA treatment. After the experimental period (total 30 weeks), immune cells were collected from the spleen, placed in culture, and stimulated with lipopolysaccharide. After 24 hours, cytokine concentrations (Th1: IFN- , IL-12; Th2: IL-4; Treg: IL-10, TGF-) were measured by the Enzyme-Linked Immuno Sorbent Assay (ELISA).

Results:
Morphological analysis showed that DMBA was able to induce carcinogenesis in breast tissue; carcinoma in situ developed in both DMBA tumour-induced groups. The group submitted to physical activity presented minor changes in the breast ducts compared to those without physical training. Regarding the Th1 response, spleen cells produced more IL-12 in the trained groups, in

both the presence and absence of the tumour ($p<0.05$), while the production of IFN- did not statistically differed between the 4 groups. Concerning the Th2 and Treg response, similar results were obtained for the synthesis of IL-4, IL-10 and TGF- : the trained groups showed a reduction of these cytokines relative to the untrained groups. A significant reduction of IL-4 was measured between the control group and the group with training, while a significant lower concentration of IL-10 was observed in the group non-tumour/trained versus tumour/non-trained ($p<0.05$). Also the expression of TGF- was significantly lower in the tumour group with physical activity versus the tumour/non-trained group ($p<0.05$).

Conclusions:
This study suggests that physical activity can have an added value along with cancer treatment. Physical activity promoted an increased expression of Th1 cytokines and reduced the synthesis of Th2 and Treg cytokines. This will promote polarisation of the immune system towards an antigrowth effect on tumours.

Keywords:
immune response, cytokines, cancer breast, physical activity.

O 10
Solitary extramedullary plasmocytoma of the thyroid: a case report.

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Solitary extramedullary plasmacytoma (SEP) is a rare malignant neoplasm arising from plasma cells. SEP most commonly occurs in the upper respiratory tract, oral cavity and salivary gland. Thyroid gland is rarely affected and less than 77 cases of SEP of the thyroid gland have been reported to date. SEP of the thyroid most commonly occurs in patients with Hashimoto thyroiditis.
A 78-year-old woman with no significant medical history presented with a rapidly enlarging palpable thyroid mass. Computerized tomographic (CT) scan of the neck and chest showed enlargement of both the thyroid lobes that appeared hypo-dense and showed several nodules. Infiltration of cervical vessels was reported. Laboratory tests were normal including serum protein level with no monoclonal gamma globulin pick. Fine Needle Aspiration Cytology (FNAC) was performed and showed a monotonous population of lymphoid cells, interpreted as suspicious for a lymphoma. A surgical biopsy was performed. Histopathological examination showed an infiltrating neoplasm composed of atypical tumor cells characterized by abundant cytoplasm and eccentric nuclei. At immunohistochemistry, tumor cells revealed diffuse reactivity for CD138 and predominant staining for immunoglobulin kappa light chains. Pan-cytokeratins, TTF1, thyreoglobulin, calcitonin, CD20 and CD79a were negative. Clinically, a complete multiple myeloma workup was negative. On this basis, a definitive diagnosis of SEP was made.
Radiotherapy (40 Gy) was performed. At 6 months follow-up, the patient showed good clinical conditions without evidence of multiple myeloma.

In conclusion, SEP should be considered in the differential diagnosis of a rapidly enlarging thyroid nodule. SEP must be distinguished from involvement of thyroid in multiple myeloma, inflammatory pseudotumor plasma cell variant, mucosa-associated lymphoid tissue lymphoma, and medullary carcinoma. Clinical correlation and immunocytochemistry are crucial in avoiding pitfalls.

O 11
Next generation sequencing is feasible on FNA specimens and improves diagnosis of thyroid nodules.

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The assessment of thyroid nodules is still a common clinical problem. Thyroid fine needle aspiration (FNA) is the standard preoperative tool for diagnosis of thyroid nodules. However, the limitation of this procedure consist in the high rate of indeterminate or suspicious cytological diagnosis which lead to unnecessary surgical intervention.
A number of studies have shown that molecular testing of FNA specimens can significantly improve the accuracy of the preoperative FNA diagnosis and the use of molecular marker is now recommended in the American Thyroid Association Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer. However, there are many molecular markers to test, which can require a large amount of DNA, is time consuming, and expensive.

In this study we evaluated the benefits of next generation sequencing (NGS) for helping diagnosis of FNA samples. We retrospectively analyzed 34 indeterminate or suspicious FNA samples for which surgical resection was performed ; histological diagnosis being considered as gold standard. DNA from these 34 samples was obtained either from cell block of from Diff-Quick stained smear and subjected to targeted NGS with the Ampliseq Cancer Hotspot Panel, which allowed us to analyze 2850 known cancer-related mutations.

Mutations in BRAF, NRAS and KRAS that are known to be involved in thyroid cancer biology were detected in 7 FNA samples. The presence of a mutation in one a these genes was a strong indicator of cancer since 5 (71%) of mutation positive FNA had malignant diagnosis after surgery. Moreover

NGS allowed us to detected in the same experiment rare or low frequency mutations in other genes such as p53, that can have a prognostic impact.

This study demonstrate that thyroid FNA specimens can be successfully analyzed by NGS. The detection in these specimens of different mutations known to be involved in thyroid carcinoma biology can improve sensitivity of the diagnosis in thyroid FNA samples.

O 12

Identification of putative precursor lesion of papillary thyroid carcinoma by cyclin D1 overexpression and p38 MAPK phosphorylation.

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Running title: Precursor lesion of PTC

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Introduction:

Papillary thyroid cancer (PTC) is the commonest endocrine malignancy. Though significant progress has been made to understand the pathways involved in the tumorigenesis of PTC, no precursor lesion has been identified yet. The present study aims to identify and understand the precursor lesion of PTC and its immunohistochemical (IHC) / molecular markers.

Materials and Methods:

Thirteen cases of metastatic PTC, papillary microcarcinoma and follicular variant of PTC (FVPTC) were identified from a histological review of 510 cases. In addition, 13 cases of a subset of follicular adenomatoid nodules with focal areas showing nuclear features characteristic of PTC, identified as putative PTC precursor lesion, were also analyzed. Immunohistochemical analysis of galectin-3, HBME-1, CK 19 and the proliferation markers Ki 67 and cyclin D1 was performed. Lesions were analyzed for cyclin D1 gene amplification by fluorescent in-situ hybridization.

PTC also frequently carries several genetic alterations in genes coding for proteins that activate the Mitogen-activated protein kinases (MAPK) signaling pathway, which plays a key role in the regulation of cell growth and

differentiation. The role of MAPK pathway activity in PTC was investigated by performing ERK, JNK and p38 phosphorylation by using IHC.

Results:
All putative precursor lesions showed immunolabelling of cyclin D1, Ki 67; 11/ 13 cases showed immunolabelling of CK 19; 10/13 cases showed immunolabelling of HBME-1 and 4/13 cases showed immunolabelling of galectin-3. Surrounding adenomatoid areas showed no to faint focal staining in all thirteen cases of cyclin D1, HBME-1 and galectin-3. A low rate of cyclin D1 gene amplification was identified in a significant proportion of cells in the putative precursor lesion as compared to surrounding benign adenomatoid areas.

ERK and JNK activation was seen in 50 and 35 percent of PTC cases with immunolabelling in less than 10 percent of cells. p38 MAPK phosphorylation was seen as abundant cytoplasmic immunolabelling in 55% of PTC cases and 60% of putative precursor lesion cases. A one way ANOVA test showed significant difference between the ERK, JNK and p38 phosphorylation ($p<0.01$).

Conclusions:
Increased expression of cyclin D1 and amplification of its gene along with immunolabelling of HBME-1 in areas showing cytological features of PTC within follicular adenomatoid nodules suggest that these areas could correspond to a precursor lesion of follicular variant of PTC.

Increased expression of p38-MAPK cascade in PTC variants indicate that it is functional in PTC. p38-MAPK hyper-expression in the precursor lesion can act as a potential complementary marker. However, its role in the tumorigenesis of PTC will be further investigated by performing Western blots, the results of which will be presented in the congress.

O 13

Immune reconstitution inflammatory syndrome in progressive multifocal leukoencephalopathy after highly active antiretroviral therapy.

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Highly active antiretroviral therapy (HAART)-induced immune restoration is beneficial for patients with AIDS-related progressive multifocal leukoencephalopathy (PML). However, in rare instances, an immune reconstitution inflammatory syndrome (IRIS) may cause paradoxical clinical deterioration.

We report the case of an AIDS 40-year-old male with a CD4+ T cell count of 52/mm3. HAART treatment was initiated. Shortly after the patient presented a cognitive deterioration. CT scan revealed multiple bilateral hypodense lesions associated with mass effect. The proposed diagnosis was encephalitis. The patient worsened clinically and radiologically while CD4+ T cell count increased. JC virus PCR on cerebrospinal fluid samples was positive. The patient died shortly after. Gross examination showed multiple necrotizing lesions disseminated in the white matter of the cerebral hemisphere. Histopathological analysis revealed massive myelin destruction with macrophage accumulation. Bizarre astrocytes and rare oligodendroglial inclusions (positive for SV40 antibody) were observed. One multinucleated giant cell was detected. The most striking feature was an intense infiltration by lymphocytes. These were either diffuse in the cerebral parenchyma, or forming perivascular cuffs. Most lymphocytes were CD8+ T lymphocytes. CD20+ B and CD4+ T lymphocytes were rare.

In conclusion, this case illustrates a fulminant inflammatory leukoencephalopathy associated with HAART-induce immune restoration in AIDS-related PML.

O 14

Familial presentation of a Granular cell astrocytoma and an Oligodendroglioma.

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Introduction:
Familial aggregation of gliomas has been reported in 5% of cases. Genome-wide association studies have identified some glioma risk variants. Amongst them, the variants located at 8q24.21 and 11q23 were specifically associated with risk of IDH-mutated gliomas.

Material and Methods:
Two gliomas occurring in a non-consanguineous family were analyzed by immunohistochemistry, FISH for 1p/19q, array CGH and sequenced for *IDH1/2*. For one of the proband, blood was tested for *IDH1/2* and *TP53* mutations and genotyped with Genome-Wide Human SNP Array 6.0

Results:
The probands, son and father, lived in the vicinity of powerlines for 20 years. The son was diagnosed with a low-grade glioma of right temporo-parietal lobe at 27 years of age. A near total resection was performed five years later because of tumor progression. A diagnosis of anaplastic astrocytoma, with features of granular cell astrocytoma was made. Tumor cells were positive for Olig2, p53; focally for vimentin, GFAP and were negative for CD68. FISH revealed deletion of 19q13. CGH demonstrated additional losses of 4q, 5q, 6q, 8q, 9p and gain of 11q. A somatic R132H-*IDH1* mutation was identified. No germline mutations in *IDH1/2* or *TP53* genes were found. On genotyping, only two of the identified single-nucleotide polymorphisms at glioma risk loci could be tested due to array probe set availability. The son was homozygous for the risk allele G for SNP rs2736100 (*TERT*, 5p15.33) and lacked the risk allele for rs498872 (11q23). He died two years post-surgery.

The father was diagnosed with low-grade glioma of left parietal lobe at the age of 47 years. He underwent complete tumor resection 14 years later due to tumor progression. A diagnosis of anaplastic oligodendroglioma was made. Tumor cells were positive for GFAP and internexin-alpha; negative for vimentin and few expressed p53. FISH revealed co-deletion of 1p36/19q13 and CGH additional losses of chromosomes 14, 15 and 18. A somatic R132H-*IDH1* mutation was found.

Conclusion:
We report the familial occurrence of R132H-*IDH1* mutated WHO grade III gliomas, one being an astrocytoma with granular cell morphology and the other an oligodendroglioma. The genotype of the son showed the susceptibility allele for SNP rs2736100 (*TERT*, 5p15.33), associated with an IDH-mutated glioma, contrary to its more frequent association with IDH-wild-type gliomas.

O 15
Are there specific biomarkers of glioma stem cells?

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Introduction:
Despite advances in surgical and adjuvant treatments, the prognosis of patients affected by GBM remains extremely poor, with an average survival of 14 months. GBM recurrences are likely due to resistant tumoral cells able to reseed cancer and give rise to the full differentiated pattern of the original tumor. This small group of cancer cells is thought to belong to a rare fraction of self-renewing, multipotent tumor-initiating cells named glioma stem cells (GSC). The identification of specific biomarkers of GSC would provide not only a powerful tool to detect and isolate them from the tumor but also to develop targeted therapies. This work aimed to identify new biomarkers potentially specific of GSC.

Material & Methods:
We carried out a comparative analysis based on the identification of inter-study concordances and discordances to identify the genes that exhibit differential level of expression between GSC-enriched conditions versus differentiated cancer cells ones. We compared the gene expression profile from independent studies using DNA chip microarray technologies. The selected genes were differentially expressed in at least two independant studies. The signification level was based on the binary logarithm of the ratio between expression in GSC-enriched conditions and in differentiated cells ones with signification threshold of log2<-2 or >2. We next addressed the expression level of 2 of the selected genes using immunohistochemistry and semi-quantitative analysis on a retrospective serie of 18 GBM and 3 non-tumoral brain tissues.

Results:
From microarray data published between January 2000 and February 2013, only three studies aimed at comparing gene expression between GSC-enriched GBM cultures and differentiated GBM cultures. Of these, thirty-two genes were fished out. Among them, 8 genes *(OLIG2, PTPRZ1, ID4, OLIG1, CCND2, AQP4, NCAN and SOX2)* were highly overexpressed in GSC-enriched conditions compared to GBM differentiated ones. Using immunohistochemistry for SOX2 and OLIG2 in human GBM tissues, we observed a dramatic expression (>25%) in the nuclei of tumor cells, respectively in 7 cases out of 18 (39%) and 15 cases out of 18 (83%)

Conclusion:
If the selected genes are putative biomarkers of GSC, we noted a discordance between the hypothetical few number of GSC in GBM mostly estimated at 2-5% as stated in the literature, and the broad protein expression of SOX2 and OLIG2 throughout our series of GBM tissues. This discordance raises questions about the definition of "cancer stem cells".

O 16

Knowledge, attitudes and acceptability of human papilloma vaccination amongst primary school girls (9 years and above) in minakulu sub-county oyam district

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Abstract-Cervical cancer is a serious health concern in Uganda that can be prevented by early Human Papilloma Virus (HPV) vaccination. Sound knowledge and positive attitudes highly influence acceptability and uptake as the vaccine becomes available. Acceptability studies are thus mandatory to highlight potential barriers and guide immunization policies. This descriptive analytical study determines knowledge, attitudes and acceptability of HPV vaccination amongst primary school girls aged ≥ 9 years in Minakulu Sub County, Oyam district-Northern Uganda. Systematic sampling of 415 pupils and 5 purposively selected key informants was conducted using semi structured questionnaires. Quantitative data was analyzed using SPSS 16.0. Directed content analysis of themes of transcribed qualitative data was conducted manually. Of the 415 respondents, majority 82.9% (n = 344) would accept and recommend an HPV-vaccine, majority 57.6% (n = 239) had not been vaccinated. 39.5% (n = 164) were not sure of the site where the HPV vaccine is administered, 45.3% (n = 188) believed it is harmful to the body, 29.9% (n = 124) had never had of HPV vaccine. 9.6% (n = 40) disagreed when asked whether cervical cancer affects only females while 9.2% (n = 38) were not sure. There was generally limited knowledge about cervical cancer and HPV vaccine that requires massive community sensitization to improve on vaccine uptake amongst the targeted population.

Key Words; Knowledge, Attitudes, Acceptability, Primary School, HPV-Vaccination