ENGAGe - ENGOT
Clinical Trials Project

STUDY BOOK 3
Genetics and Pathology
for the participants of the Clinical Trials Project

ENGAGe
ESGO | European Network of Gynaecological Cancer Advocacy Groups

ENGOT
European Network of Gynaecological Oncological Trial groups

ESGO
European Society of Gynaecological Oncology

Introduction

Two study books have already been written in the ENGAGe effort to educate gynaecological cancer patients and patient advocates from several European countries to a level where they can give the patient perspective in connection with the design and implementation of a clinical trial.

This study book is the third step in the education of 'patient experts' in a collaboration between ENGOT and ENGAGe.

The third study book is based on the webinars by two geneticist and one pathologist as you will see on the ensuing pages.

The training is still ongoing and will be followed by several webinars in 2024 with various topics about new methods of treatment for gynaecological cancer patients.

The intent of the Clinical Trials Project is to enable patient experts to understand the layout of a clinical trial and in that way be capable of reading and commenting on any trial within their own area of disease.

The webinar is a joint project between two ESGO networks, ENGAGe and ENGOT.

ENGAGe is the European Network of Gynaecological Cancer Advocacy Groups.
Learn more at **engage.esgo.org**

ENGOT is the European Network of Gynaecological Oncological Trial groups.
Learn more at **engot.esgo.org**

Featured speakers:

Florentia Fostira, PhD Clinical Laboratory Geneticist INRASTES, National Centre for Scientific Research "Demokritos", Athens

Carien Creutzberg, professor of radiation oncology at Leiden University Medical

Prof. Xavier Matias-Guiu, President of the International Society of Gynecological Pathologists (ISGYP). Chairman of Pathology, Hospital Universitari de Bellvitge and Hospital Universitari Arnau de Vilanova, Spain. Professor of pathology at Universities of Barcelona and Lleida, Spain.

Moderators:

Prof. Jalid Sehouli, Charité – Universitätsmedizin, Berlin, Germany

Birthe Lemley, Head of the Clinical Trials Project, EEG member of ESGO ENGAGe

Head of the Clinical Trials Project and writer of this report:

Birthe Lemley, EEG member of ESGO ENGAGe

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ENGAGe would also like to thank all participants of the ENGAGe-ENGOT Clinical Trials Project.

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1st webinar

Webinar on Genetics

May 16, 2022

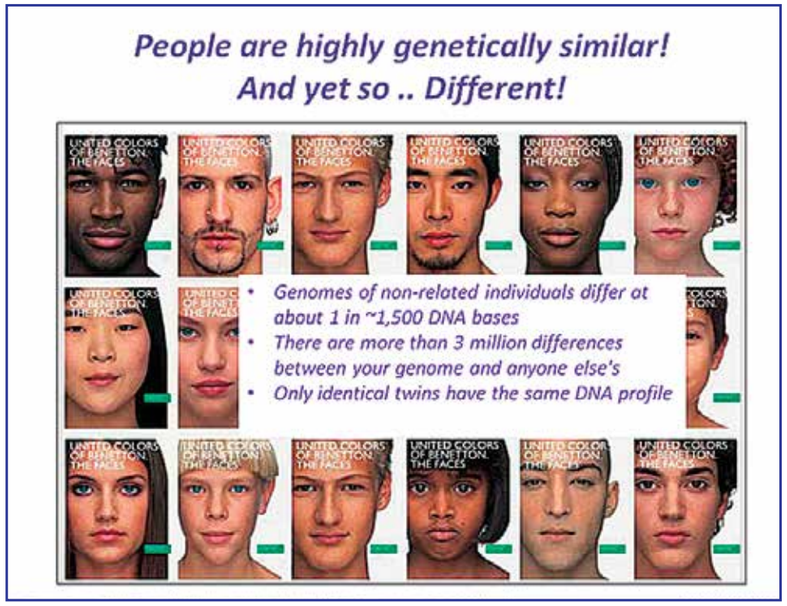
Ovarian Cancer: Hereditary Aspects, BRCA1/2 and beyond

Endometrial cancer: update on the molecular classification and genetic aspects

The first webinar was on the hereditary aspects in ovarian cancer – BRCA1 and BRCA2 by Florentia Fostira, PhD Clinical Laboratory Geneticist, ErCLG

Welcome to the two speakers **Florentia Fostira, PhD Clinical Laboratory Geneticist and Carien Creutzberg, professor of radiation oncology at Leiden University Medical** by Birthe Lemley.

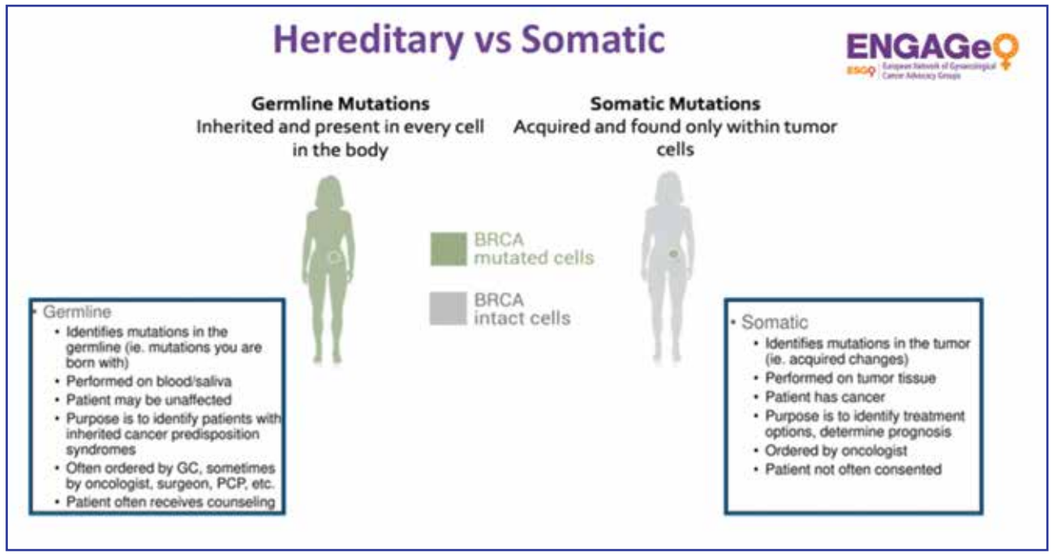
Florentia: Good afternoon, everybody. I would like to thank you for this kind invitation. It is always a pleasure to talk about science in front of a broader audience and in the next few minutes I will be talking about the hereditary aspects of ovarian cancer. I am going to be talking about genes, mutations, and signatures in ovarian tumours that can be associated with therapeutics. You have probably seen this advertisement some years ago, which actually shows the diversity that is associated with human beings. Each two individuals that are not related have approximately three million different letters in their DNA.



So, these differences in the DNA are what makes us unique and special. And this is what decide our hair and eye colours. And these DNA changes are what I will be talking about in the next half an hour or so.

The first thing that I would like to really clarify is that when it comes to DNA, we are talking about DNA and DNA mutations, and we have two large pools.

The first pool is the germline pool, which is actually our constitutional DNA.So, this is the DNA that we were born with, and we cannot change it, no matter what. So whatever DNA changes, DNA mutation or DNA variant (all these are synonymous words) we have in that DNA is probably inherited from our parents, and we can pass it on to our offspring. So, if we want to get the germline constitutional DNA, we might want to get blood or saliva from an individual, and if we are going to have genetic testing, we might have some consultations, a genetic counselling session, and we to have to give our consent in order for a lab to test our germline DNA. **The other big pool of DNA is the tumour DNA, the somatic DNA.** That DNA is not in molar cells, it is just in specific cells. That means that if we have a tumour, that DNA will be extracted from the tumour cells. So, these tumour cells are generally stored safely in the pathology lab after we have had a biopsy or we have had surgery, and they are stored for many years. If we get tumour DNA, this is going to give us some results that might be associated with therapy. So, the tests that are associated with tumour DNA do not cause a genetics consultation and do not need our consent because it is not something that is in our constitutional DNA, it is something that is very specific in our tumour DNA.



So, since we have got this straight, we might want to first of all focus on the hereditary aspects of ovarian cancer. We now know that up to 35% of ovarian cancer patients will have a mutation, a DNA variant in a very important gene in their germline, in their constitutional DNA. The majority of these variants would be in the two genes that you might have heard of in the past BRCA1 and BRCA2 but we do have mutations in other genes that work with BRCA1 and 2 like RAD51C, RAD51D or BRIP1 and I am going to give you some information about these genes in the next slides, but we do have mutations in some genes that are called mismatch repair genes and are associated with the specific ovarian cancer subtype endometrioid ovarian cancer. So, if we want to test all of our patients that have been diagnosed with

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ovarian cancer, we will probably find up to 21% mutations, germline mutations in BRCA1 and BRCA2 genes and somatic tumour BRCA1 mutations in an additional 6% of the patients. And we might get pathogenic variants in additional genes as high as 5% and we might get MMR mutations in less than 1% of the patients being tested. So, you will see that the most important clinically actionable genes that are associated with hereditary ovarian cancer are BRCA1 and BRCA2.

Prevalence of pathogenic variants in key genes

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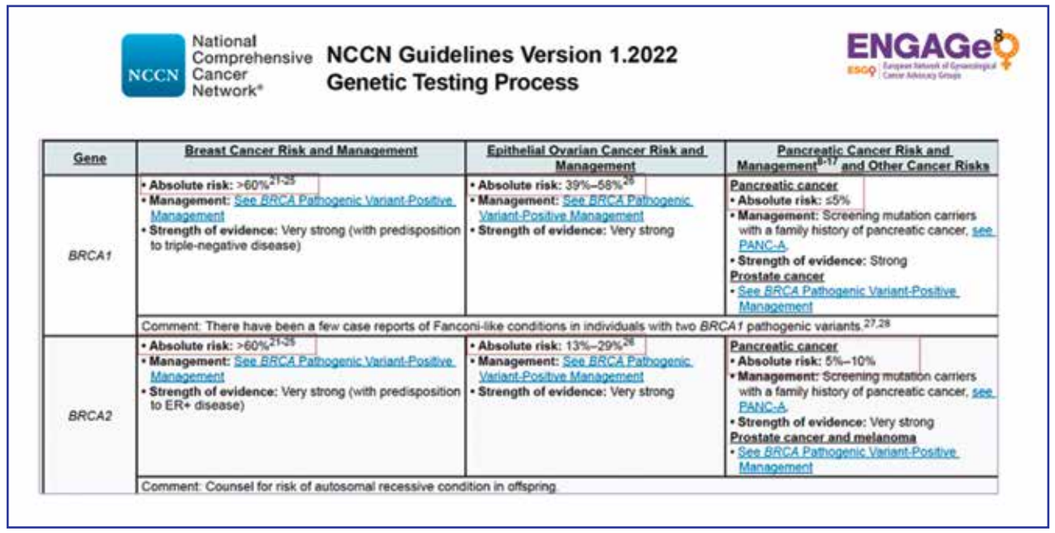
	Epithelial ovarian cancer
Germline *BRCA1* & *BRCA2* pathogenic variants	15%-21%
Somatic *BRCA1* & *BRCA2* pathogenic variants	6%-7%
Pathogenic variants in other genes	3%-5%
Pathogenic variants in MMR genes	0.5%-1%

So why is it important to identify these germline variants? Because we do know that when these genes are not working properly - have a defect as we say - this is a strong predisposing factor for a woman to get ovarian cancer at some point in her life. We know that women in the general population that do not have any gene defects have approximately one percent risk of getting ovarian cancer. But if a woman carries a BRCA1 variant, she has a risk of about 40 to 50 percent to get ovarian cancer at some point in her lifetime. With BRCA2 the number is a bit lower, close to 18% and RAD51C and RAD51D are clinically really important genes but are much rarer compared to BRCA1 and 2 and their risk is as high as 12%. BRIP1 is an important gene but is not as strong as the others so women that carry a BRIP1 mutation will have an 8% lifetime risk of getting ovarian cancer and PALB2 is also a candidate for an ovarian cancer gene but is not as strong. Women, who carry PALB2 mutations, will have an approximate 2 to 3 % risk of getting ovarian cancer.

So, a faulty gene in BRCA1 or 2 carries a high predisposition for ovarian cancer but we also need to remember that beyond ovarian cancer, women that carry BRCA1 and 2 mutations will have a high risk of getting breast cancer as well. This risk is as high as 70% during a woman's lifetime, and we must never forget that men, who carry pathogenic variants in BRCA1 and two, might get cancer as well. Obviously, they will not get ovarian cancer, but they might get male breast cancer, prostate cancer, or pancreatic cancer. So, this information is quite important for men as well.



National Comprehensive Cancer Network®

NCCN Guidelines Version 1.2022
Genetic Testing Process

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Gene	Breast Cancer Risk and Management	Epithelial Ovarian Cancer Risk and Management	Pancreatic Cancer Risk and Management[9-17] and Other Cancer Risks
BRCA1	• **Absolute risk:** >60%[21-25] • **Management:** See *BRCA* Pathogenic Variant-Positive Management • **Strength of evidence:** Very strong (with predisposition to triple-negative disease)	• **Absolute risk:** 39%–58%[26] • **Management:** See *BRCA* Pathogenic Variant-Positive Management • **Strength of evidence:** Very strong	**Pancreatic cancer** • **Absolute risk:** ≤5% • **Management:** Screening mutation carriers with a family history of pancreatic cancer, see PANC-A. • **Strength of evidence:** Strong **Prostate cancer** • See *BRCA* Pathogenic Variant-Positive Management
	Comment: There have been a few case reports of Fanconi-like conditions in individuals with two *BRCA1* pathogenic variants.[27,28]		
BRCA2	• **Absolute risk:** >60%[21-25] • **Management:** See *BRCA* Pathogenic Variant-Positive Management • **Strength of evidence:** Very strong (with predisposition to ER+ disease)	• **Absolute risk:** 13%–29%[26] • **Management:** See *BRCA* Pathogenic Variant-Positive Management • **Strength of evidence:** Very strong	**Pancreatic cancer** • **Absolute risk:** 5%–10% • **Management:** Screening mutation carriers with a family history of pancreatic cancer, see PANC-A. • **Strength of evidence:** Very strong **Prostate cancer and melanoma** • See *BRCA* Pathogenic Variant-Positive Management
	Comment: Counsel for risk of autosomal recessive condition in offspring		

This is a photo from the NCCN Guidelines, so NCCN is the National Comprehensive Cancer Network. These are guidelines that are being updated regularly, based on the new data that are coming along, and it is quite helpful to have new versions of genes or therapeutic interventions or whatever comes along after a scientific evaluation. So based on NCCN, women that carry BRCA1 and 2 pathogen variants as already mentioned have a high lifetime risk of getting breast cancer and really high risk of getting ovarian cancer. BRCA1 seems to be a stronger gene when compared to BRCA2 - not only because of the numbers but because of the age at diagnosis. So, woman that carry BRCA1 variants tend to get diagnosed earlier as compared to BRC2 women.

We must never forget that these genes are associated with pancreatic cancer risk as well. The numbers are not as high as for ovarian and breast cancer but again it is quite important to remember this, as this information is very important, especially in families that have relevant family history. So, we always use this type of information in a genetic counselling session.

I have already told you that **RAD51C and RAD51D are two clinically really important genes in ovarian cancer disposition**. You will see that breast cancer risks are much lower compared to BRCA1 and BRCA2 and ovarian cancer risks are again lower when compared to BRCA1 and BRCA2. But we should keep in mind that due to the lack of good surveillance techniques detecting an ovarian cancer early, NCCN suggests that women that carry RAD51c and RAD51d pathogenic variants might want to think about having a prophylactic salpingo oophorectomy at the age of 45 to 50 after childbearing due to their increased risk of getting ovarian cancer. One might think that 10% or 12% is not very high but it is 12 times higher than in the general population since the general population's risk is 1%.

So therefore after 2014 most medical and scientific societies have decided that all woman that are diagnosed with epithelial ovarian cancer should be offered genetic testing and genetic counselling - not only because you can see that there is a high prevalence of pathogenic mutations in the number of genes but because this information is quite critical for additional clinical interventions as we will discuss a bit further on.

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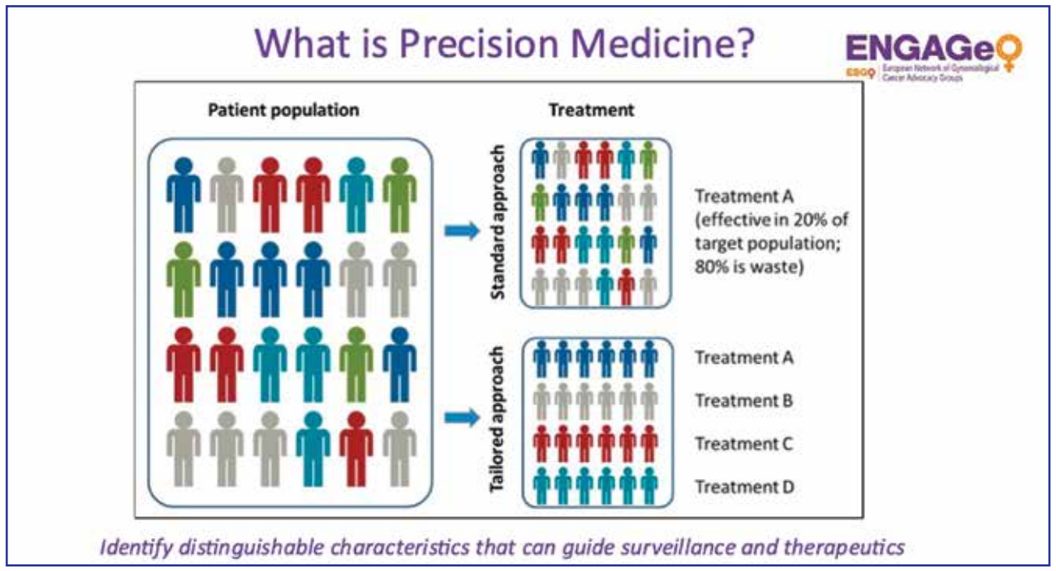
So, there are no selection criteria when it comes to ovarian cancer because we now know that probably half of the women that are diagnosed with ovarian cancer will not have a significant family history, and we now know that approximately two-thirds of the women that get an ovarian cancer diagnosis will be more than 50 years old. So, the age criterion is out, the family history criteria are out. So just a diagnosis of ovarian cancer at any age. We will have patients that will get a diagnosis at the age of 80 or 85. They are eligible to be offered genetic testing.

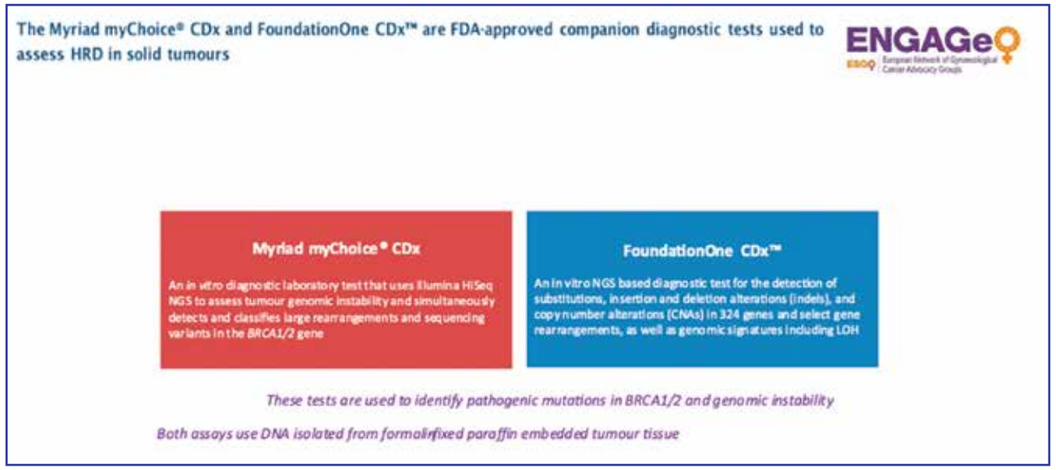
The good news is that we have been able to identify a sensitive pool for ovarian cancer, which is called **homologous recombination**. So, what is it? **It is just a repair pathway within a cell that is meant to protect us from getting harmed**. Every day our DNA replicates and in some cases this DNA will accumulate errors. So, a homologous recombination pathway is a repair pathway that will repair these errors. In a case where someone carries a BRCA1 pathogenic variant, BRCA1 is not working properly, so this pathway is not working properly. This is what is called homologous recombination deficiency. It means that this pathway, the repair pathway - which is a good pathway - does not work properly, and this is a problem for the cell.

This is one thing, the other thing is that we now know that if we are able to identify this homologous recombination deficiency, we might be able to identify good candidates that can be offered targeted therapy, implementing a new drug that is called a PARP inhibitor. So, this drug got an FDA approval back in 2014 as maintenance therapy for ovarian cancer patients but nowadays this drug is being offered to breast cancer patients, to prostate cancer patients and to pancreatic cancer patients as well. So ovarian cancer provided the knowledge and opened the driveway for additional tumour types to enter this targeted era of PARP inhibitors. So, this is all in the scope of **precision medicine**.



So, back in the day we had a patient population - this could be your ovarian cancer population. You can see that ovarian cancer patients are shown here with different colours. So back in the day when we did not have any biomarkers or targeted therapies, we would just give treatment A to the whole load of this population. And what would happen? Some of these patients would have a good response, some of them would not. But what does precision medicine do? It just identifies these different colours and provides targeted therapy based on its colour. So, this is how PARP inhibitors work. You have to find the colour, it is here HRD homologous recombination deficiency, and you give the anti-colour, the anti-blue, the anti-grey or the anti-red. So how do we do that? We need DNA. DNA from the tumour or from the blood, so tumour DNA or germline DNA. And now we have a number of technologies based on the next generation sequencing and we can simultaneously analyse and detect new mutations in a large number of genes. Not only BRCA1 and BRCA2 but additional genes that are associated with homologous recombination. So, depending on the lab, where you are doing your test, you can get tested for e.g., two genes or maybe 500 genes, again information for a really big number of genes.

You might also have heard of the two really important tests that are FDA-approved companion diagnostic tests used to assess HRD in solid tumours. They are myChoice and FoundationOne tests. Both of these tests are being performed on tumour DNA, not germline DNA, but they can actually detect additional phenomena in the tumours that might be associated with the HRD, which in our case is the biomarker for getting a PARP inhibitor.



I don't want to get into details of this because it's quite complicated, but I just want to point out that these tests are designed to detect mutations in a number of genes but are also designed to detect a number of other phenomena like what is happening at the end of the chromosomes which are called telomeres or what is happening with the loss of heterozygosity. So, they are accumulating all these data in order to provide a score. This score is an indicator of whether there is HRD or not.

So, what will happen then? If we go and test only the germline DNA for BRCA1 and 2, we find a mutation in about 15% of the ovarian cancer patients. If we get germline DNA tested for all the homologous recombination genes, we will find mutation in approximately 23% of our patients. Whereas if we test tumour DNA, we might be able to get both germline and two more mutations in BRCA in 22% of the cases. And if we do tumour testing, we might get as high as 35 percent. If we combine these phenomena

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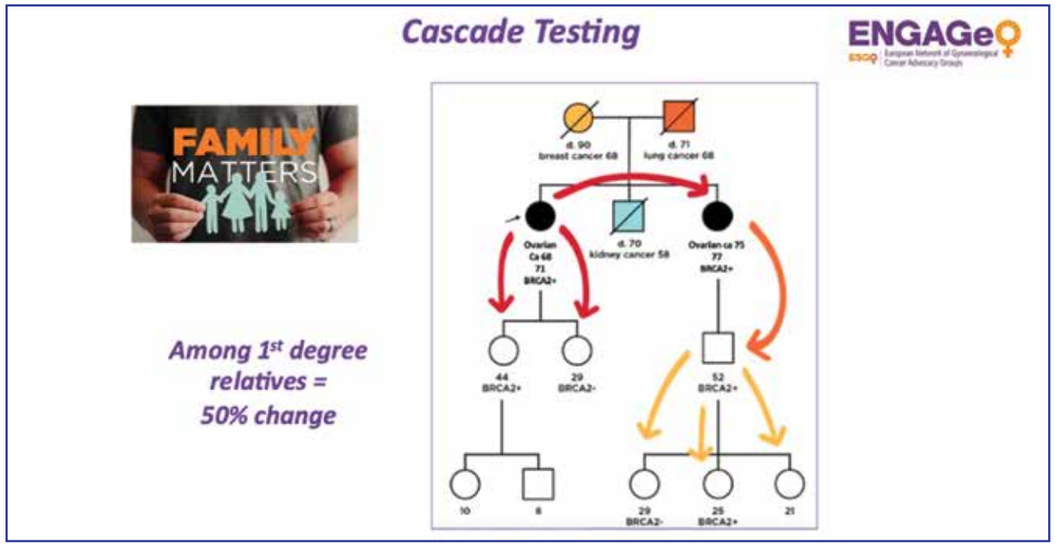
that I have already mentioned, we might be able to offer this targeted treatment to as high as half of the ovarian cancer patients. We need to balance out what we really want to do and what we really want to identify in order to provide information and to provide optimal treatment options for all our patients.

The best way to go, which is what is mentioned in the ESMO guidelines as well, is to perform both tests. So, test both germline and tumour DNA. In some cases, it can be quite tricky and difficult since not all European countries have a policy in place to reimburse both tests. Some countries do not have any reimbursement scheme in place, but most countries will reimburse at least one test. But remember that the optimal thing to do if we want to do the best for our family, for the inheritance, and for the therapeutics, would be to do both tests, germline, and tumour DNA.

So, when we were talking about ovarian cancer, epithelial ovarian cancer, we would in most cases be focusing on high grade serous ovarian cancer because this is the most common type of epithelial ovarian cancer, and this is the type that will have the majority of mutations in BRCA1 and 2, in homologous recombination genes and so on. We know that low grade serous subtypes have a really low prevalence of such mutations whereas mucinous cases almost have no possibility of having a BRCA1 or BRCA2 mutations. On the contrary, we know that in endometrioid ovarian cancer cases approximately 10 to 15% of endometrioid cases will have mutations in the mismatch repair genes, which is an important information because these patients will be able to be offered immunotherapy, which is an important therapeutic aspect for ovarian cancer as well. So just going towards the end of this presentation, I just really want to focus on the family aspect of genetic testing. We should always remember, when we identify a genetic mutation, that this is not an individual thing. This information is applicable to multiple family members.



On the right hand of this slide, I am just showing you a graph of a family pedigree. The circles are the females, the squares are the males, and these two black dots are two sisters who have been diagnosed with ovarian cancer. One at the age of sixty-eight, the other at the age of 75. So, they were both found to carry a BRCA2 mutation, a germline BRCA2 mutation, so their family relatives, their offspring have been offered cascade testing as we say. They have been offered testing for the specific BRCA2 variant. And as you can see, this lady with the arrow had two daughters, one at the age of forty-four that

carried the BRCA2 variant and the other at the age of 29 that did not carry the BRCA2 variant. Imagine how important this information is. Imagine, this 29 years old girl here does not have to do anything because her risks of getting breast and ovarian cancer are almost similar to the general population's risks. Whereas her sister who had been found to carry the BRCA2 variant is at increased lifetime risk of getting both breast and ovarian cancer.

Going to the other lady here, on the righthand side, she only has a son. She does not have any daughters, but this information is important for her son as well, because as already mentioned he might get prostate or male breast cancer or pancreatic cancer. But most importantly this guy has three daughters, so he can be the transmitter of this important genetic information to his daughters. So, this information is quite critical both for himself and also for his daughters. So, it is quite important to acknowledge and to understand what hereditary predisposition means, even for an individual that has already been diagnosed with cancer if there is a DNA change that is written on your constitutional DNA. There might be a high risk of getting a second or a third primary cancer diagnosis so it's quite important. We have been talking about the therapeutic innovations which are quite critical, and these therapeutic innovations are associated with improved survival. And what we should never forget is how important this information is for the whole family, as I have already shown you.

This is my last slide, and I just want to remind everyone that it is not just DNA, it is not just about genes. We can alter our cancer risk. Eat healthier, have a normal BMI, quit smoking, start exercising and drink less alcohol.

Thank you for your attention and I will be happy to take any questions!

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Questions
from the Audience at 1st webinar

Birthe:

Thank you so much for this excellent presentation; that was really great! Can I ask the first question?

- **Of course!**

Birthe:

■ **You were talking about PARP inhibitors, and you know in some countries, they give the PARP inhibitor to the patient no matter whether she is HRD positive or HRD negative. I know in Germany they do so and in other countries. Do they do that in Greece, and would you comment on that?**

- Yes. So that is a great question. Niraparib has arrived in Greece like less than a year ago now, so for many years since 2014 and so on we only had Olaparib in Greece so you had to had to have proof of HRD to get access to a PARP inhibitor, but as you already said there are a number of studies, which prove that there might be some benefit for women that are not HR deficient. Women with proficient homologous recombination can also benefit from the treatment.

- I will tell you though that we do know that BRCA1 and 2 or HRD has a prognostic value in these cases as well. So even if you can have access to a PARP inhibitor, it is quite important to know your HRD status because that can add significant prognostic value to the effect that the PARP inhibitor will have on you. So, one thing is access, and another thing is how well you will respond to the treatment.

XY:

■ **Can I ask a question? Hello, and thank you for your very exciting presentation. Could you tell me what the difference is between Olaparib and Niraparib? Are there any significant differences? And another thing is, do we already know, who will benefit from having the PARP inhibitor? Isn't that kind of a grey zone where we haven't found all the genes of the tumour?**

- Another great question. All these 3 PARP inhibitors have the same concept. So, the basis is the same. But as you can understand, they have different pharmacodynamics as we say, so this is the difference. But we do not have a study yet, which has a direct comparison between them, so that would be great, that would give us really important information about their pharmacodynamics as well. But all these three PARP inhibitors have the same outcome as to the BRCA genes; the best responders would be women that have germline BRCA1 and BRCA2. These would be the excellent responders.

What we do not understand up to now is how some women, who do not have germline BRCA1 and BRCA2, are excellent responders. So, in my opinion there are some additional genomic phenomena that we have not been able to identify yet. I would say your best-case scenario would be to have a germline BRCA1 and BRCA2. That would be your better chance to have a good response to a PARP inhibitor.

Birthe:

- Okay, can I comment on that? We do know that some of the women in the HRD negative population, as we call it, also have a benefit, but it seems that we don't know yet who they are, because from the NOVA trial and from the PRIMA trial we saw that some of these patients had a benefit. But if you look at the whole group - all 50% of them, the response was not very great. But the individual patient might have a response. So, I think that you also mentioned the Myriad test. I would argue as a patient that maybe we should have a better test. We should try to find out if there are women among the other 50% that we know will benefit, but we don't know yet, who they are, and I think that's the problem today with the PARP inhibitors.

Florentina's response:

- You know I am on the same page as you. In my opinion the Myriad test is the best test we have to date because it is probably one of the few tests that have been tested on a large scale. Well, it is not an optimal test because we know, we are missing a lot of information. So even though the Myriad test is working on three types of mutations, keloid and telomeric imbalance to give you the score, this is probably not enough to get the optimal number of patients that will get benefit from PARP inhibition. Something that is quite important and has been proven on a cell culture is the methylation, the epigenetics, which is not in many of these tests, yet. So RAD51C methylation or BRCA1 methylation might give a signal that can be associated with PARP inhibition.

Birthe:

Thank you very much. I would just say I have just seen that Prof. Sehouli is on board.

Prof. Sehouli:

Yes, I am sorry for the delay but patients first. It is wonderful, nice to see you.

XY:

I have a question; can you hear me? Thank you for the great presentation.

■ **I wanted to ask, I am a BRCA1 mutated patient myself and I've been taking Olaparib for three years now and everything is going well, and I go for check-up for my breasts, and I asked the genetic counsellors if they thought Olaparib would be a benefit or would decrease the risk of having breast cancer. They did not have an answer the last time I asked them, which was about a year ago or so. But I saw that Olaparib is offered to some breast cancer patients, and I was wondering if it makes sense just to lose some weight or to go to a prophylactic mastectomy as I wanted to do before and I am now in doubt.**

Florentina:

- So just to clarify something. You had an ovarian cancer diagnosis, and you had a BRCA test and that was germline positive BRCA1?

Yes.

- Okay. So, I will give you a very important tip. So, there are two really large studies that have been evaluating the breast cancer risk of BRCA1 and 2 germline positive women that already had an ovarian cancer diagnosis. Your 10-year risk of getting breast cancer is 8%. So, did you get that? So, you had an ovarian cancer diagnosis, right?

Five years ago, yes.

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- Okay, so 5 years plus 5 years your breast cancer risk is 8%. It is really low. But for the next upcoming years after those ten years you do not know, right?

Florentina:

- We have new studies! So, what these studies say is that if there is a woman who had an ovarian cancer diagnosis, do not offer her any prophylactic mastectomies or anything, at least for the first 10 years after her diagnosis because the cancer risk is not that high!

Prof. Sehouli:

- But it is always very important to separate between prevention and treatment. That is a key story. Theoretically it can be that BRCA positive diseases can be treated earlier if you have ongoing treatment. Nevertheless, all examinations regarding breast cancer should still be ongoing independent of the drug. And from the scientific point of view, I can tell you it is very complicated to show evidence that this will prevent breast cancer because you need many, many events for the evidence-based analysis. So, I will say to be clear; NO; there is no evidence if you use Olaparib for ovarian cancer that this will prevent breast cancer. That will be my key story. I think Florentina you will support this message.

Florentina:

- Yeah, 100%. But would you agree with me that her breast cancer risk after her ovarian cancer diagnosis at least for the first 10 years is not that elevated for her to go and have some preventive surgery?

Prof. Sehouli:

- Yes, that is true but nevertheless if you suffer like a patient, dealing with numbers is very difficult. That is the key problem, more psychologically. And even though I am a scientist - for a woman with cancer, it is only yes or no. It is not 3%, 8%, so it is yes or no. That is the reason. I will say if somebody has ovarian cancer and in the next 3 to 5 years you have no relapse of ovarian cancer, it is very good to think about prevention, for everything; for colon cancer, heart diseases, for melanoma and breast cancer. But I think in the first 2-3 years after ovarian cancer surgery is a very frail story. So, I would not recommend that a patient with ovarian cancer stage IIIC underwent 10-hour surgery then to make even in this case prophylactic mastectomy that will not be my recommendation. So, step by step but after 3-4 years to think what is the best way that I am still healthy.

Okay, thank you for your answer. You said this gene mutation also increases the risk of pancreatic cancer?

Florentina:

- Yeah, I am just going to clarify that. So BRCA2 mutation are mostly associated with an increased risk of pancreatic cancer. I know, you do not like numbers, but I am going to give you a number.

I do like numbers; I am a scientist at the end. Another kind, but...

- So pancreatic cancer is for BRCA 2 carriers about 5 to 10% lifetime risk, so you do understand that it is not that high. For BRCA1 (you said you were a BRCA1 carrier), the pancreatic cancer is 2 to 3% lifetime risk and as I told you already, this is strongly associated with family history as well. So, if you do not have any pancreatic cases in your family history, it is highly unlikely that you get something like that in the future.

Birthe:

- There are other questions, and we have to move on.

XY:

■ **I have a question about immunotherapy. You told me, somebody can have benefits of immunotherapy, but I did not hear quite well which group you meant.**

Florentina:

- Yeah, that is the endometrioid histology that have MMR mutations. It is the Lynch syndrome group. That was the one.

XY:

■ **I have another question; if you do not know how to treat the cancer, would you still have benefits from genetic testing?**

Florentina:

- You know for me the major benefit of genetic testing is the family aspect always. I do find that this is a major aspect of the whole thing, so even if you do not have a way to treat the patient, genetic testing might reveal something that might be offered to asymptomatic relatives, and that has prevention mechanisms around it.

Birthe:

- Do you want to ask a final question, XY?

XY:

■ **Yes, a short question. Do you have any kind of evidence about the combination of PARP inhibitor with other kinds of treatment for ovarian cancer and non-BRCA? High-grade serous non-BRCA.**

Florentina:

- I am not an oncologist so I don't have all these regiments in my head but from a gene wise approach I will tell you that we do have some data for RAD51C and D. Patients can get some good responses if they have mutations in these genes but I wouldn't know the drug combination in chemotherapy or anything like that but I would say that RAD51C and RAD51D are really good candidates for PARP inhibition.

Birthe:

- Thank you.

Florentina:

- Thank you, it was my pleasure.

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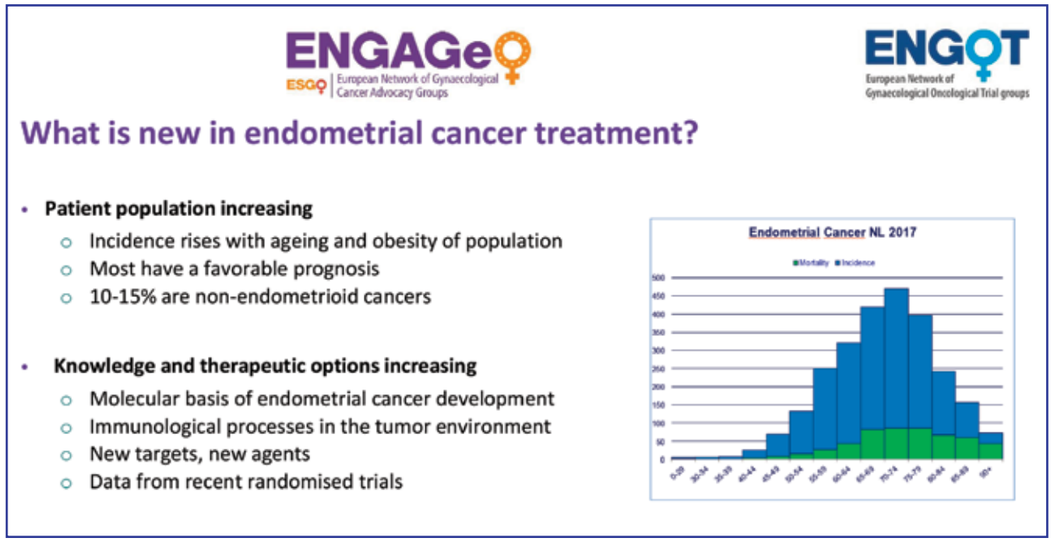
Birthe:

I would like to welcome Professor Carien Creutzberg, whom I have already seen in the audience here. She is a professor of radiation oncology at Leiden University Medical in Leiden, in the Netherlands. And the title of her presentation is Endometrial cancer: Update on the molecular classification and genetic aspects. Thank you so much for joining us and welcome.

Carien:

- I will share my screen and start right away. So, as you already mentioned in the introduction, **I will try to give you a brief update on the molecular classification and genetic aspects of endometrial cancer.** These are in fact two big topics, but I will try to do this as clearly as I can. In the past decades of endometrial cancer treatment, we have in most countries seen that the number of women, who have endometrial cancer, has been increasing - mainly due to the aging of the population with more elderly women in the population and more obesity, which is also a risk factor for endometrial cancer. Most of the women have a favourable prognosis and on the right-hand graph you see the women that were diagnosed with endometrial cancer with a peak incidence around 70 to 75 years of age and the greens are those who died of endometrial cancer, so the majority will be cured.

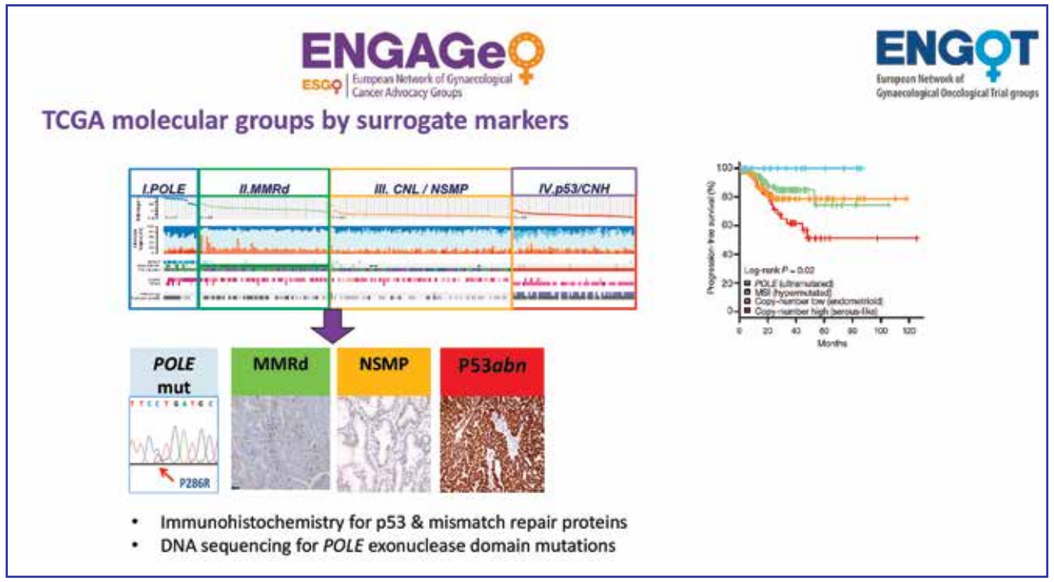


About 10-15% of non-endometroid cancers will in general have a somewhat less favourable prognosis. But in the most recent years, we have seen a molecular basis of endometrial cancer development, which has put a new light on all the risk groupings and the thinking about risk. We know more about immunological processes; we have discovered new targets for therapy with new agents, and there have been more trials reporting data.

So traditionally, this was before the molecular groups. We said that risk groups were based on important factors, such as stage, grade, low-grade, well-differentiated or high-grade, less differentiated histological

types and the endometroid vs non-endometroid and combinations of those factors resulted in a low-risk, intermediate and high-risk group, and the low-risk group is the largest one, about half of all women. They have a good prognosis and are cured by surgery alone. So usually there is not much debate on the modality of treatment but the intermediate risk and high-risk cancers, which are about the other 45 to 50%, have a somewhat higher risk and most of these women get adjuvant treatment after surgery.

And then in 2013 there was a big collaboration in the United Sate on the cancer genome atlas where they did whole genome sequencing of various cancers, and this was the publication of endometrial cancer, and they discovered four different molecular groups. This meant that the endometrial cancers could be divided into four groups, and they are here ordered by the number of mutations.



The ultra-mutated group, which looks like an aggressive cancer with many mutations in a micro-scope, is called **POLE** (polymerase epsilon mutated), and the key striking thing is that these patients have a very good prognosis with progression-free survival of the same study. And those types of cancers barely have recurrences. Then there was **MSI**; micro satellite instability or hypermutated group; this is also a group that is of interest because of Lynch syndrome. They are different by mismatch repair deficiency, and the patients have an intermediate prognosis. Then we have the copy number low group; that is the large group of mostly endometrioid cancers. This is the classical endometrioid low-grade cancer, and they also have an intermediate prognosis. And there is the copy number high group. This one is serous and resembles a little bit ovarian cancer; the serous ovarian cancers are driven by P53 mutations, and they have the most unfavourable prognosis of all cancers. So, these four molecular groups were a new finding and published in 2013.

After the publication, several groups began to look for these four molecular groups, and they looked for how to define these in the normal pathology tissues because pathology tissues used in the daily clinic are not freshly frozen and we do not do whole genome sequencing. We do more limited sequencing, and we have paraffin-embedded tissues and there are markers, which you can find on the normal pathology tissues, which indicate which molecular group the cancer belongs to. For this we use immuno-

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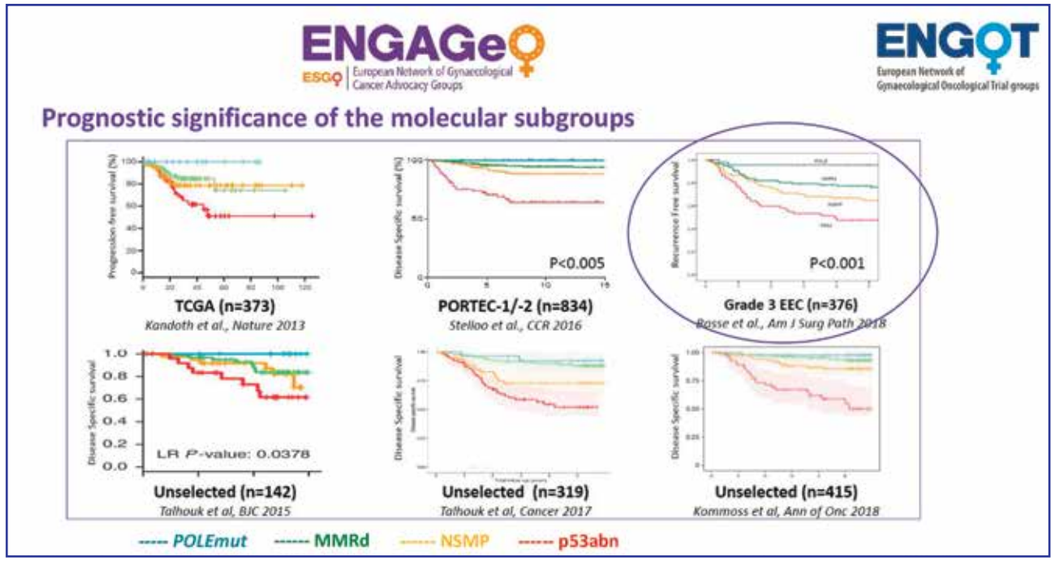
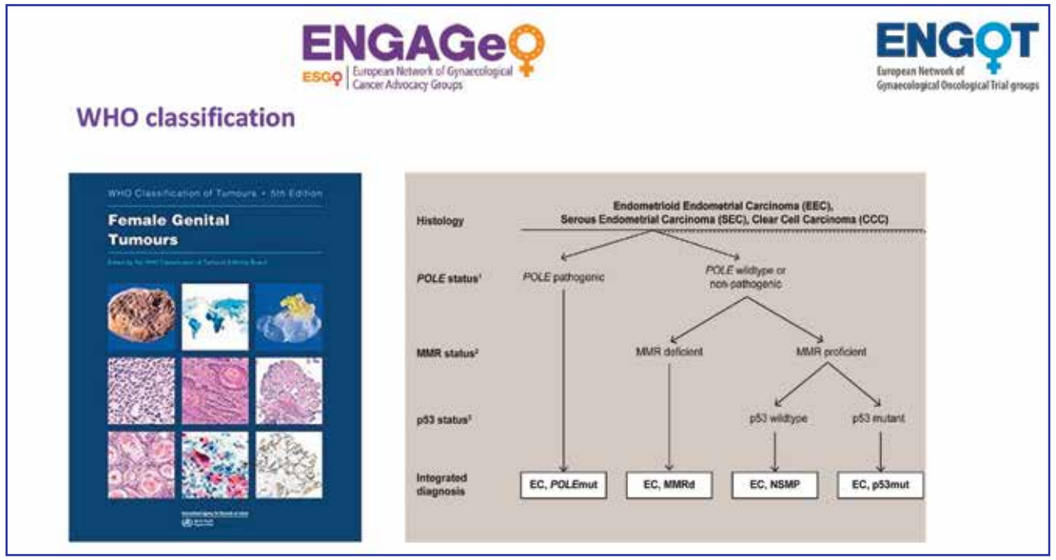
histochemistry, which is a normal, fairly routine staining method to see if one of the mismatch repair proteins is not expressed, and that would identify the type of mismatch repair deficiency. We can do P53 immune histochemistry to see if there's abnormal expression of P53, which would be the copy number high, or P53 mutant group, and we should do DNA sequencing for the detection of pathogenic POLE mutations. So, with the use of these methods, which you can do in normal, regular pathology tissues, these groups identified four molecular groups in normal pathology tissues and found the same differences in prognosis. So, we can find the four molecular groups by using these markers in the routine pathology tissues.



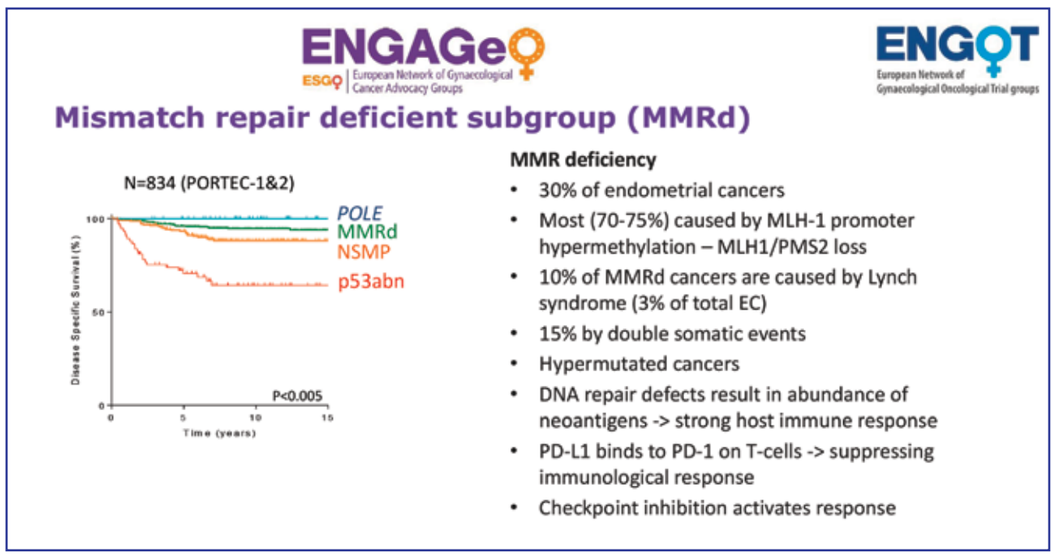
And here are examples of the progression-free or recurrence-free survival curves which were found in the different groups. This is the graph of the cohorts of the PORTEC-1 and -2 trials with intermediate to high intermediate endometrial cancers, and this is the graph from the Vancouver group who had a series of cancers of all stages and types, and this 3rd graph is an important group that was an international collaboration with only grade three endometrial cancers, so the group of cancers which were previously thought to be grade 3, not well differentiated, so more aggressive, and in all of these studies we found similar differences in recurrence-free survival curves for the four molecular groups, with the POLE-mutant cancers always being on top and with a very good prognosis with only very rare recurrences.

The P53 mutant group, which is unfavourable, have the highest risk of recurrences and the lowest survival and the other two are in between. So even in this group, which was previously a higher risk group, we now see that there is a big difference between the four molecular groups.



Since late 2019 this has also been introduced in the World Health Organization's classification of endometrial cancer. So, POLE pathogenic cancers, mismatch repair deficient cancers, P53 mutant cancers and the others which are copy number low or P53 wildtype or of no specific molecular profile, which is NSMP.

In the recent guidelines, which were introduced by ESGO together with ESTRO and the pathology organization ESP, we did introduce these molecular groups in the risk classification, but because we know that not all centres and countries have access to these tests, we also had the risk groups for molecular classification unknown. We introduced the risk group for molecular classification known, with POLE mutant cancers moving to lower risk and P53 to higher risk. So, this is now being introduced both in categorizing risk of prognosis and also in treatment recommendations.



The above slide is from the PORTEC trials 1 & 2.

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One of the four groups, which I showed you, is the mismatch repair deficient subgroup. And this is the curve of PORTEC-1&2. That is intermediate risk of high intermediate cancers, and they had a fairly favourable prognosis.

The MMRd cancers are 30% of all endometrial cancers, so generally one woman out of three has mismatch repair deficiency as the cause of endometrial cancer. But most of these - about three quarters - are not caused by any germline or inherited condition. They are caused by hypermethylation of the promoter, which is a local event in the tumour. About 10% of the mismatch deficient cancers are caused by Lynch syndrome and I'll come back to that, but if you can do the simple calculation that if one third, one in three of the women that have endometrial cancer, have mismatch repair deficient cancers, and one in ten of those women have Lynch syndrome, that means that 3% of all endometrial cancers are caused by Lynch syndrome. So, keep in mind, it is an exceedingly small minority of the women with endometrial cancer, and then if you look at the 75% and then the 10%, we are still not addressing 15% and those are not inherited mutations in endometrial repair genes but there are two somatic changes in the mismatch repair genes.

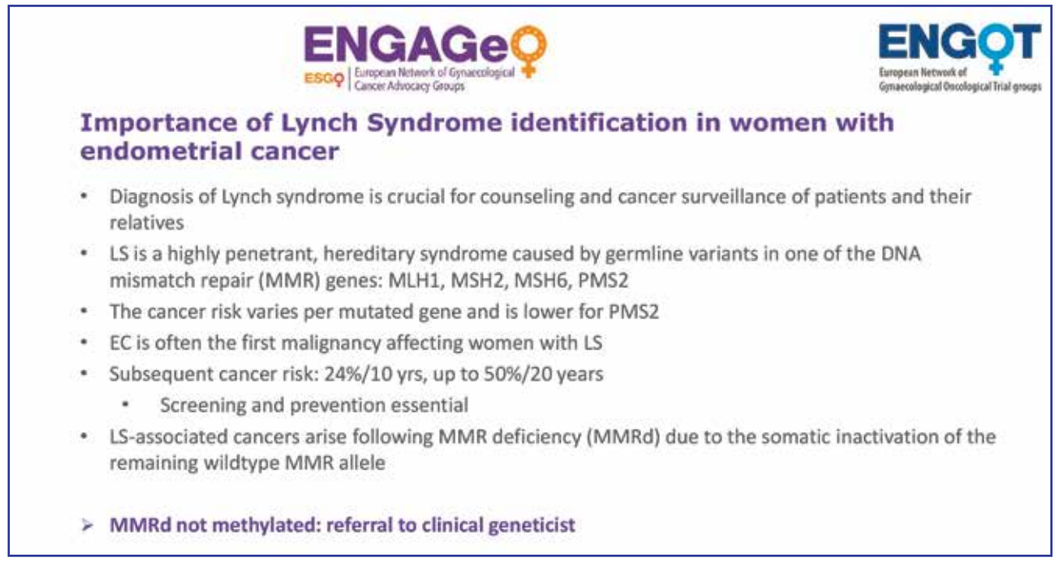
So that is all local changes in the cancer itself, not genetic but a double event in the tumour. This is also because they are hypermutated cancers. They have lots of mutations, so if you have a mutation, you get another one and subsequently another one, but it is all in the cancer itself. All of those DNA repair defects and fragments of mutated DNA segments act as a neo-antigen, so an antigen that can be recognised by the body as not right. Something that is not right elicits an immune response, and then your body is often able to kill the cell via its immune system but there are also mechanisms in the tumour to suppress that immune response, and that is where now this recent development of checkpoint inhibition is of interest to reactivate the immune response and try to kill the cancer cells.

This is the main cause as I said of mismatch repair deficiency. So first we do the MMR immunohistochemistry, and then in 30% of all endometrial cancers we find a mismatch repair deficient cancer. The question whether this cancer can be inherited (Lynch Syndrome) depends on which of the mismatch repair proteins is not expressed. If it is MLH1 and/or PMS2, then it is usually a cancer caused by hypermethylation, and then we are doing a methylation essay. As I said 75% of all MMRd cases are caused by methylation, and then it is a methylated mismatch repair deficient cancer. If we find loss of the other mismatch repair proteins MSH2 or MSH6, or isolated loss of PMS2, then we refer the patient to the clinical geneticist for further evaluation. Up to half of these cases are caused by Lynch Syndrome, and if the clinical geneticist finds a germline mutation, then it is an inheritable mutation and that would confirm Lynch syndrome.

So, what is now the importance of a Lynch syndrome identification in women with endometrial cancer? Of course, if you diagnose Lynch syndrome, and it is the first cancer of these women, then this will have consequences for further counselling and cancer surveillance - not only of the patient but also of her relatives. It is very penetrant, which means that it is a directly inheritable syndrome in the germline with a mutation in one of the four mismatch repair genes. The risk of getting a cancer varies by which genes mutate, most often MSH2 or MSH6 are mutated but if it is only PMS2, the risk is somewhat lower. Endometrial cancer is often the first malignancy affecting women with Lynch syndrome. So, it can be what we call index cancer - the first cancer which is diagnosed in the scope of the Lynch syndrome.

Subsequent cancer risks for those women are about 20-25% in 10 years and up to even 15 and 20 years. So, screening and prevention are essential. Lynch syndrome associated cancers are developing because you inherit one mutation in a mismatch repair gene from one parent but in the course of your lifetime the second not affected gene copy, which you inherit from the other parent, gets silenced or also

mutated and then you can develop the cancer. So, if we find a mismatch repair deficient cancer, which is not methylated, we would refer the woman to the clinical geneticist for further germline testing and counselling – both for the woman, who is diagnosed with Lynch syndrome, and her family members.



There are a number of well-known Lynch associated cancers, and colon cancer is the most frequent and most well-known Lynch associated cancer. All women and men with colon cancer get this mismatch repair testing to see if they have signs of Lynch syndrome. In endometrial cancer it is the second most common Lynch associated cancer, and that is 20% to 60% lifetime range in Lynch carriers. So, in mutation carriers colon cancer screening is generally recommended by colonoscopy. Stomach cancer screening is recommended by gastroscopy and helicobacter pillory testing. In women screening with ultrasound of the uterus and biopsy from the age of 35 have not really proven effective, so usually the main cancer prevention matter is to consider a prophylactic hysterectomy, and if you want to prevent ovarian cancer also do something right after childbearing age.

So, this is usually what is recommended, also of course both women and men are made aware of other potential cancer risks. I mentioned ovarian cancer, lifetime risk up to 35%, stomach cancer up to 15%, small bowel cancer is a much rarer cancer, urinary tract cancers like ureter cancer, renal pelvis cancer, bladder cancer are rarer 1-15%, and very rare only a few percent are cancers like bile duct cancer or pancreatic cancer and certain types of skin cancers.

If someone is diagnosed with Lynch syndrome, then other family members can be screened but of course at the end it is their own choice if they want to be screened to find out if they have also inherited the mutation. As you know every cell have two copies of each gene. One you inherit from your mother, and the other one from your father, and the Lynch syndrome gets inherited in a dominant way. So, you have a risk of 50% of inheriting the gene with a mutation and 50% of inheriting the gene without the mutation. So, a child has 50% risk of having the copy with a mutation. This is why it is important for children but also for sisters, siblings, parents, aunts, etc. to have the choice of being screened for the same mutation. Because if someone has not inherited that copy, then they have no increased risk and don't have to do all of those screening measures. So, it is really important for everyone to know what the consequences of Lynch syndrome are.

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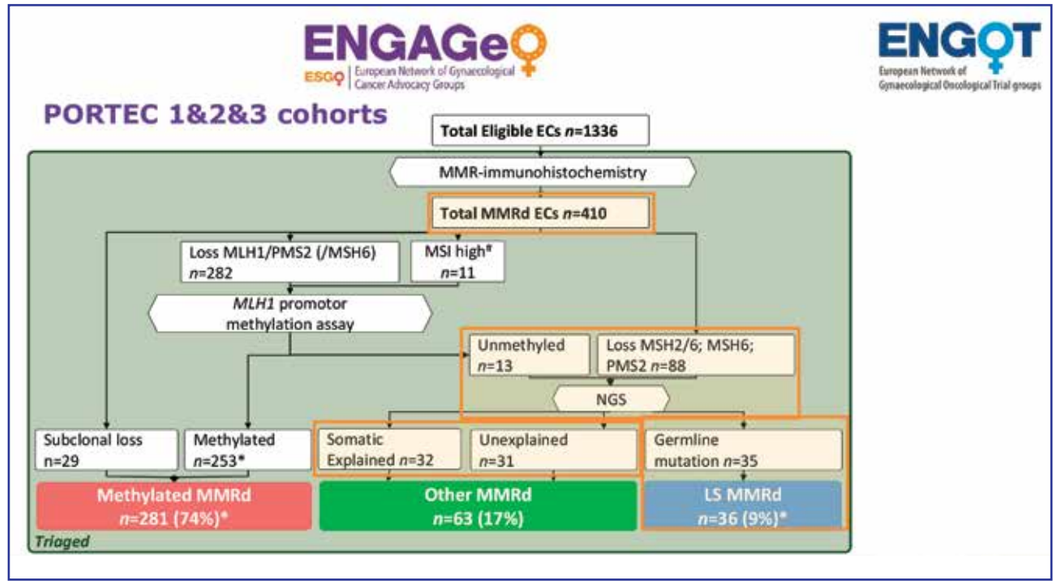


We did a big analysis of the PORTEC 1&2&3 cohorts, which are three trials with a total of 1300 women with endometrial cancers. We tested them all for mismatch repair deficiency and we found 410 with mismatch repair deficiency, so indeed that is 30%. Then we did further testing if this was a hyper-methylated cancer or if MSH2 or MSH6 had loss of expression, or isolated PMS2 loss, and for those we did next generation sequencing for all of the mismatch repair genes and in this big analysis we found 36 women with Lynch syndrome, 3% of the total cohort of endometrial cancers and about 9% of the mismatch deficient repair cancers. 74% had methylated mismatch repair deficiency and the other 17% had other local tumour-related mutations. So, we confirmed that out of all endometrial cancers one in three has mismatch repair deficiency, and about 10% are caused by Lynch syndrome so that is 3% of the total cohorts.

These are the types of genes that were affected in our cohort and half of them were MSH6. This is more than in analysis of Lynch Syndrome in colon cancer patients. This maybe because the endometrial cancer cohorts are relatively older, and more often relatively older women have MSH6; 17% were MSH2, 28% were PMS2 and only 5% were MLH1 not hypermethylated.

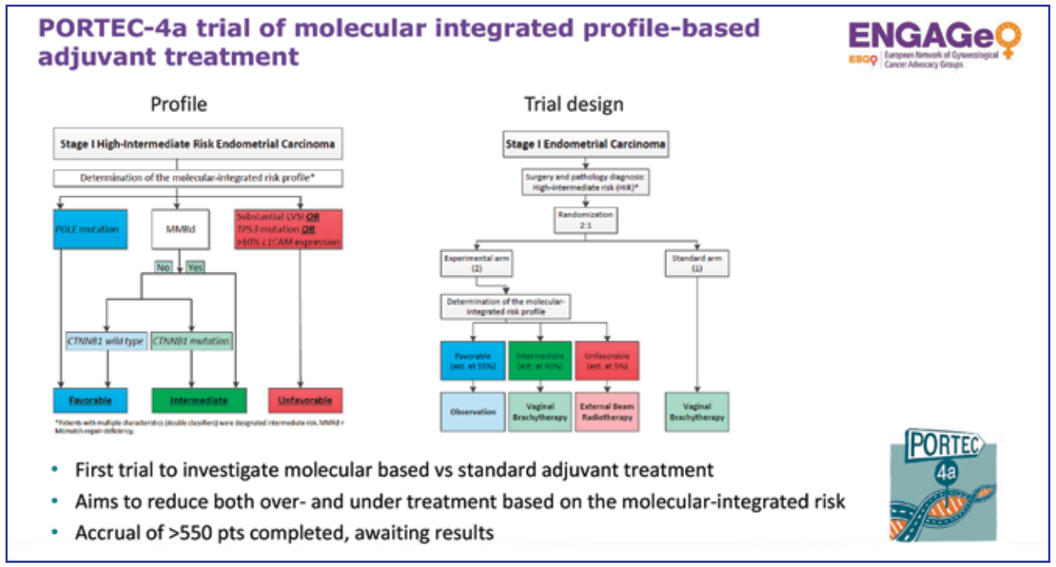
We found that of all the women with methylated mismatch repair deficiency - and as I said 9.5% Lynch syndrome - the women with Lynch syndrome but also those with the double somatic events were younger (median about 60 years of age) while those with methylated metabolic deficiency were 76 years of age median. So that was significantly different. That means that Lynch syndrome and also the none-methylated cancers affect younger women than the others. And we found a small difference in recurrence-free survival and also in overall survival, where those with methylated mismatch repair deficiency - the older women with no Lynch syndrome were at a somewhat higher risk of recurrence and somewhat lower survival than those with Lynch syndrome.

The second primary cancers, which were found in our total cohort of 1300 cases, were not really that much different, although second primary cancer are more frequent among the women with Lynch syndrome - 11% while they were 3-5% in the other women, so it was a slightly higher risk of a subsequent cancer after endometrial cancer, and it was more often in a Lynch syndrome diagnostic cancer - colon cancer and we had one with ureter cancer. So of course, these are still small numbers but it is more often

than the other women that had 5% subsequent cancers and of those colon cancer was most frequent but that was only 5%, compared to 11% Lynch syndrome women.

So, what is the impact now of the molecular groups and now I am not talking specifically about Lynch syndrome but molecular groups in clinical practice and in treatment. We did an analysis of the 10-year results of the PORTEC-2-trial for our intermediate risk endometrial cancers, which were randomized to external beam radiation or vaginal brachytherapy. And also, in the 10-year analysis we analysed the molecular groups, and again we found this clear difference in outcomes between the POLE tumours, those with mismatch repair deficiency, those with no specific molecular profile and the P53-abnormal cancers, which had the worst prognosis. Only the very small group that had either P53 or substantial lymph-vascular invasion, or L1-CAM overexpression had a somewhat higher risk of pelvic recurrence than those, who had vaginal brachytherapy, while the large group of women without those unfavourable factors had similarly very low pelvic vaginal recurrence rates with either external beam or brachytherapy, so maybe only this small group with those factors would benefit from external beam radiotherapy.

And we used that in the PORTEC-4a trial - a trial that recently closed for completed accrual - to do a trial where we introduce the molecular groups to determine the best adjuvant treatment for the first time. In this trial we recruited women with high-intermediate risk endometrial cancers, and they were deemed unfavourable if they had P53 mutation or substantial LVSI or L1-CAM overexpression. The profile was favourable if POLE was found or none of the other factors, and if the case had mismatch repair deficiency or beta catenin mutation, then it was intermediate.



So, in this trial we randomized 2:1: Two women to the experimental arm and one woman to the standard arm of vaginal brachytherapy, and here in the experimental arm if the profile was favourable, we did no adjuvant treatment, so we spared this large group of women extra treatment. We gave external beam radiation to the small group with unfavourable factors, and the intermediate group received the same vaginal brachytherapy as the standard arm. This is the first trial of molecular vs standard treatment in the world. More than 550 patients have been included, and we are awaiting results. It aims to reduce both too much treatment but also too little treatment and designate the appropriate treatment based on the molecular risk group.

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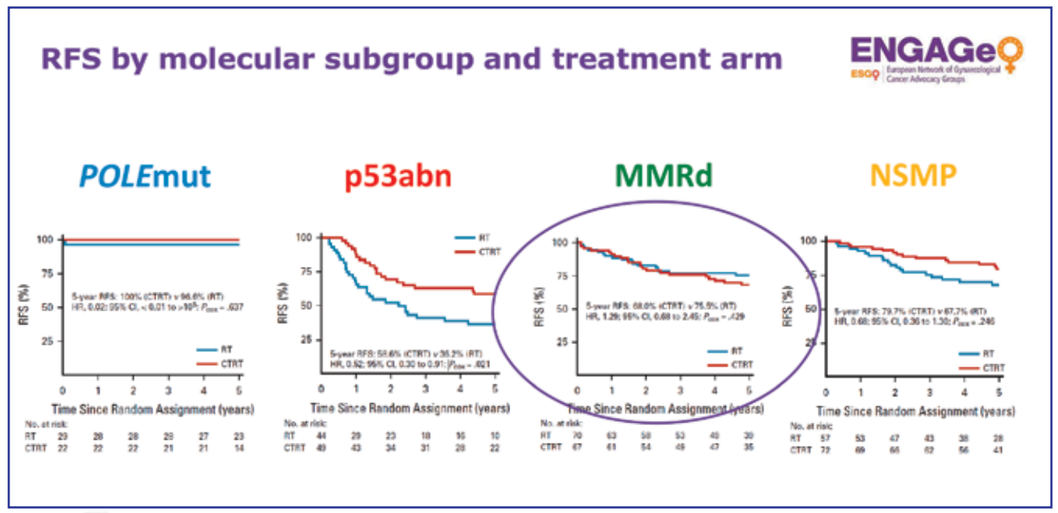
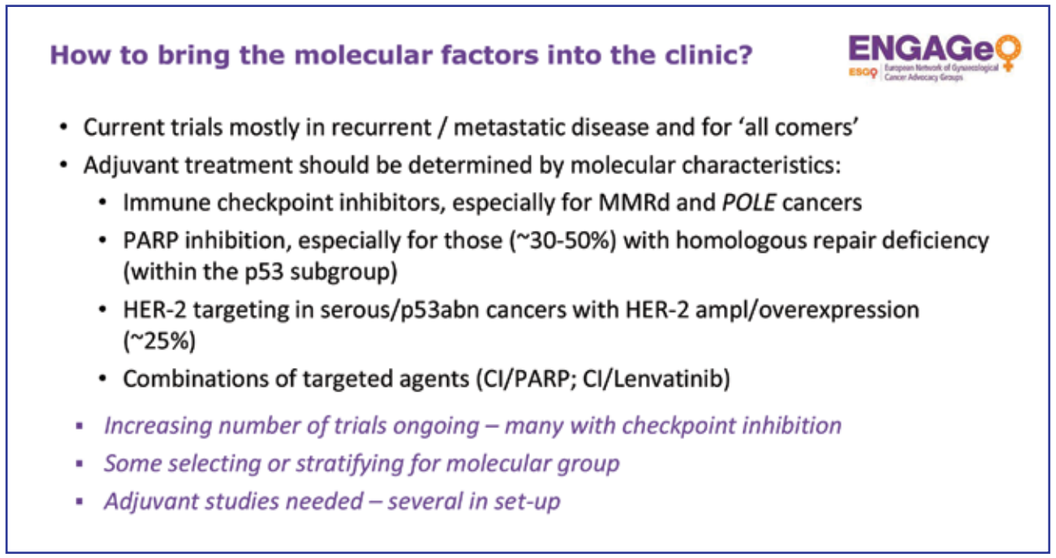
We also did a similar analysis after we completed PORTEC-3, which was a trial for higher risk endometrial cancer with more grade 3, more advanced stage and more non-endometroid cancers, and we compared radiation alone - external beam radiation – in the one arm, with chemotherapy together with radiation and adjuvant chemotherapy in the other arm. This was similar chemotherapy to the one ovarian cancer patients receive. In general, we found a 5% better survival with the addition of chemotherapy, but this benefit was mostly found in the women with stage III cancers or those with serous cancers.

Therefore, we thought after the trial that the best recommendation for the addition of chemotherapy would be to women with serous cancer or stage 3 cancers. But when we did this trial where there was also quality of life analysis, we could see that adding chemotherapy causes more adverse events, more side effects during and shortly after treatment but also after 3 and 5 years. Slightly more women in the chemoradiotherapy arm had adverse events compared to the group that only received radiation, and the majority of the side effects were neuropathy symptoms like tingling or numbness or feeling of weakness, and of course we would like to prevent such extra toxicity by selecting the appropriate women for the addition of chemotherapy. And if we looked at the four molecular groups and compared this to PORTEC 1&2 (which were the high intermediate risk cancers, more favourable than PORTEC 3), we did indeed find differences in the frequency of the molecular groups. One in three had a mismatch repair deficiency, which was not very different from the PORTEC 1&2, but in PORTEC-3 we had fewer women with no specific molecular profile but more P53 mutant cancers and more POLE cancers.



When we looked at the prognosis in this high risk group again, we found a significant difference between the four molecular groups, so even in this higher risk cohort there were some with a really good prognosis, and mainly the P53 mutant cancers had the poorest prognosis.

So again, it was really very important to look for the molecular groups, and then we further looked per molecular group in the treatment arm, and we found that POLE cancers in both groups had almost no recurrence, only one recurrent cancer. So, you can question any adjuvant treatment here, while the P53 abnormal cancers were the cancers which benefitted strongly and significantly from the addition of chemo. A little bit like the sensitivity of ovarian cancer to carboplatin/paclitaxel. So here, chemotherapy should be the standard treatment together with radiation therapy. But in contrast, the mismatch repair deficient cancers in the PORTEC-3 cohort did not have a benefit from chemotherapy, so this might be the group where immune checkpoint inhibition would be a better treatment than chemotherapy. And for the group without a specific molecular profile, the benefit of chemotherapy was similar to what I showed you for the whole cohort, but less significant because it's only a small group, so here chemotherapy seems to help somewhat on the prognosis.



So how to bring these factors into our daily clinical treatments? Currently, the clinical trials which are being done and have been done, are mostly in recurrent disease, and often women, who have this metastatic or recurrent disease, have not yet been selected by molecular group. And we think that more and more treatment both for metastatic disease, but also adjuvant treatment should be determined by molecular characteristics because as I showed you, immune checkpoint inhibition seems very efficient for mismatch repair deficient cancers and also for the rare POLE cancer with recurrence. In the meantime, the most recent trials have shown strong benefit of immune checkpoint inhibition, given together with chemotherapy, in first line treatment of women with metastatic disease.

PARP inhibition might be attractive within the group with P53 cancers because they are similar to the ovarian cancers - 30 to 50% may also have homologous repair deficiency.

HER-2 targeting might also be attractive within the serous P53 abnormal cancers because about one in four also have HER-2 overexpression and might benefit from HER-2 inhibition.

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And some combinations of targeted agents are already being studied like a checkpoint inhibition with a PARP inhibition or checkpoint inhibition with a multi-target VGEF inhibition.

Increasing numbers of trials are currently ongoing. Many use checkpoint inhibition. Currently, some are now selecting or stratifying for molecular group or at least collecting tissues. We need more adjuvant treatment studies, and this is one of an international collaboration where we try to develop a platform of trials with new agents for each molecular group separately, so chemotherapy with or without PARP inhibition for P53 cancers, radiation therapy with or without a checkpoint inhibition for mismatch repair deficient cancers. For NSMP cancers with higher stage disease the trial will compare chemoradiotherapy with hormonal treatment because these are the cancers with a 95% oestrogen and progesterone receptor positivity, so we could use hormonal treatment to have less toxicity and similar outcomes if we select ER and PR positivity. **For POLE mutant cancers, we believe that we could do with less treatment because they have such a good prognosis in all of the cohorts so far. These are new trials.**

SO, TO CONCLUDE MY TALK:

Treatment of women with endometrial cancer will be more and more individualized based on molecular risk factors. This may lead to less toxic treatments and use of better targeted treatments based on the molecular characteristics.

About 30% - one in three women - with endometrial cancer have mismatch repair deficiency and about 10% of those women with mismatch repair deficiency have Lynch syndrome which adds up to about 3% of the total number of women with endometrial cancer. And we now believe in screening for Lynch syndrome based on analysis of the women with mismatch repair deficiency. Then we would do a methylation test if applicable, and a referral of only those women that have suspected Lynch. This would be more efficient than using age and family history-based criteria because in the cohort I showed of women with Lynch syndrome, 17% of these women were over 70 years of age and would not have fulfilled the criteria for age-based screening or for family history. **So, it is a much more efficient way to include all women and it is easy to do.**

So that is the end of the presentation. Thank you for your attention. I am open to any questions you might have.

Birthe:

- Thank you so much. That was very interesting. Maybe I have the first question for you.

Do you think that this group of women of endometrial cancer need a lot of testing to put them into the right group and into the right treatment? It becomes almost individualized treatment. Do you see that as a problem in Europe?

- I personally do not see it as a problem that a treatment becomes more individualized because my experience with counselling women is that they like being told that they are individualized basically but of course, yes, the pathology labs would need the resources and techniques to do this testing. In PORTEC 4 we collaborated with several countries and had good, well-equipped pathology labs doing the same tests in all of those countries, but the budget is sometimes a big discussion. It is sometimes also strange because in some cancers like lung cancer and brain cancer, molecular testing has been introduced without

any discussion about the costs and every pathologist is just doing this. Nowadays if I speak about the Netherlands, every pathologist would also screen for mismatch repair deficiency in colon cancer and I think in many of the European countries and, now because what we know about endometrial cancer is relatively new, in various places they still have to think about it and also because it is now in the WHO criteria and in the ESGO guidelines, it is getting more and more well-known and bigger pathology labs can do this and in the end if you look not only at the costs of the pathology test but of preventing treatment, which has higher cost than doing the pathology tests - if you treat a woman with highly toxic but not effective chemotherapy or unnecessary radiation therapy, you save much more money than you spend by doing the pathology test.

And the second question is that the immunohistochemistry is more easily available and less expensive, and many labs have now started doing at least the testing for mismatch repair deficiency and for the P53. **The POLE mutation because of DNA sequencing is more expensive** and several groups are now also in the process of developing more focused, cheaper POLE tests. So that will also come in a couple of years. I know I taught you a lot, but you can read it back at leisure.

Birthe:

- Sounds really good and important to that disease.

Any questions from the audience? No questions, fine. Thank you so much.

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2nd webinar

Webinar on the work of a pathologist

January 31, 2023

The role of the pathologist in the diagnosis of gynecological cancer

Presented by Prof. Xavier Matias-Guiu

- President of the International Society of Gynecological Pathologists (ISGYP).
- Chairman of Pathology, Hospital Universitari de Bellvitge and Hospital Universitari Arnau de Vilanova, Spain.
- Professor of pathology at Universities of Barcelona and Lleida, Spain.

Birthe Lemley introduced the professor and thanked him very much for having taken the time to talk to the patient advocates in the Clinical Trials Project

What is a pathologist?

Professor Xavier Matias-Guiu started by mentioning an article from the journal called **The Pathologist**, which was written by his friend the pathologist José I. López. The name of the article was **The Invisible Doctor**. José I. López wrote: ***"The pathologist is the invisible doctor because the patient never sees him/her."*** **But professor Xavier Matias-Guiu said that he would try to convince us that the pathologist is very important for the patient and so is the patient for him.**

The professor continued as follows: **the pathologist is the medical doctor that determines the diagnosis of diseases based on the microscopic examination of tissue samples.** We call them biopsies, or surgical specimen, or cells (Cytology), and we also perform molecular tests on these samples. We do not perform molecular tests on blood - only on tissue samples.

The patient usually never meets the pathologist, but particularly in cancer the pathologist is responsible for the diagnosis of the lesion. Technically, a patient does not have cancer until the pathologist makes such a diagnosis based on a biopsy or cytology.

The pathologist not only makes the diagnosis of cancer, but as you will see, he also ascertains the type of cancer that the patient has. This is very important because there are many different types of tumors. And they have different prognosis and different kinds of treatment. The patient is always in the center of the pathology practice.

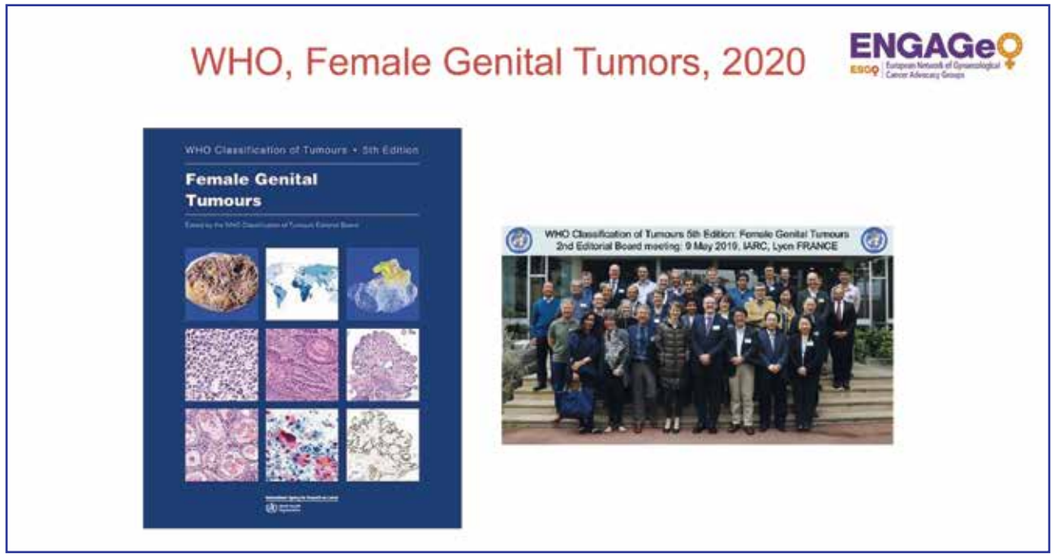
The professor also mentioned that sometimes if the diagnosis was very difficult, he would remember the patient's name afterwards.

Pathology starts with the clinical history of the patient and ends with a pathology report that is sent to the clinician. There will then be a meeting of the multidisciplinary team where the pathologist tries to answer all the questions raised by the members of the multidisciplinary team.

The final result is the pathology report. It contains a diagnosis that is based on a microscopic examination and a molecular analysis of tissues. We try to answer all questions raised by the clinician. The pathology report is standardized. We are following different rules.

In the International Collaboration on Cancer Reporting (ICCR) – there are rules of how to report ovarian, endometrial, and cervical cancer - any type of cancer. We gather a group of experts and discuss for several months. At the end we do the reporting. There is a template for that.

We have a classification - WHO, Female Genital Tumors, which is updated every 5 – 10 years.



The end of our work is a report, which is shown to other clinicians managing patients with gynecological cancer - surgeons, medical oncologists, radiation oncologists, radiologists, nurses, and pathologists. Together we discuss the best clinical management of the patient. The role of the pathologist is crucial in MTB - the multidisciplinary tumor board. We make decisions together.

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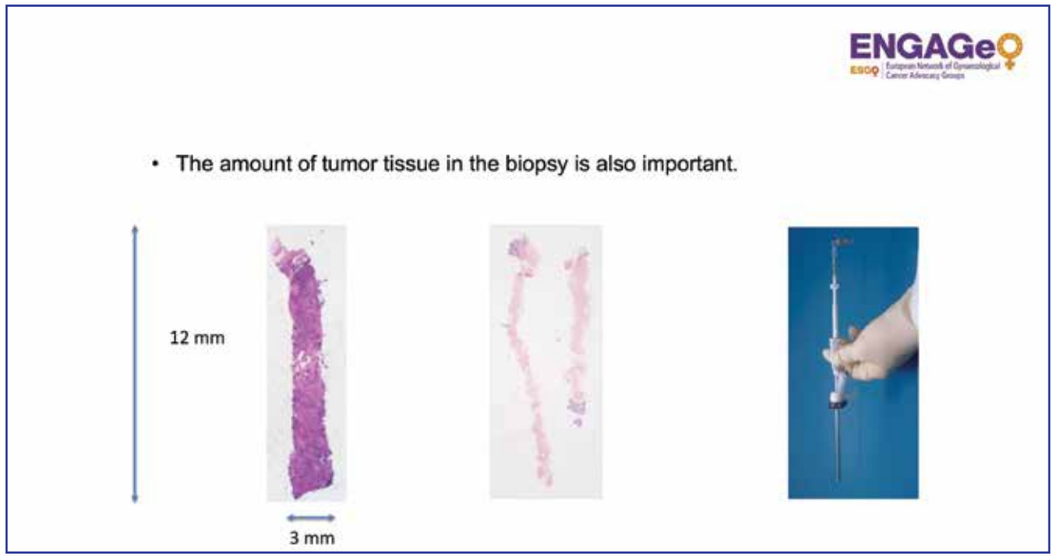
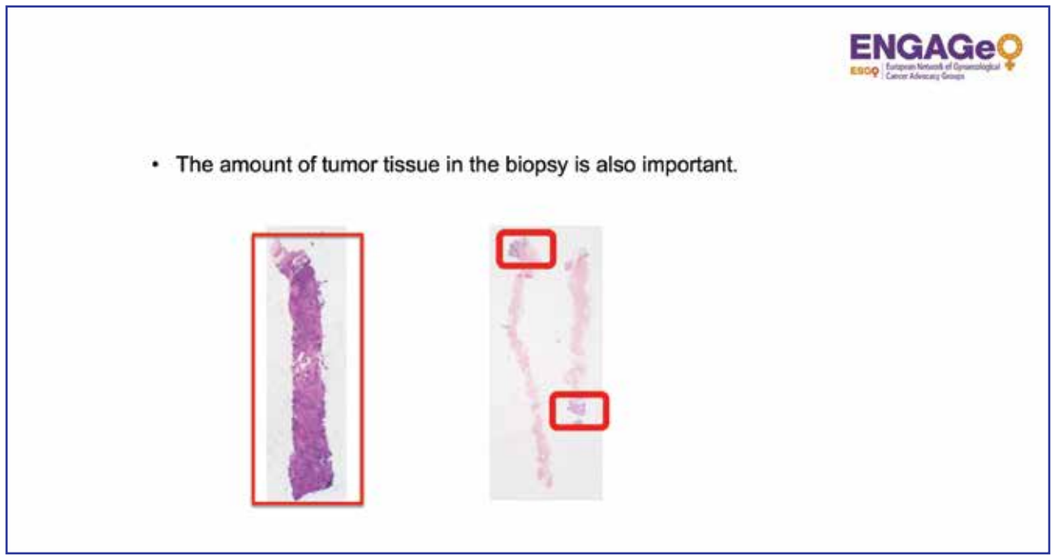
Another important issue, which you are probably not aware of, is handling the small piece of tissue. Because the tissue clinicians take, and that we then have to take care of, is sometimes very small. We have very complex machines to take care of this. At the end there is a slide (the section) and paraffin blocks, which contain the tissue of the patient. This is kept in our pathology lab for many years. So, if there is any question raised in five or ten years after the initial surgery, we can address that, because we have the material. This is very important.

Below is a picture of a slide (the section) and paraffin blocks, which contain the tissue of the patients, and an example of how they are being stored.

The amount of tumor tissue in the biopsy is also important. Here you see a biopsy of two patients taken by the radiologist.



One biopsy is 3 mm in width and 12 mm long. In the other case, there are two different smaller biopsies. And the radiologist has the feeling that these samples are ideal. Pay attention to the difference. In the first fragment of cancer - everything is a tumor. But in the next sample - two thin fragments are not all tumor; they have only small parts of tumors in them. That means we must work a lot to find information, which clinicians need from these small fragments of tumor. Diagnosing a tumor in a small biopsy can be tricky if the most informative area is not contained in the biopsy.



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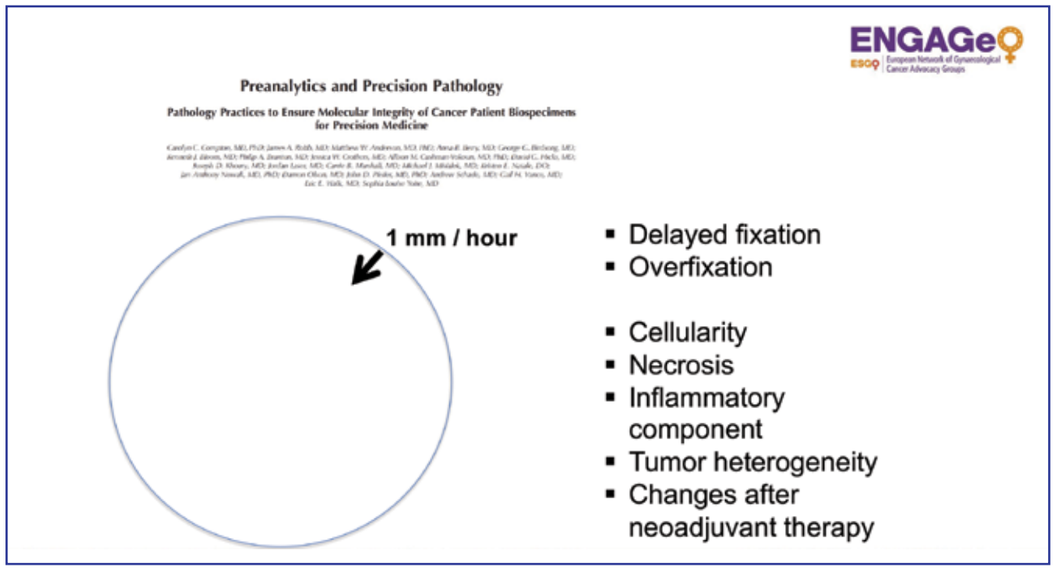
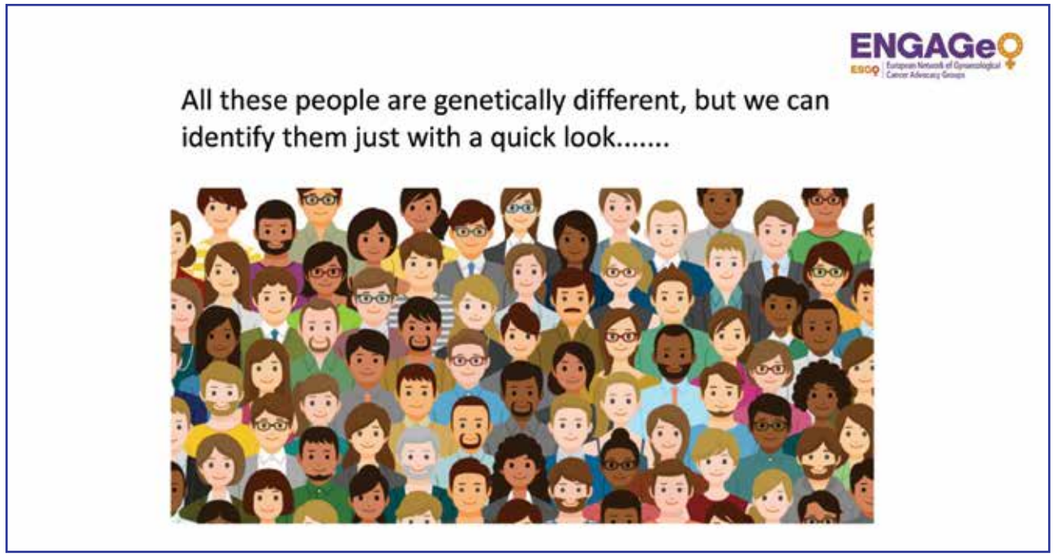
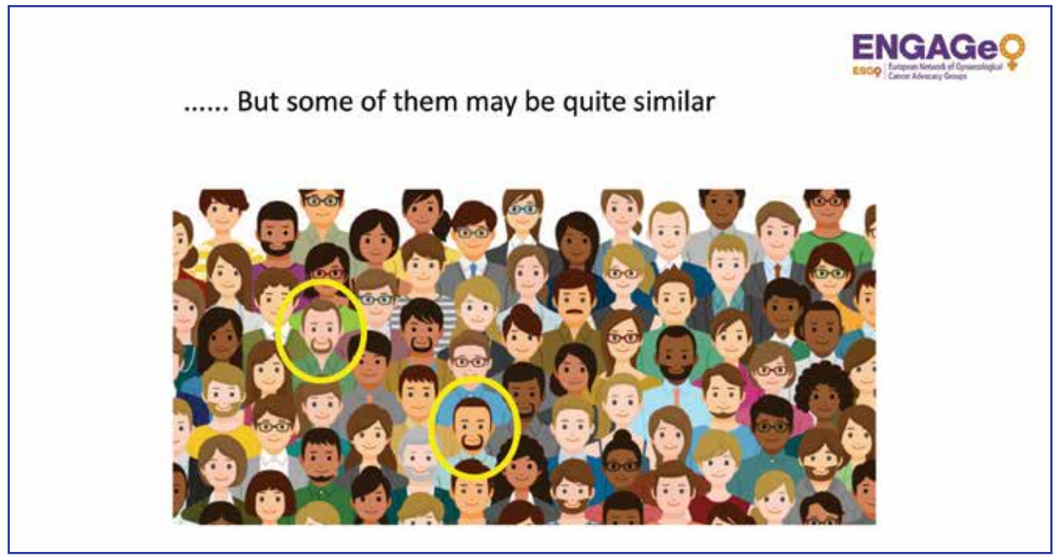
When the patient is surgically operated in the operating room, the specimen – the ovary, the uterus, should come from the operating room to the pathology department. Here time is very important. The longer it takes, the worse for the specimen and the worse for the patient. Sometimes sending those samples from the operating room to the pathology department may take one day. And the tumor is changing its form, the penetration speed for the fixative (formaline, important for handling the sample) is 1mm / hour. This goes against the patient because this is information that we lose.



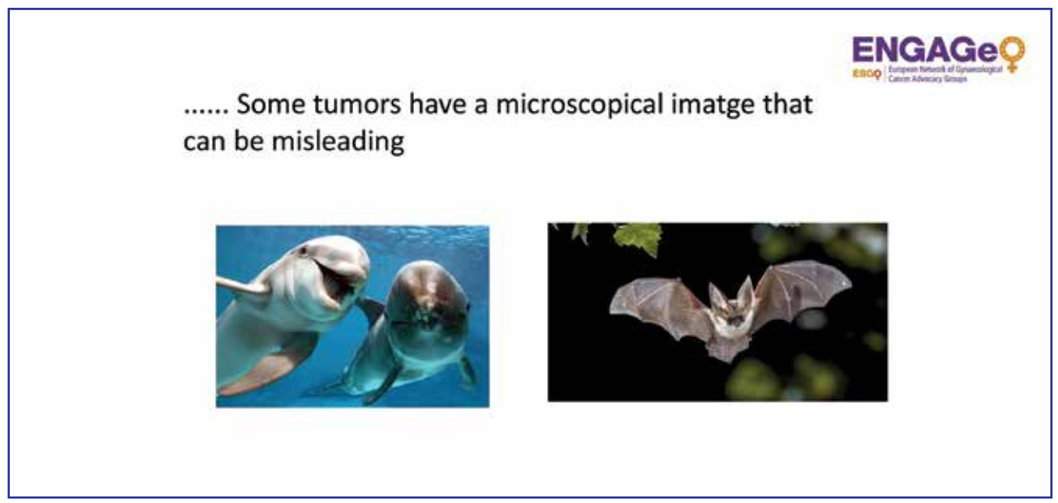
We use the microscope. Microscopical images allow us to see if there is cancer or not and we state the type of cancer that the patient has. Diagnosis of cancer is not always easy. Pathologists are sometimes fighting to make the right diagnosis.

I am going to show you how difficult this is on those few cartoons. For example, here you have several people. By only looking into their faces, you can identify them.

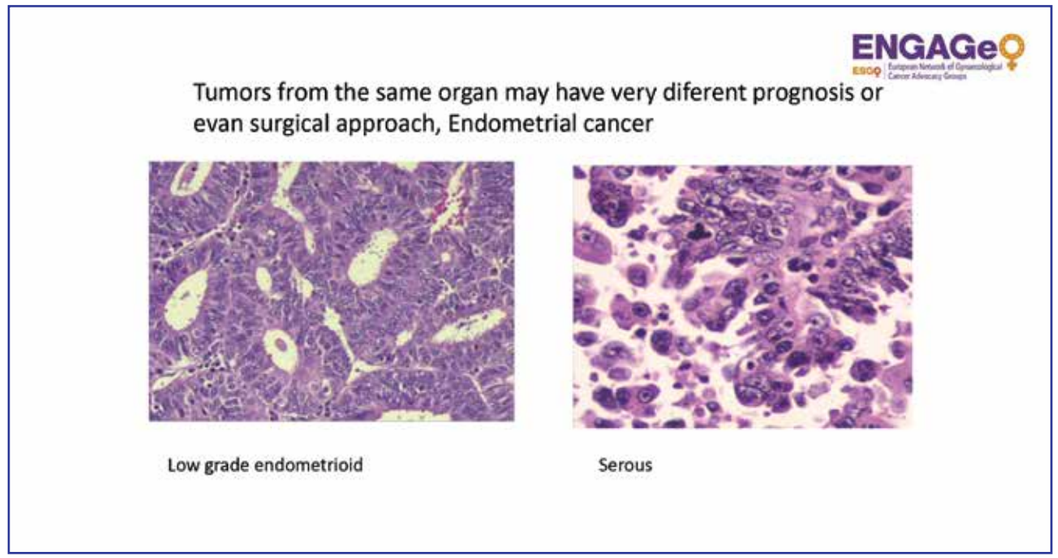




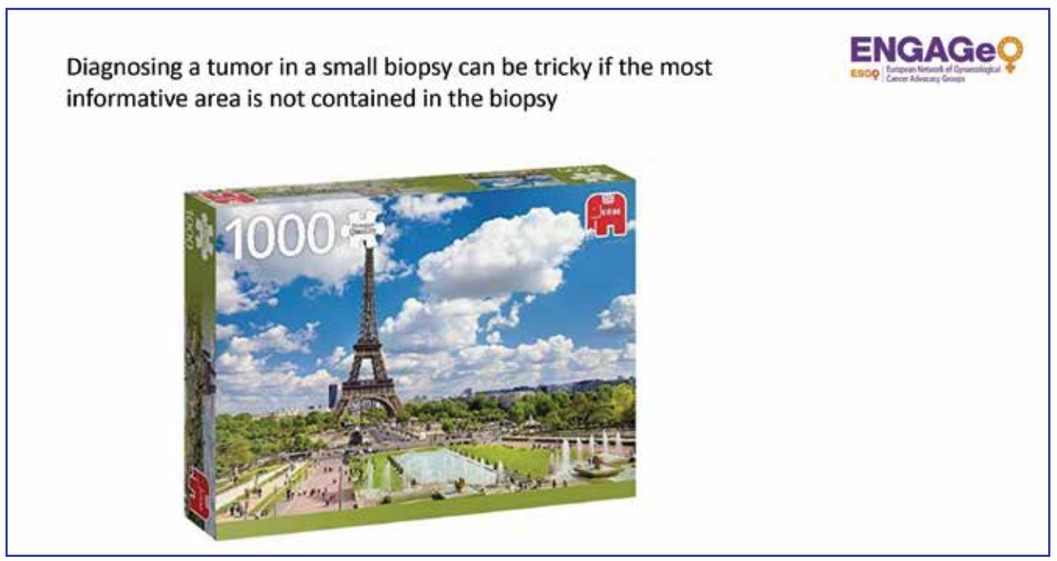
That's what we do. Looking through the microscope, we can easily identify two different tumors. But look what happens in the picture above, two people can be very similar, and yet being different. **Sometimes two tumors that are different look very similar.** That is the problem we have. And even more, like in nature, there are animals, who look like one thing and are another. That is the same with tumors. Dolphins look like fish, but they are mammals. Bats look like birds, but they are mammals. The same happens to us.



Tumors from the same organ may have a very different prognosis or even surgical approach. Our tool here is the microscope. Look at these two samples of endometrial cancer of two patients. Just by looking through the microscope I know that the one on the left – the low grade endometrioid patient – has a 70% chance of being alive in five years. The patient on the right – the serous endometrial patient – has only 40%. So, this is the power of morphology, which is sometimes difficult especially when the samples are not very representative.

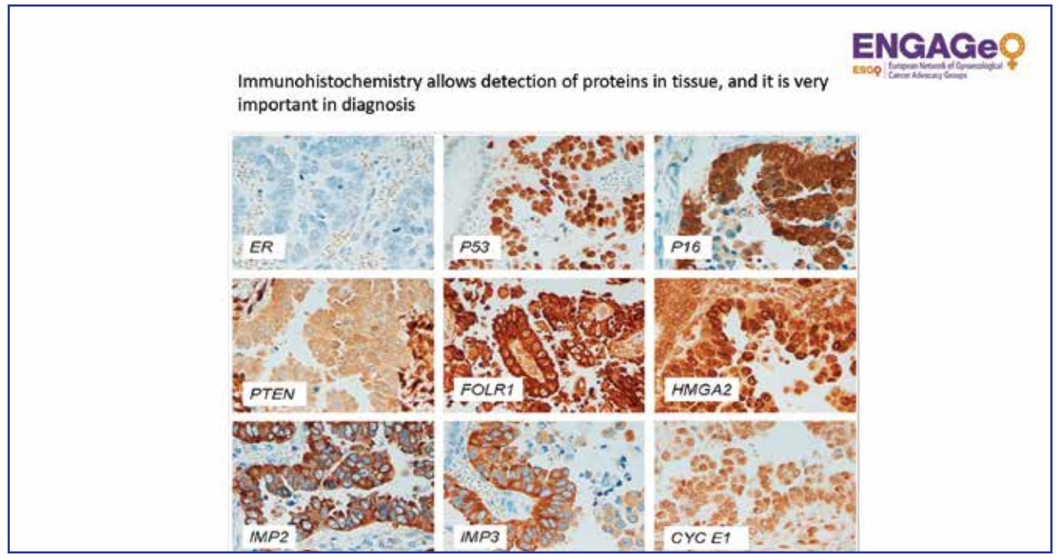


I am going to use another example. **A tumor is like a puzzle that has several pieces. And the biopsy is just one piece of the puzzle.** So here you have the puzzle of Paris. If we take 2 pieces, and on one we can see part of the Eiffel tower, only watching we know that this is Paris. But what happens if we take the piece which corresponds to the sky? Problems. So, we are lucky, because clinicians usually take the part with the Eiffel tower, but sometimes they pick the sky. But even in this case we have tools to see that this is the sky from Paris.



We must use other techniques - detection of Proteins / Immunohistochemistry and detection of DNA or RNA sequences (Molecular Pathology).

Detection of proteins in tissue is very important in diagnosis. For example, this is an endometrial carcinoma. I have used different antibodies to identify different proteins. If you see brown staining in these nine pictures of antibodies, it means that they are positive. If the staining is light-brown, it is slightly positive. By doing that, we know that the sky is from Paris. So, this is what we do every day.



Some of these proteins or DNA/RNA alterations are called biomarkers. They allow distinction of different types of tumors, but also help in deciding upon the appropriate treatment. We have many biomarkers. Let me anticipate something. There is no perfect biomarker.

Digital pathology is an opportunity, leaving the microscope aside, scanning pictures and working on a computer. And this is fantastic. It is used in a limited number of labs, but in five years it is going to be general. In the picture on the next page, it is me in Bangkok, far from Barcelona, looking at the endometrial cancer on a laptop. So, this is the power of digital pathology.

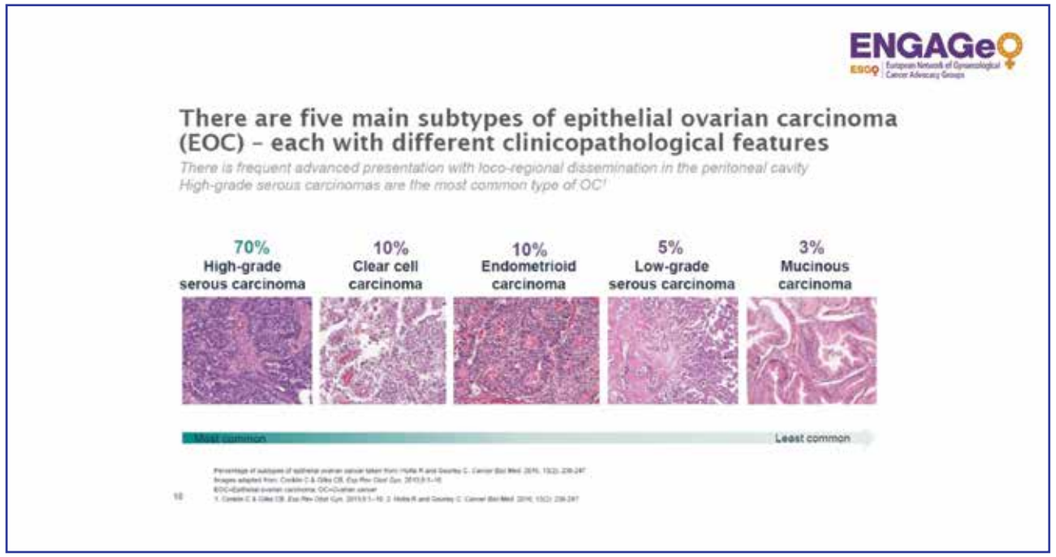
Conclusion of this first part – What is a pathologist?

- The pathologist not only makes the diagnosis of cancer, but also diagnoses the type of cancer that the patient has.
- Tissue management is crucial.
- The pathologist integrates microscopical and molecular data into a standardized report.
- The pathologist plays an important role in the Multidisciplinary Tumor Board.

Ovarian cancer

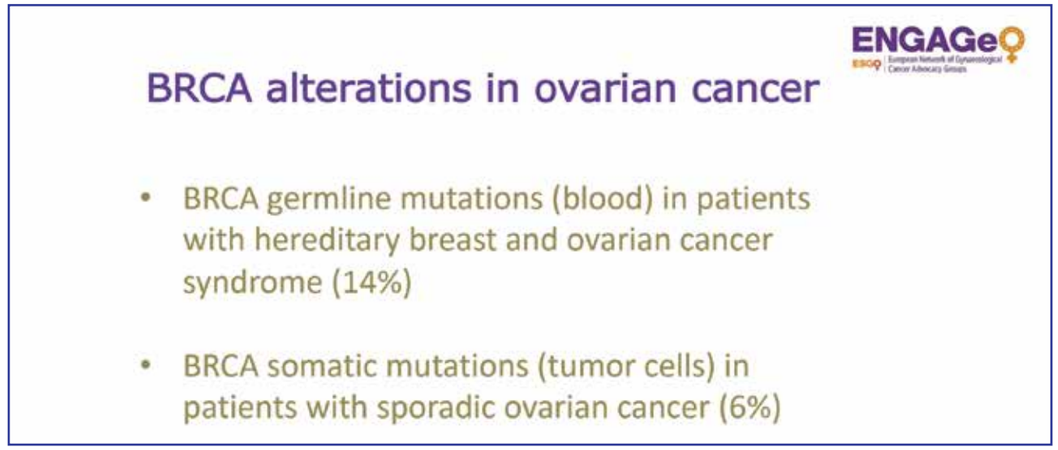
Now I will address the role of pathology in ovarian cancer.

Let me go to the **WHO classification of tumors**. Every five to ten years we meet in Lyon. More than one hundred pathologists work on updating the classification of Female Genital Tumors.



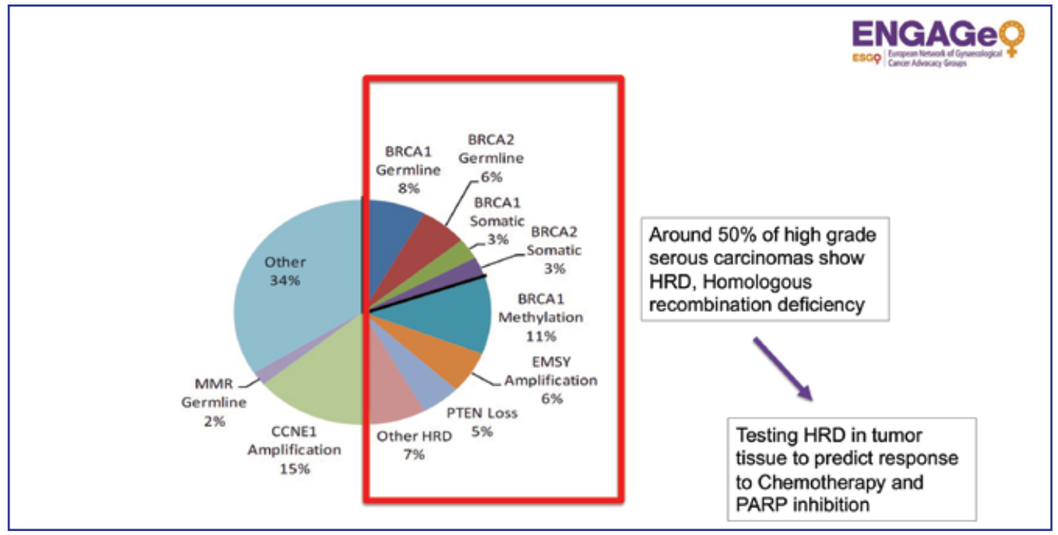
Above we have the five most important types of ovarian cancer. These 5 tumors I can recognize in the microscope. They have different prognosis, also different chemosensitivity. That is why it is important not to make only the right diagnoses of ovarian cancer, but also specify the type of ovarian cancer the patient has. The most frequent one is high-grade serous carcinoma, which is the most aggressive type of tumor. Let us go into that. In the majority of cases morphology – looking into the microscope is enough. If not, we have the proteins. This is good quality control for examining tissues.

And now we go to HRD. I have been working in ovarian cancers for more than 30 years, and more than 25 years ago it was clear that a proportion of the patients with ovarian cancer belonged to families that had the tendency to develop breast and ovarian cancer. The way to identify these patients was to look for germline mutations of two genes - BRCA 1 and BRCA 2 in the blood. Germline mutations are inherited from parents, and they are in the tumor. That is what we were doing 25 years ago. We saw that around 14% of patient had these BRCA1/2 mutations. It was fantastic.



So, at the beginning we were looking for the mutation in BRCA1/2 in the blood. That was done by geneticists not by pathologists, to identify those families. Later, it was clear that patients who belonged to these families had a better prognosis. So those tumors responded better to chemotherapy. It was the other reason for looking for these mutations. Not only to identify these families but also to help in predicting the prognosis. It was clear that ovarian cancer not only had BRCA germline mutations, carrying the tumor in the blood, but they also had somatic mutations (6%), which are present in the tumor cells in patients with sporadic ovarian cancer. Both have a good prognosis.

So, we started to analyze not only the blood but also the tissue, looking for any patient that have a BRCA mutation. It was fantastic because we could identify 20% of patients with ovarian cancer that had a better prognosis and could benefit from a specific treatment. But there was a problem, these BRCA mutation tumors have good prognosis because they have a molecular phenomenon, which is Homologous Recombination Deficiency – HRD. It's not only occurring in those 20% of tumors but in 50%. So, these are good news. That's why tests for HRD started. Tumors with HRD have better prognosis and respond better to platinum-based therapies and PARP inhibition. Homologous Recombination Proficiency is a mechanism that allows normal cells to repair some DNA lesions.



A couple of years ago I was honored to become a member of the panel that ESMO organized to make recommendations about how we should test for HRD. We analyzed several tests, and it was clear that there were two tests that were better. The reason was that they were validated in clinical trials. So, the performance of these tests allowed us to distinguish between patients that responded to PARP inhibitors, and those who didn't respond. Myriad my Choice CDx Plus is one test, the popular one. Foundation Medicine NGS assay is the other test. These two tests analyze alterations happening in the tumor that occur when the tumor has HRD.

Myriad my Choice CDx Plus provides scores that go from zero to one hundred. Clinical trials, basically the PAOLA1 clinical trial, allows us to identify the score 42 that was separating patients that could benefit from PARP inhibition in comparison with patients that didn't benefit. That has been the recommendation. Of course, this is not perfect. And we do not know very well what happens in the tumor that has a score of 43 and the tumor that has a score of 41. Are they really different? Well, this is not perfect, but it works.

Now we have many other tests. Why is that? Because Myriad my Choice CDx plus is expensive and is performed in the US and also in some satellite labs. We are not controlling these tests in our hospitals. There are many academic centers and some companies that have created tests. And we, all of Europe, are now in the process of validating these tests. Their technical level should be compared with Myriad, but also tested in the samples. We are using the tumor tissue samples from the patients that participated in the PAOLA1 trial (the one that validated Myriad my Choice CDx) for validating the new tests. There are chances that these tests will be implemented within a short period of time in Europe.

Pathological evaluation of the tumor tissue specimens

Let me go back to the role of the pathologist. Pathological evaluation of the tumor tissue specimens used for assessment of somatic molecular alterations is essential. But sometimes our tests are not informative. The problem is that the tissue has not been handled appropriately. So, the samples should be handled appropriately by the pathologist so that the test can be informative. This is also an important task.

The pathologist's conclusions for ovarian cancer are:

- Histologic assessment (pathologist role) is important.
- HRD is important in prediction of prognosis and management (response to platinum-based therapy or PARP inhibitors)
- There is a general transition from out-source tests (Myriad and Foundation) to in-house tests (ongoing)
- There is no "perfect" biomarker, there is always a tumor, that is not following the rules.

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Endometrial cancer

There are seven histologic types of endometrial carcinoma, the most frequent tumors.

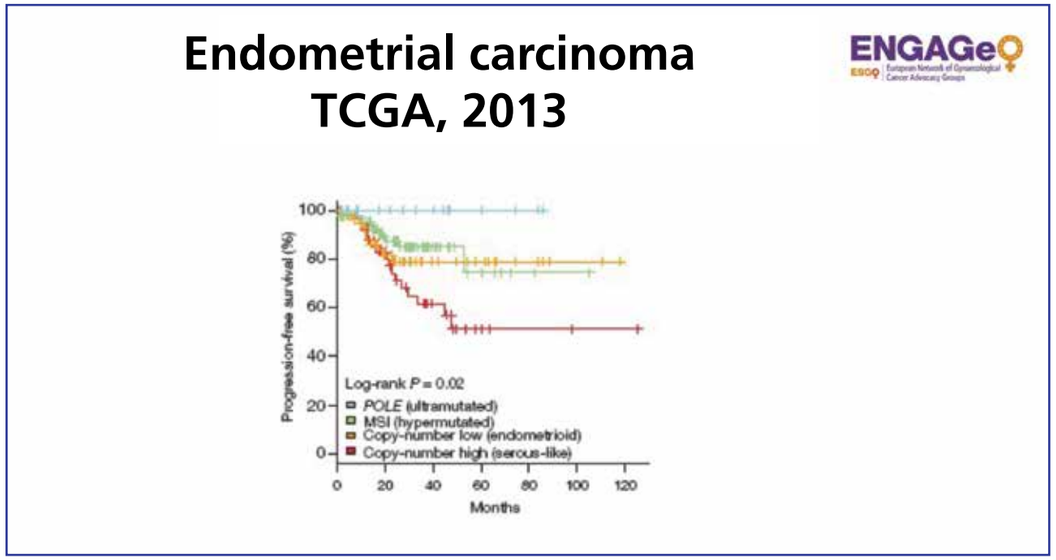
In the ovary it is a little bit easier than in the endometrium. In the ovary those markers, the brown staining is more precise. In the endometrium it is a little more tricky. Fortunately, the vast majority of tumors, 70%, are low-grade, i.e., with good prognosis. There are 30% that are high-grade. Unfortunately, endometrial cancer is far more frequent than ovarian cancer.

Pathologic classification has some limitations:

1-Poor interobserver reproducibility in high grade carcinomas.

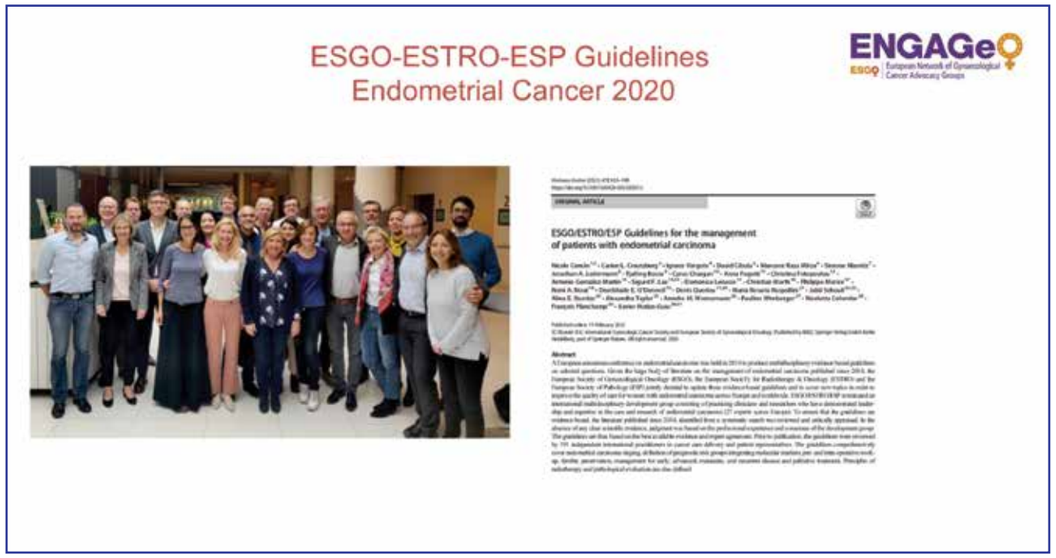
2- Some histotypes are heterogenous regarding prognosis (grade 3 EEC)

We had good news 10 years ago in a journal called Nature. A collaboration of scientists came up with the **The Cancer Genome Atlas – TCGA** by analyzing a huge number of tumors using many different complex techniques, which resulted in the classification of endometrial cancer in four groups. In the first one – POLE (ultramutated) all patients stay alive. The last one is called Copy-number high. It is the bad group of tumors - many patients used to die in this group. And there are two groups in between. That was achieved in a huge number of cases by performing complex techniques. And we cannot do that in every case, for example in places like Pakistan or South Africa. So, we had to look for a surrogate. Just by doing the brown staining, the protein for MMRd, p53 and mutation analysis of POLE, just this small number of tests allowed us to reproduce results of TCGA (The Cancer Genome Atlas).

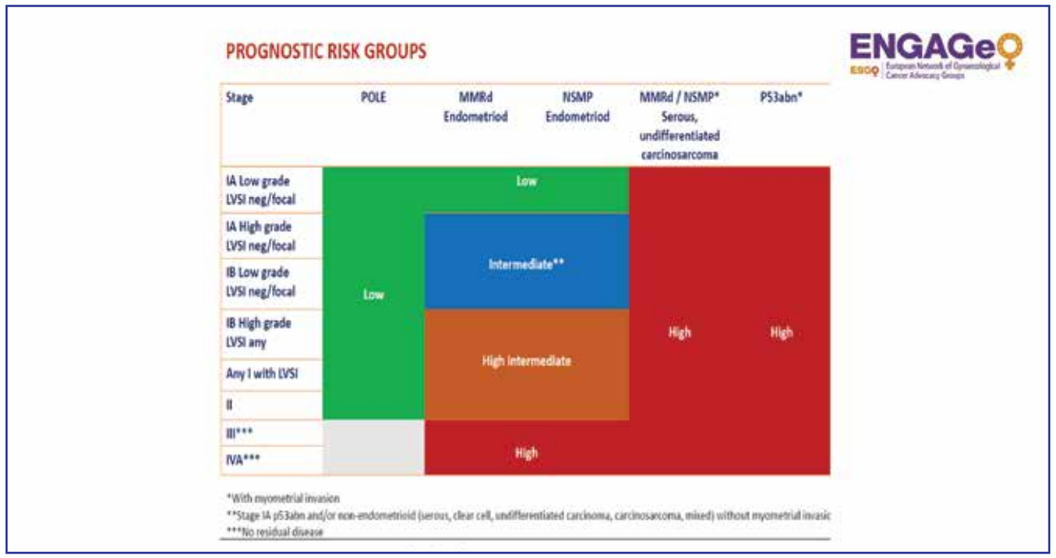


That was done by people from Vancouver and Europe. Our group also contributed and that was fantastic. We had the surrogate - the way, in which we could bring this classification into clinical practice, which is very complex. I'm not going to go into details. Just for your information, when the tumor is POLE-mut, the prognosis is very good. When the tumor has positive staining for the p53 (the brown staining), the prognosis is poor. In between we have pathology, histologic types. Molecular classification is not different, it is part of the pathology.

We are in the process of implementing that to the World. In Europe it is a much more advanced situation than in other countries, also more advanced than in the US.



Below you will find a slide describing the various prognostic risk groups:



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Is conventional pathology still important in endometrial carcinoma

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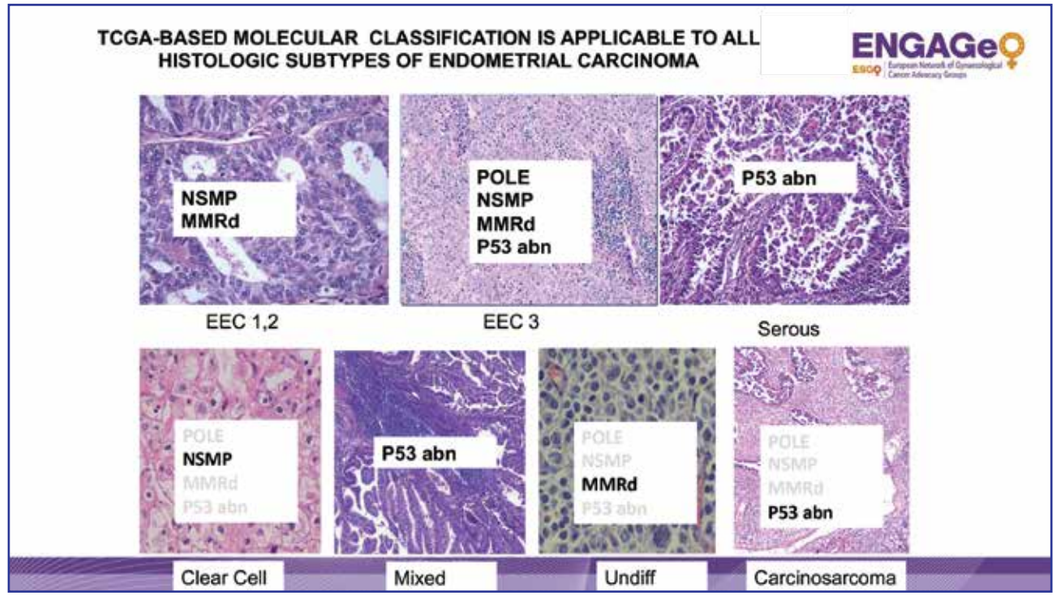
- Histologic typing is important
- Staging is important, and depends on numerous pathological findings (myometrial invasion, cervical invasion, lymph node status)
- There are other important pathological features for prognosis (Lymphovascular space invasion)
- Molecular classification subtypes are heterogeneously distributen among histologic types.



Glossary for endometrial cancer:

POLE (ultra-mutated), **NSMp** (no specific molecular profile), **MMRd** (Mismatch Repair deficient), **P53abn** (P53 abnormal), **ECC** (endometrial endometrioid carcinoma), **serous** – like in ovarian cancer.
Myometrium = the middle layer of the uterine wall
Lymphovascular space invasion (LVSI) = the presence of tumor cells within endothelial-lined channels outside the main tumor

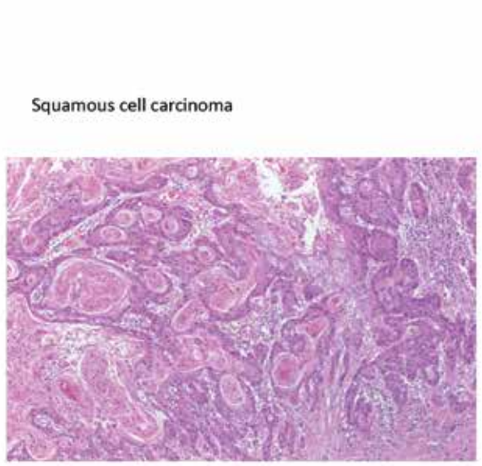
Conclusions for endometrial cancer

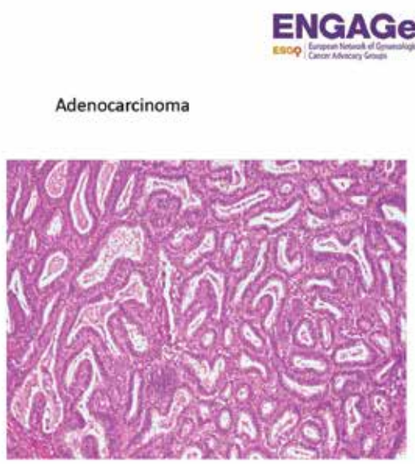
- Histologic assessment (pathologist role) is important
- Molecular classification is important

Cervical cancer

At WHO the cervical cancer classification was modified. Here again we have different tumours that respond differently to treatment and that behave differently. The most frequent is the Squamous cell carcinoma. We also have Adenocarcinoma, where the tumour grows forming glands. But more important is that WHO decided that the presence of the DNA of one virus – papilloma virus was very important in terms of prognosis. So, we amended the classification by introducing the presence or absence of human papilloma virus – HPV.

Y**ou can see that there are many different tumours, some of them are more aggressive than others. And some of them are difficult to diagnose. (So, we must play with the brown stains that you have seen.)**





- **Squamous Cell carcinoma, HPV-associated**
- **Squamous cell carcinoma, HPV-independent**
- **Squamous cell carcinoma, NOS**

- **Adenocarcinoma in situ, HPV-associated**
- **Adenocarcinoma, HPV-associated**
- **Adenocarcinoma in situ, HPV-independent**
- **Adenocarcinoma, HPV-independent, gastric type**
- **Adenocarcinoma, HPV-independent, clear cell type**
- **Adenocarcinoma, HPV-independent, mesonephric type**
- **Other adenocarcinomas**

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Cervical cancer guidelines update was standardized by ESGO. After WHO it was clear that we have to look for HPV. We can do that by looking for the DNA of the virus, but we also have a surrogate, a trick, which is called (stain) d16. So, you can do one or the other. In academic centers we are doing both. There is also another one – PD-L1 testing for the selection of immune checkpoint therapy. PD-L1 is very important in different types of tumors in the body. They are very important in lung cancer, in melanoma, in cancers of the head and neck, urinary bladder and of course in cervical cancers.

Cancers learn to become invisible to the immune system, so PD-L1 helps us to detect the tumors that have become invisible. And that is an indication for what is called immune checkpoint inhibitors.

Here you have one of them – pembrolizumab.

FDA approves pembrolizumab for advanced cervical cancer with disease progression during or after chemotherapy

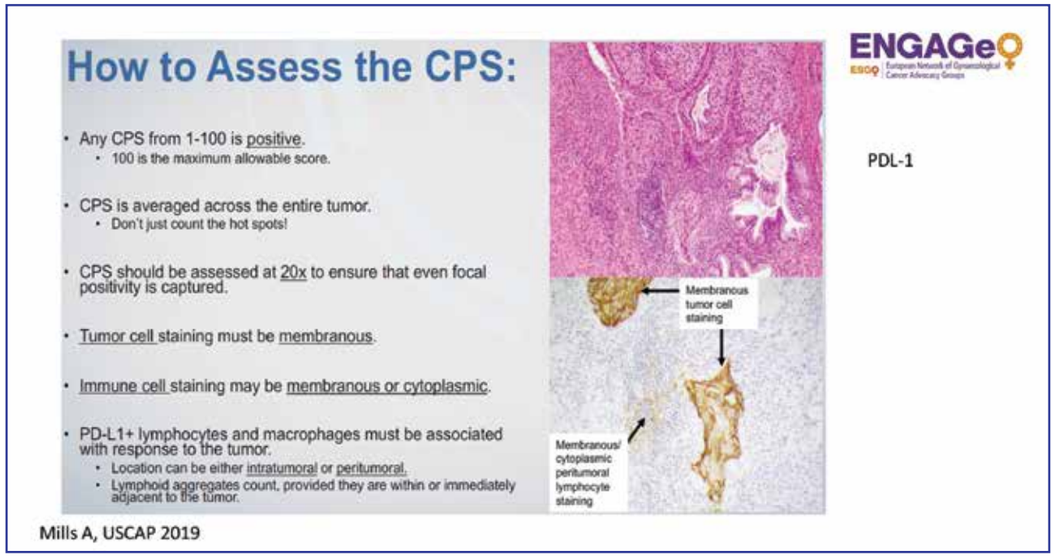
On June 12, 2018, the Food and Drug Administration approved pembrolizumab (Keytruda, Merck and Co. Inc.) for patients with recurrent or metastatic cervical cancer with disease progression on or after chemotherapy whose tumors express PD-L1 (CPS ≥1) as determined by an FDA-approved test.

European Commission Approves Merck's KEYTRUDA® (pembrolizumab) Plus Chemotherapy, With or Without Bevacizumab, for Patients With Persistent, Recurrent or Metastatic Cervical Cancer Whose Tumors Express PD-L1 (CPS≥1)

4/29/2022

Approval Based on Overall Survival Benefit Demonstrated in Phase 3 KEYNOTE-826 Trial

But there are others. FDA approved pembrolizumab for advanced cervical cancer with disease progression during and after chemotherapy, whose tumors express PD-L1. This is not easy. It is actually very complex, so we are making rules.



This picture is a presentation of pathologist A. Mills from USCAP. We, ESGO, asked her to provide rules for all of us to do the same. As you see there are many tricks to do so. We are now improving. What's very important is that we are reproducible. PD-L1 is being assessed the same way in patients with cervical cancer in Sweden, in Italy, in Greece and in different parts of the world. It is important that people with cervical cancer are benefitting from this.

Glossary for Cervical Cancer:

PD-L1 = a protein that allows some cells to escape an attack by the immune system. Extending from the cancer cell surface, **PD-L1** interacts with a protein called **PD-1** on important immune system cells called T cells. This coupling — known as an immune checkpoint — instructs the T cell to leave the tumor cell alone. Source: 2023 Dana-Farber Cancer Institute

CPS = stands for combined positive score, which is a method to measure the expression of PD-L1 protein on tumor cells and immune cells in cervical cancer1.

The professor's final conclusion:

- The pathologist is important in gynaecologic cancer.
- Tissue handling is important. We need the tissue in its best condition.
- Molecular analysis (HRD or molecular classification) has to be integrated with conventional pathologic analysis.

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After the sessions there was a period of questions and answers

One of the members in the audience, Anne, thanked the professor for having given us new knowledge about pathology in a patient language and then asked **how you could determine whether PDL-1 was positive or negative**. Xavier's answer was that that depended on how much staining there was, and also that is an area for improvement.

The professor had said that the patient's slide would be saved from 5 – 10 years. The question from one of the patients in the audience was: **What if the patient has a relapse after 15 or 20 years, is the slide then gone?**

The answer was that that depends on the country and the facilities available. At his hospital in Barcelona the tissue would still be available – even after 30 years, but that might not be the case in all countries. In the other hospital where he works, they had no more space for storing all the samples, so they had to store them at a place 50 km away from the hospital. This might be very different across Europe.

There was then a question from Maria **if the patient could get the tissue and store it at her own house.** The professor did not believe that that was a possibility as the sample has to be stored in a specific way, which would not be available in a normal household. Of course it is the patient's sample, but there had been a case where the sample had then been used for a clinical trials, and in that way no longer was available for the patient.

Birthe brought the case with HRD and ovarian cancer up again. We know that in the so-called HR proficient group, the group with a GIS lower than 42 according to the Myriad test, there are still patients that will benefit from a PARP-inhibitor, especially if they had had a good respons to platinum therapy. Xavier's answere was that there is still work going on in Europe to find these patients but it will take time. However, the Myriad test has helped us. It is a good test – although it is not perfect.

Birthe thanked Prof. Xavier Matias-Guiu for having taken the time to talk to us and for all the valuable information he gave us. Prof. Jalid Sehouli, who was present on behalf of ENGOT, also had a few questions to the professor and thanked him on our behalf.

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