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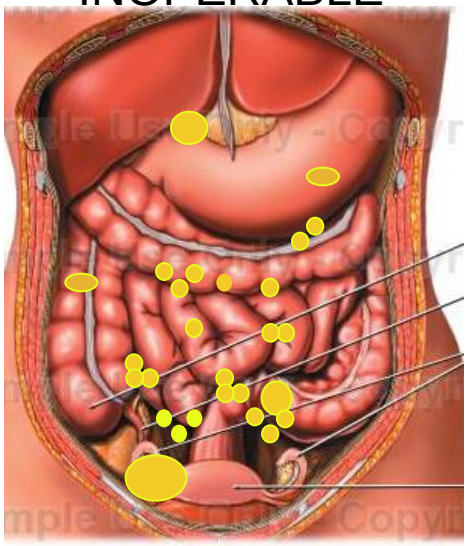
RÉPARATION DE L'ADN, METABOLISME ET INSTABILITÉ GÉNOMIQUE DANS LES CANCERS DE L'OVAIRE

THE NEOADJUVANT SETTING: TRANSLATIONAL RESEARCH OPPORTUNITY

At diagnosis:
INOPERABLE

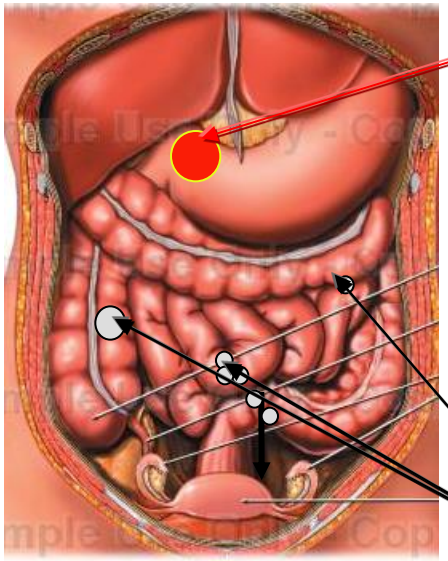


Diagnostic laparoscopy: Tumor sampling pre-treatment

Neoadjuvant Chemotherapy
(3-4 cycles)

Chemosensitive:
RR= 70%

Complete surgery



Nest of highly proliferative tumor cells

Sterilized by chemotherapy

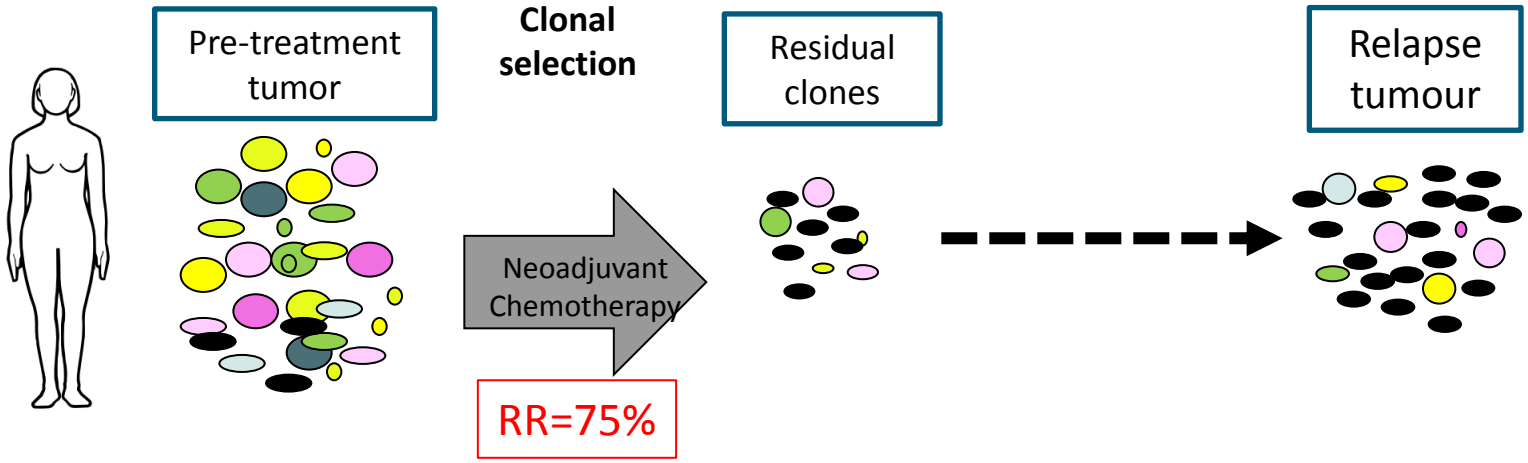
Excision of all residual disease post-chemo

Opportunity to study minimal residual disease (chemoresistant clones?)

Inform treatment sequence (maintenance)

ASCO 2016 Educational Leary A:
Translational value of neoadjuvant chemotherapy in OC

EVOLUTION OF DNA REPAIR COMPETENCY AND GENOMIC INSTABILITY WITH TREATMENT AND RELAPSE



- HGSOC initially very chemosensitive (likely due to DNA repair or HR defects) → RR=75%
- BUT invariably relapse
- Little is known about the evolution of DNA repair competency with neoadjuvant chemotherapy
- Are the surviving clones DNA repair competent? Selected by the chemotherapy?

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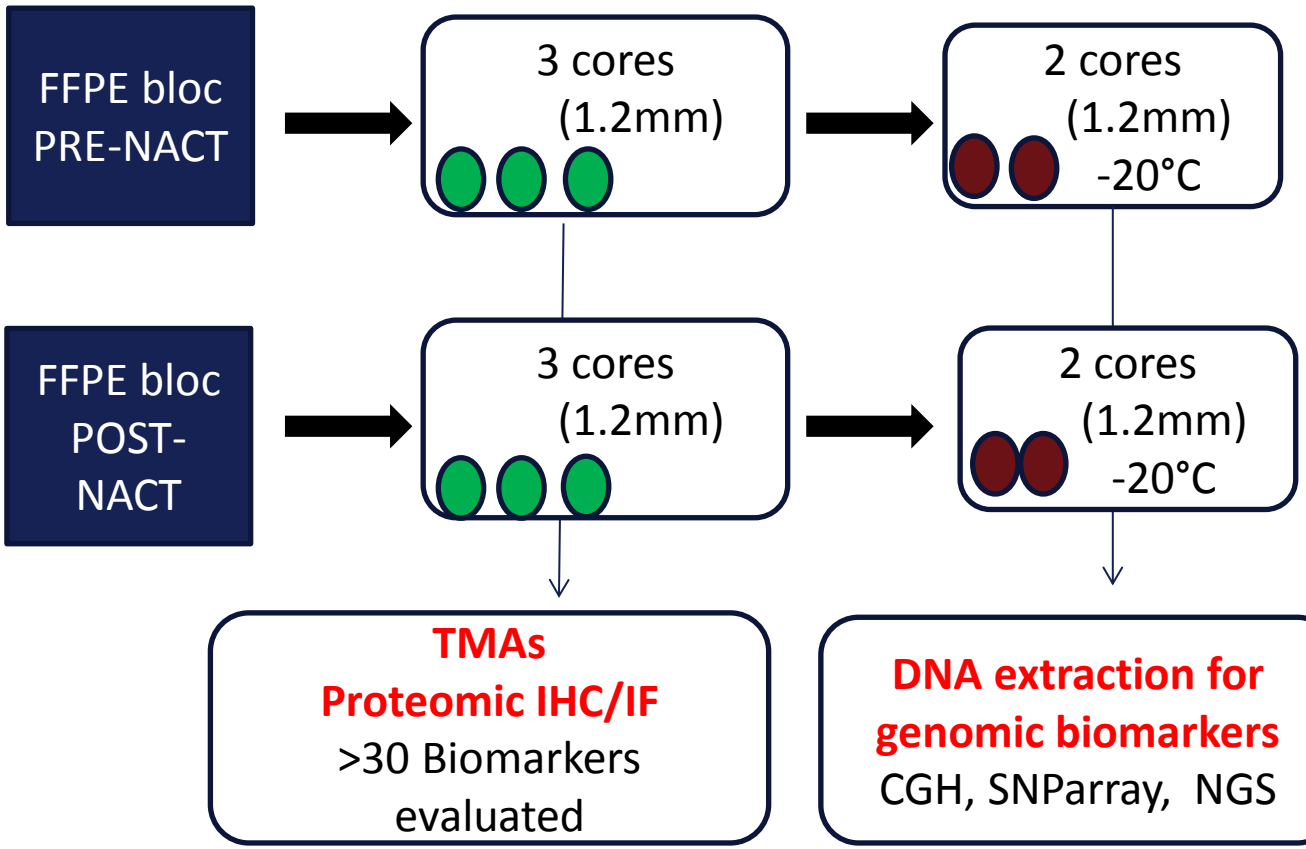
METHODS: OPTIMIZING SMALL TUMOR SAMPLES

Retrospective cohort:
>300 OC tumor samples (pre-, post-NACT and/or relapse)

Prospective cohort:
OVBioMARK
Biological Trial



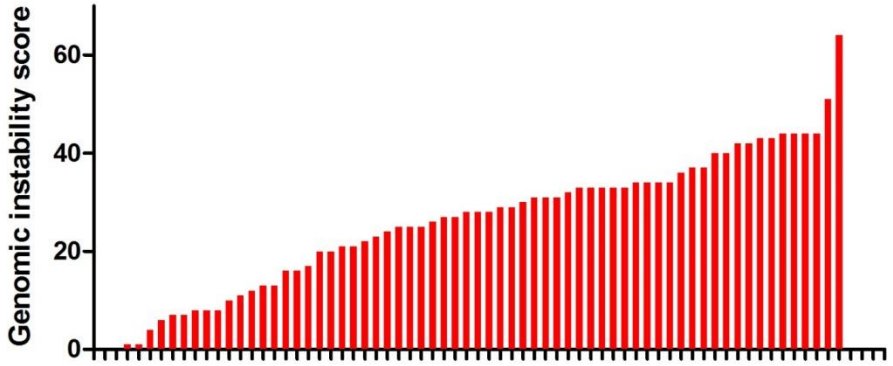
TMAs
+
DNA
+
Fresh tumor or ascites for ex vivo models
+
Blood for ctDNA

GIS AS A MEASURE OF DNA REPAIR COMPETENCY

Development of in house « Genomic Instability Score » (GIS)

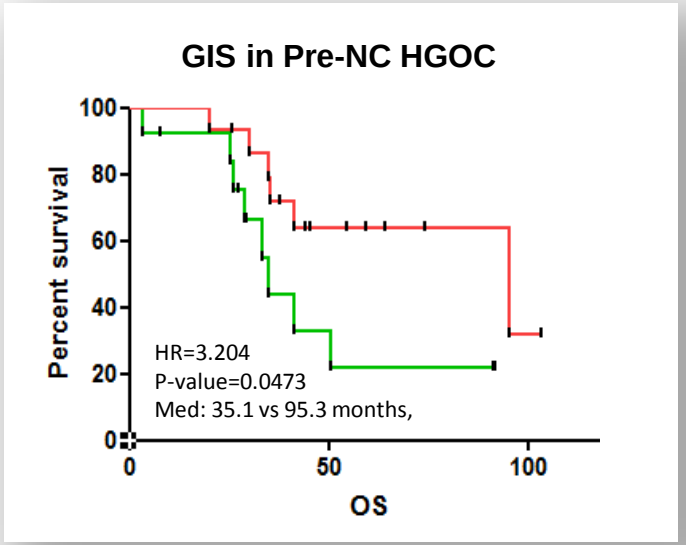
- Number and size of SCNAs
- Validated on both Frozen (CGH) and FFPE samples (SNParray)

- GIS varied greatly among OC sample



GIS significantly predictive for OS:

- OS: 95mo vs 35 mo for high GIS vs low
- HR=3.2; p=0.047



ASCO 2016

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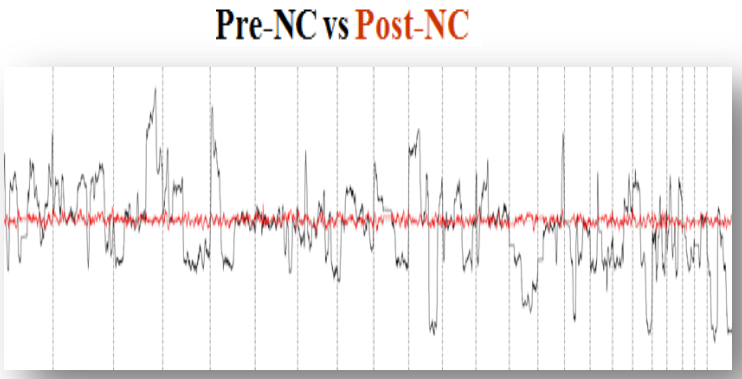
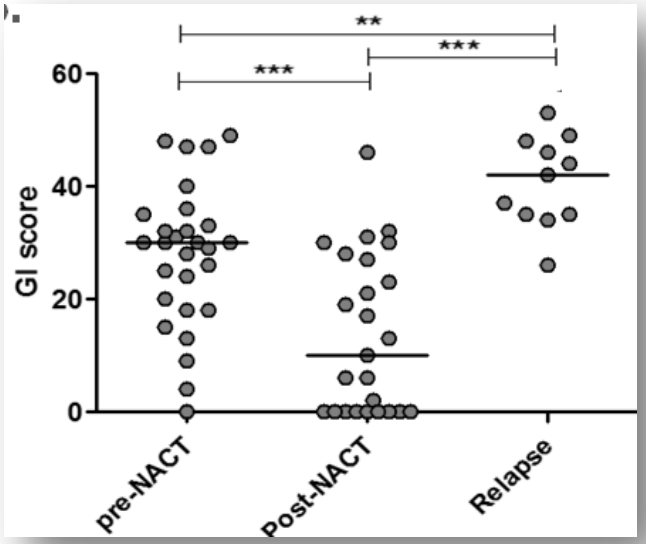
EVOLUTION OF GENOMIC INSTABILITY WITH TREATMENT AND RELAPSE

GIS significantly decreases with NACT

→ selection of genomically stable (DNA repair competent?) clones





but significantly increases at platinum sensitive relapse

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Comprehensive evaluation of DNA repair proteomic biomarkers

At diagnosis and evolution with treatment

Objective: To evaluate the evaluate the loss of key DNA repair biomarkers in tumors at baseline, Post-NACT

- as a measure of DNA repair competency and
- inform choice of maintenance PARP inhibitors

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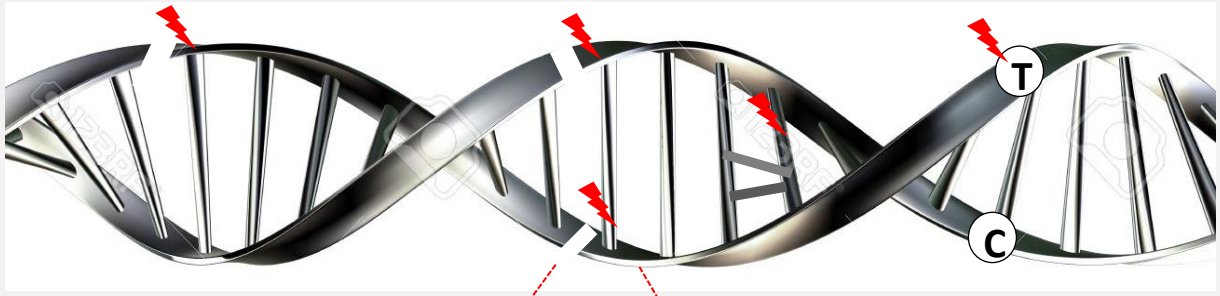
BIOMARKERS OF DSB REPAIR: CROSS-TALK AND REDUNDANCY

Types of DNA damage

SSBs **DSBs** Bulky adducts Mismatches, Indels



Choice of DSB DNA repair pathway

DNA repair biomarkers

HR

High fidelity

BRCA1/2
RAD51
FANCD2
ATM

NHEJ

Error-prone

TP53BP1
DNApk

Reviewed in Current Opinion Oncology, Leary et al

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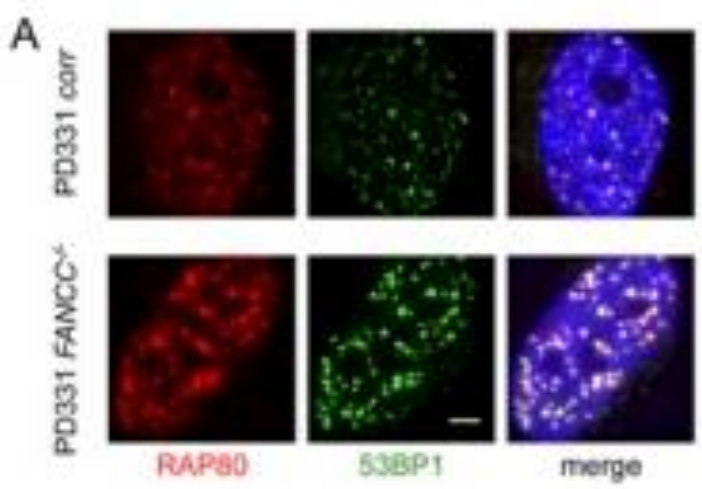
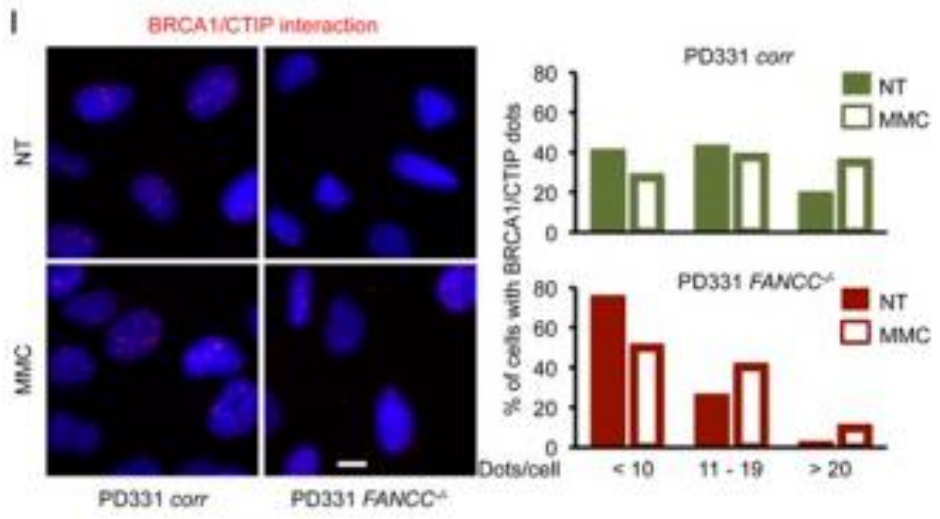
The balance between HR and NHEJ: the ‘good’ and the ‘bad’ of DSB repair



FANCD2 loss results in
- accumulation of 53BP1
- FANCD2: negative regulator of NHEJ
- Loss of FANCD2 promotes error prone NHEJ

FANCD2 loss results in
- Decreased accumulation of BRCA1 foci
- An HRD



Nucl Ac Res 2016

A significant proportion of HGOC show complete loss of DNA repair proteins at diagnosis

Explains initial chemosensitivity

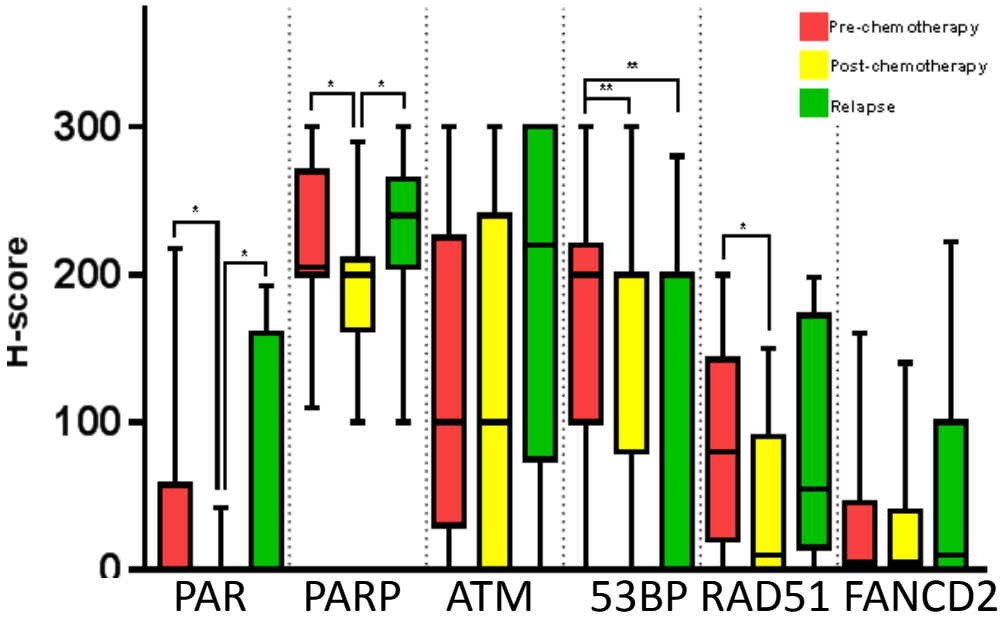
DDR markers at diagnosis:

60% PAR-negative, → Defect in DNA repair via BER

59% FANCD2-negative, 23% RAD51-negative, 20% ATM-negative → HR defect

14% TP53BP1-negative. → PARPi resistance

DNA repair protein expression is significantly altered by NACT and or at relapse



AACR DNA Repair 2016
SGO, 2018, manuscript in writing

Is DNA repair competency in residual tumor post-NACT prognostic?

Could DNA repair biomarkers in the residual tumor post-NACT inform the selection of patients for PARP inhibitor maintenance?

- Most individual DNA repair biomarkers were not significantly associated with survival
- Given the redundancy and complexity of DNA repair pathways, combined DDR biomarkers may be more informative

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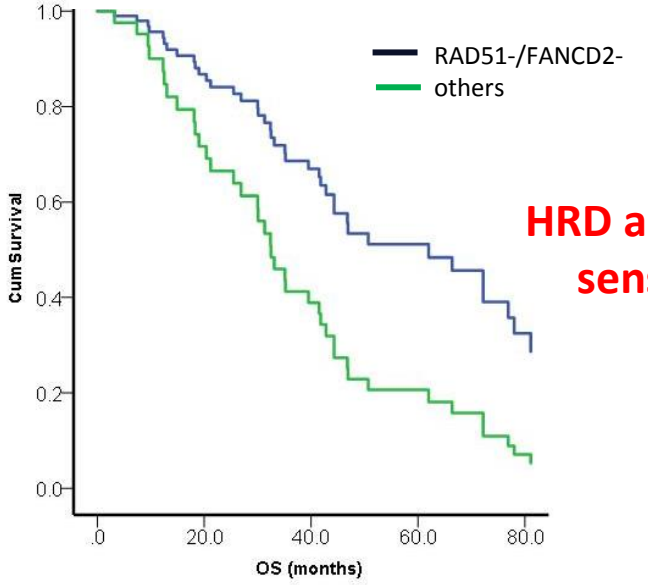
Combined DNA repair biomarkers in residual tumor post-NACT are predictive of both PFS and OS and could identify patients for PARP inhibitor maintenance

Post-NACT, RAD51-/FANCD2- predicted improved PFS (p=0.05) and OS (HR 2.35, p=0.02)

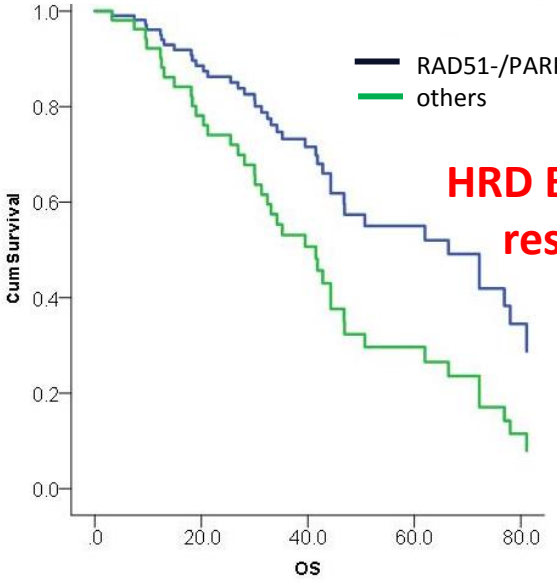
Post-NACT, RAD51-/PARP- predicted improved PFS (p=0.038) and OS (HR 2.03, p<0.034)

RAD51-/FANCD2- post-NACT



HRD and PARPi sensitive?

RAD51-/PARP- post-NACT



HRD BUT PARPi resistant?

AACR DNA Repair 2016

SGO, 2018, manuscript in writing

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CONCLUSIONS PART 1

1/ At diagnosis, HGOC is associated **with high genomic instability (GIS) and lack of DDR effectors** in most patients, which likely explains platinum sensitivity

2/ NACT had a significant impact on GIS and DDR markers but effect **variable,** likely reflecting the **heterogeneity** of HGOC.

3/ Combined evaluation of DDR biomarkers in residual tumor post-NACT

- was more significantly predictive of PFS and OS, and

- could inform **selection of patients for PARP inhibitor maintenance**

FANCD2-/RAD51-/TP53BP+/PARP-1+ → sensitive or

RAD51+/TP53BP-/PARP-1- → resistance to PARP inhibitors

Implication of metabolism in platinum resistance

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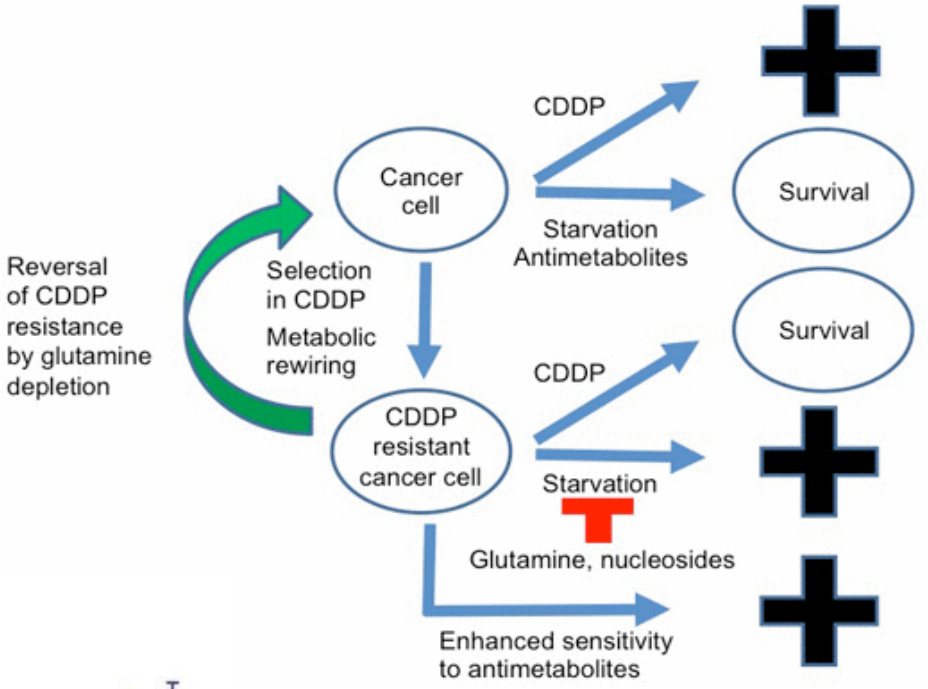
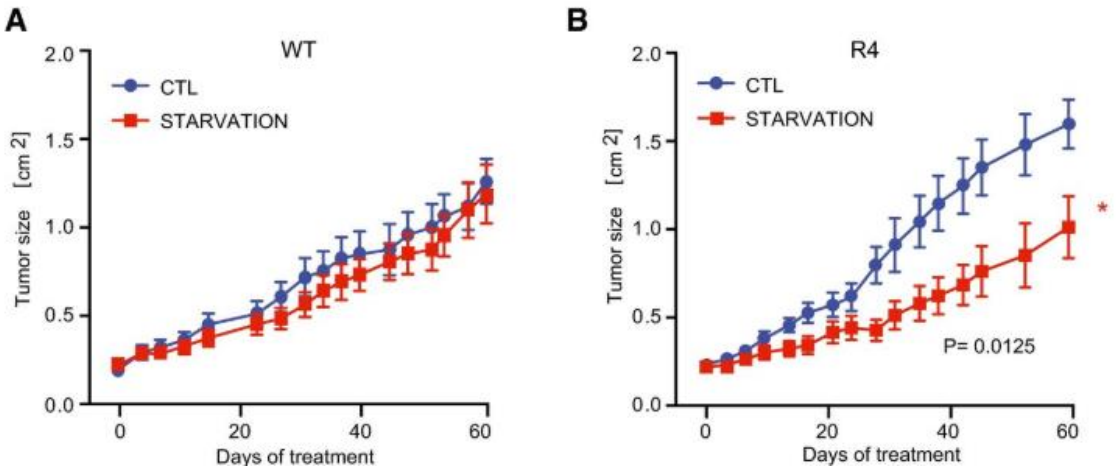
METABOLIC VULNERABILITY OF PLATINUM RESISTANT OC

- Cisplatin-resistant OC clones strong dependence on glutamine
- Glutamine depletion restored cisplatin responses in cisplatin-resistant clones





EMBO 2018

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Characterizing PARPi responsiveness in a panel of patient-derived ex vivo models

Objective : Establish a panel of ex vivo patient derived models to correlate PARPi response to candidate biomarkers of PARPi sensitivity/resistance

OvBIOMARK study

- 3D primary cultures were established from fresh tumors or ascites: creation of a tumor bank of ex vivo models **(N=30)**
- Responsiveness to cisplatin or Olaparib established for a proportion (N=18)
- Proliferation assays Cell Titer Glo®

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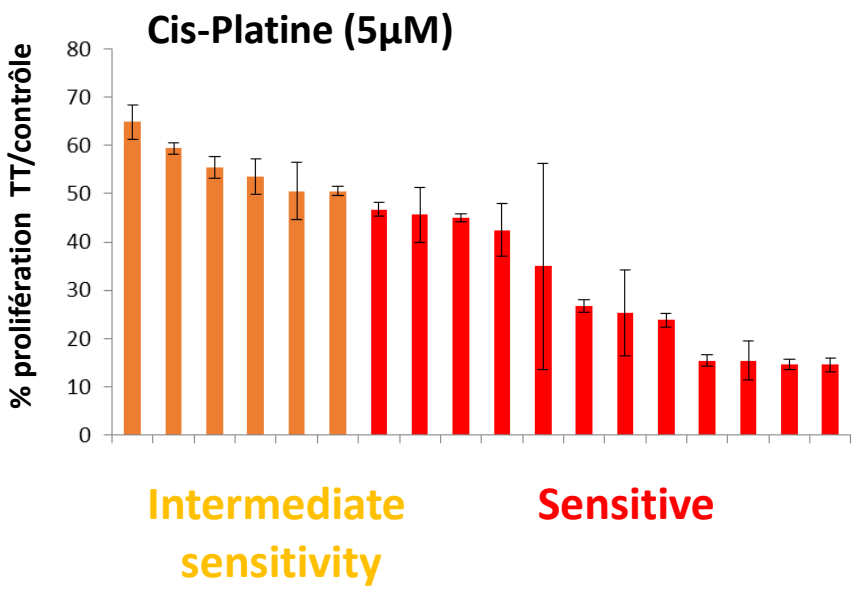
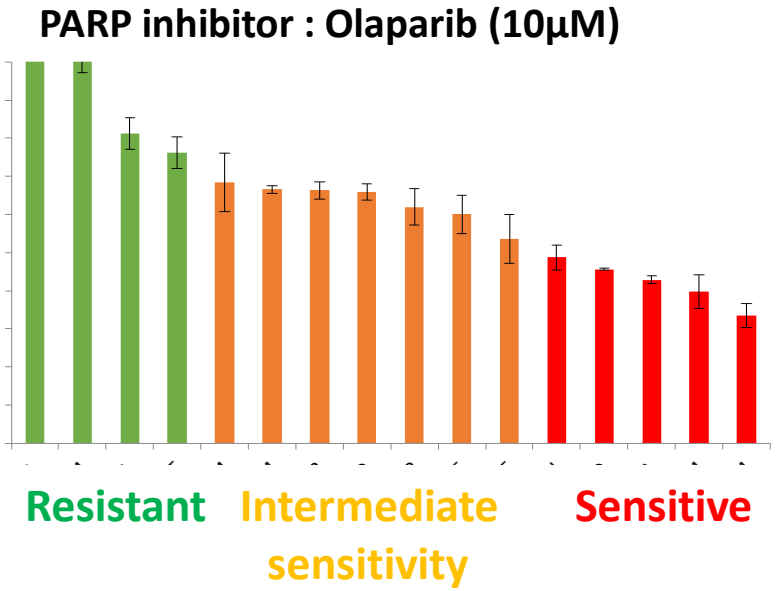
Characterizing PARPi and platinum responsiveness in our cohort of ex vivo models





1/ 100% of HGSOC models showed high or intermediate sensitivity

2/ Wider range of sensitivity to PARPi

Résistant: <20% growth inhibition
Intermediate: 20-50% growth inhibition
Sensitive: >50% growth inhibition

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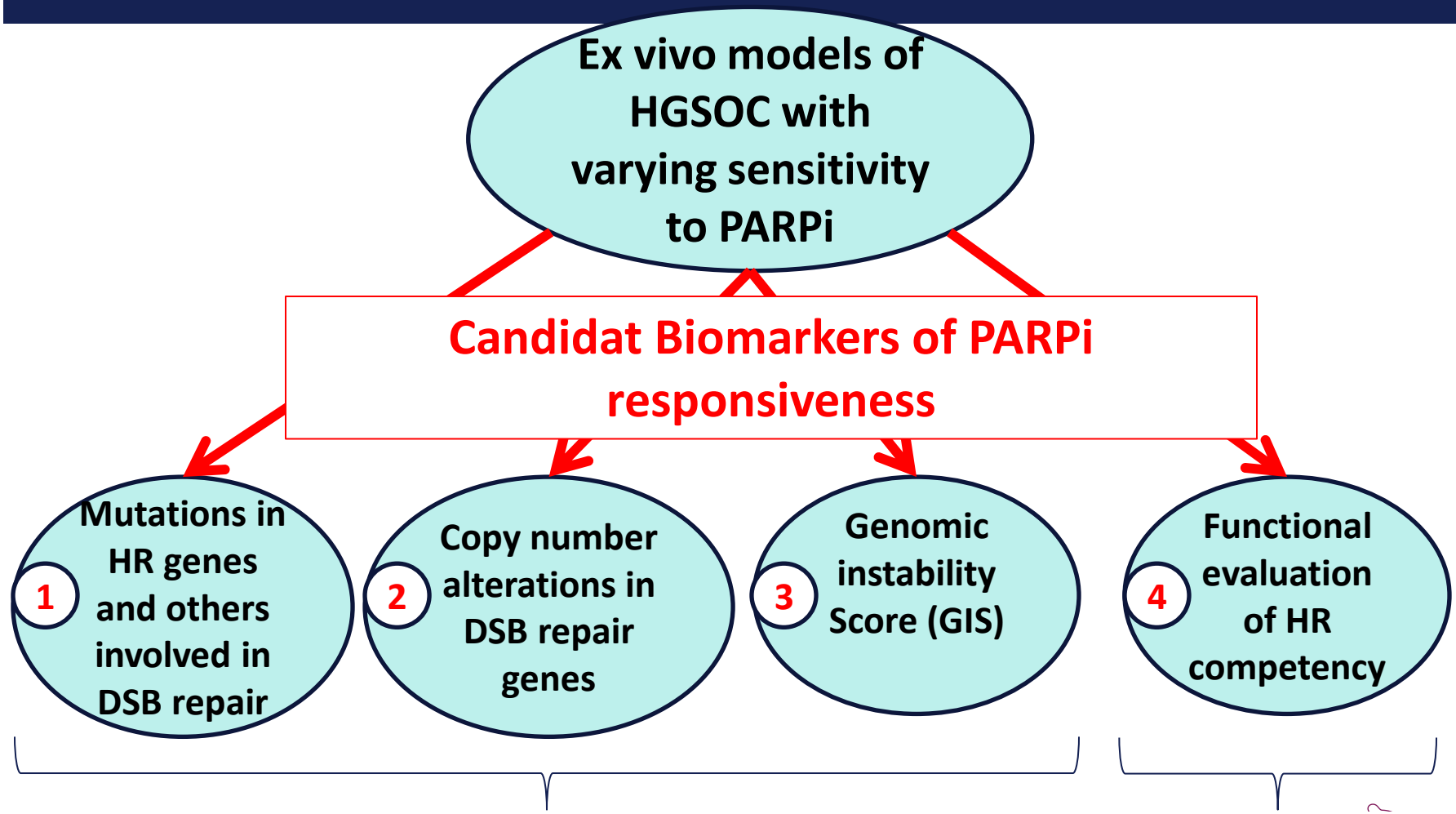
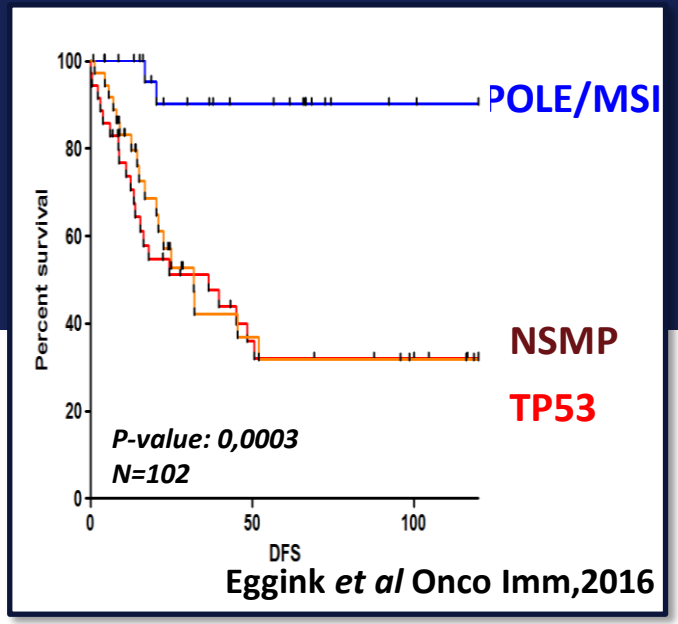
Validating the 'best' biomarker of PARPi responsiveness



DNA Damage Response (DDR) biomarkers in the closely related high grade endometrial cancers



Eggink *et al* Onco Imm,2016

Trans PORTEC

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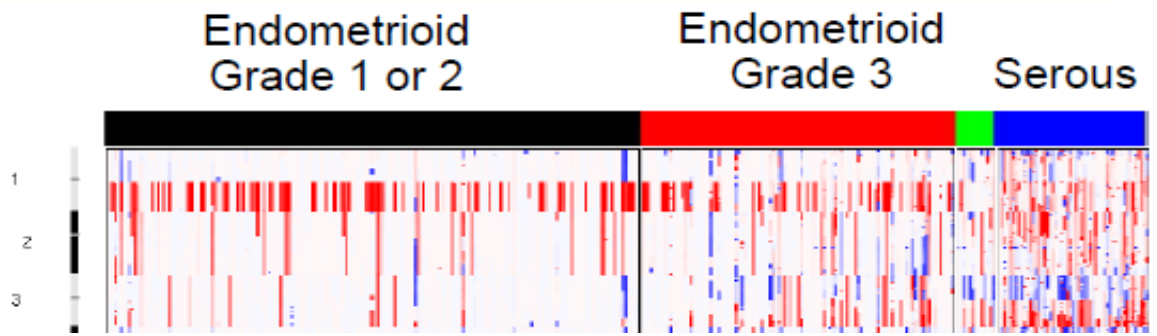
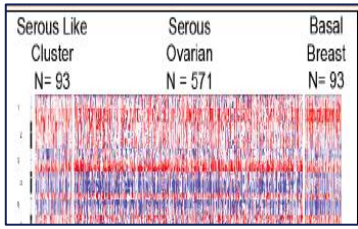
Prognostic and predictive implications of DDR biomarkers in HR-EC





Mutation load

Genomic instability

Prognosis: GOOD | POOR

TransPORTEC classfication: Ultra-mutated POLE → Hyper-mutated MSI → NSMP → P53

DDR biomarkers (DNA repair capacity): MMR deficiency | DDR biomarkers of DSB repair: HR, NHEJ ?

Targeted therapy: Immunotherapies | ?

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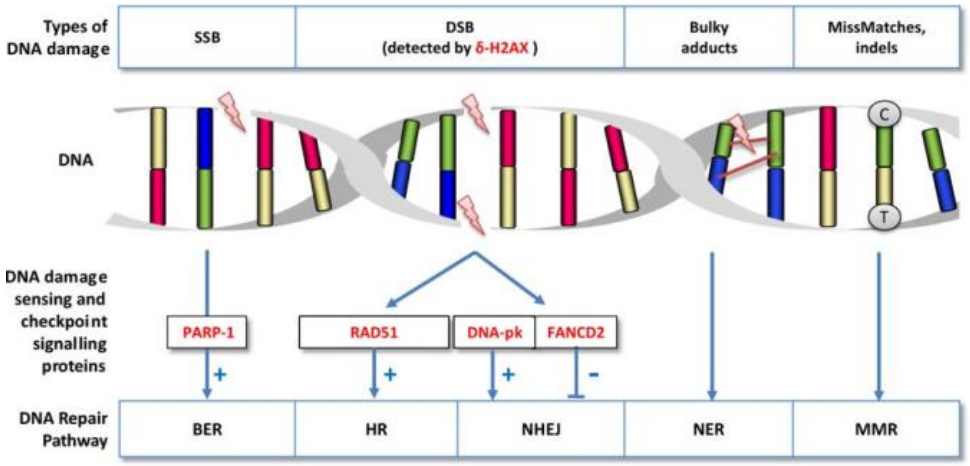
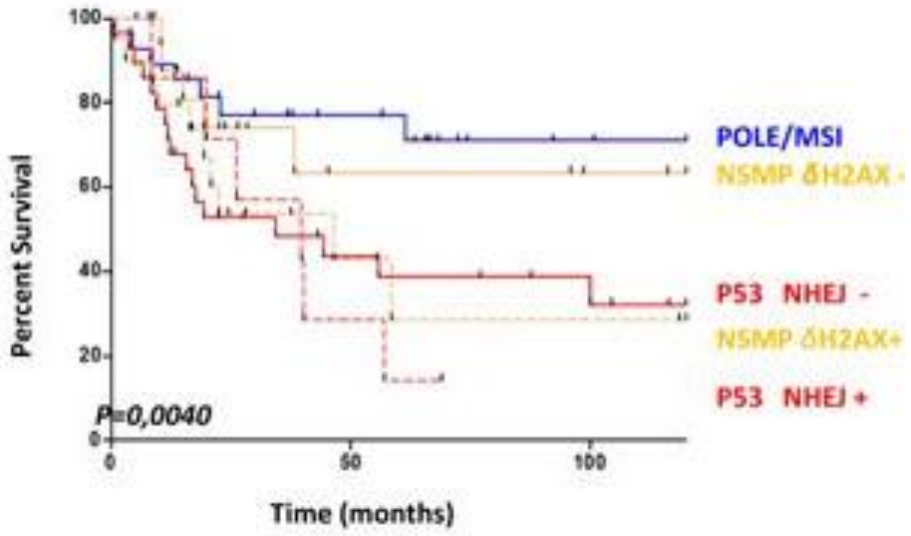
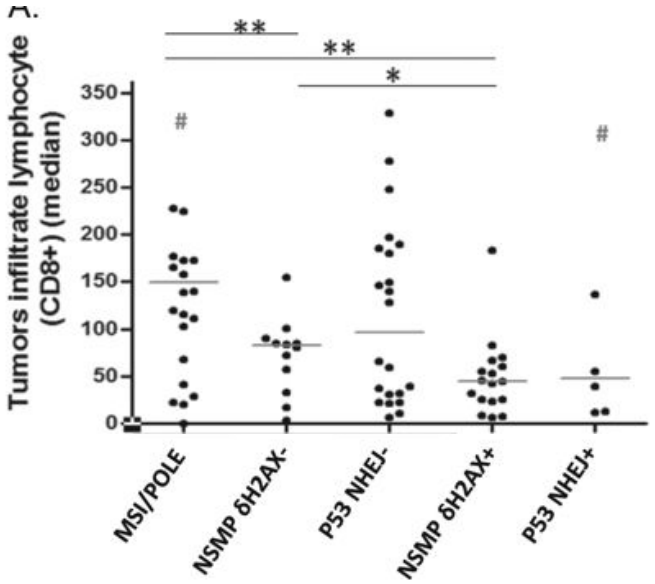
Refinement of endometrial cancer classification using DDR biomarkers



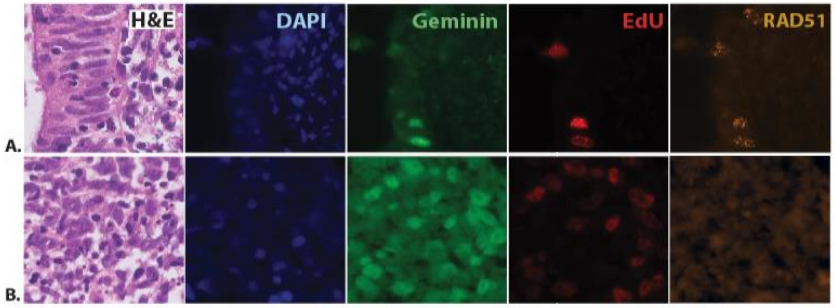




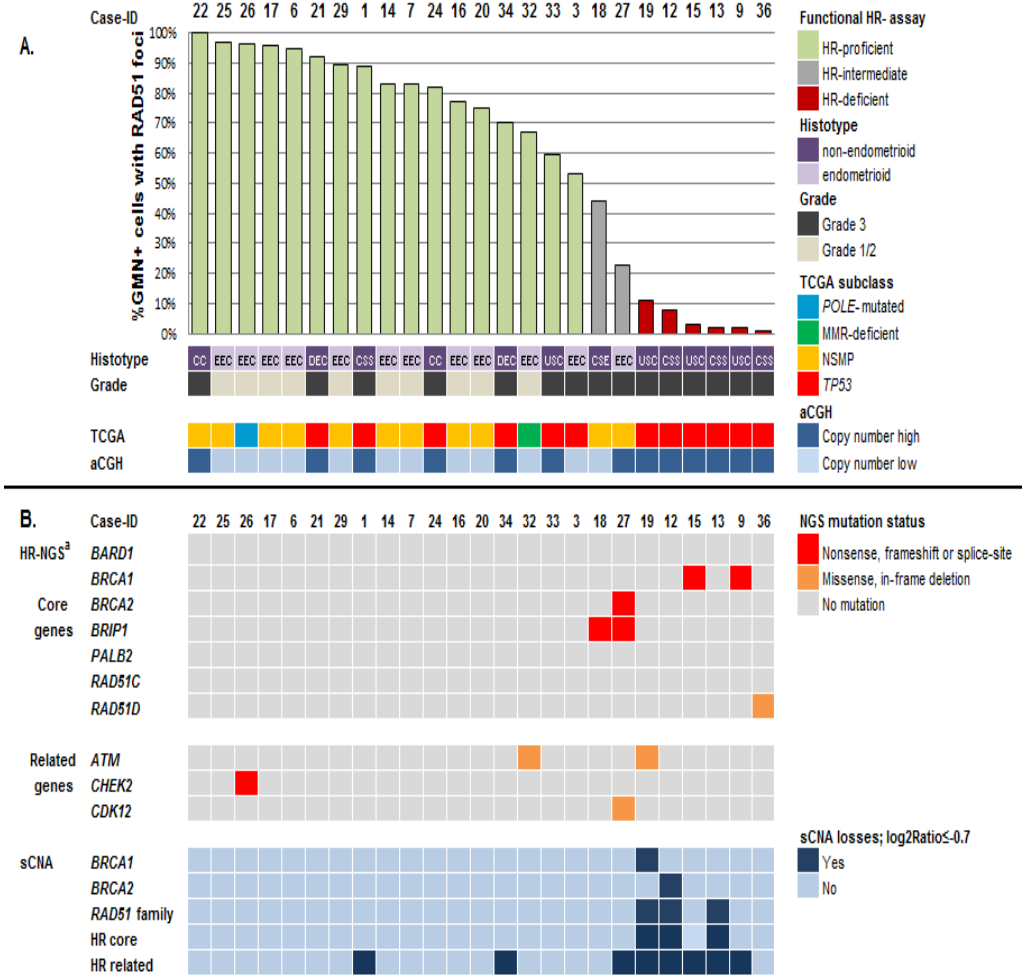
Modern Path 2018, ImmunoOnco 2017
Lancet 2016, Modern Path 2016

Homologous recombination deficiency is a frequent event in high grade endometrial cancer

Comprehensive functional and genomic characterization of HRD in high grade EC ex vivo models



Functional evaluation of HR competency (RAD51 foci) correlated with deletions of mutations in HR genes in all HRD cases



CCR 2018

Interaction between DNA repair competency and anti-tumor immunity

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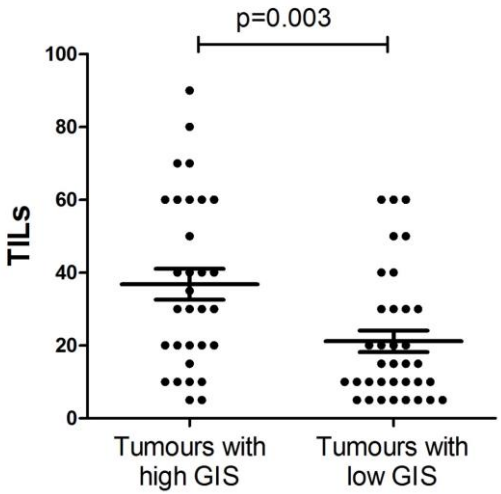
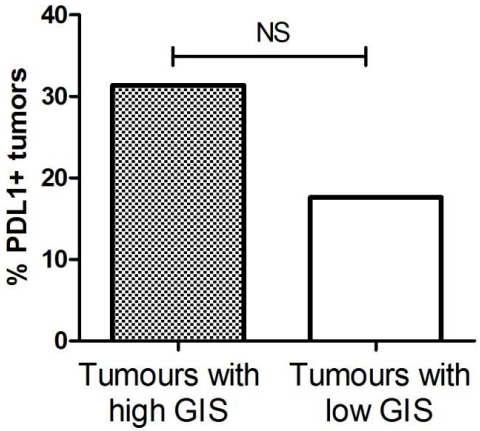
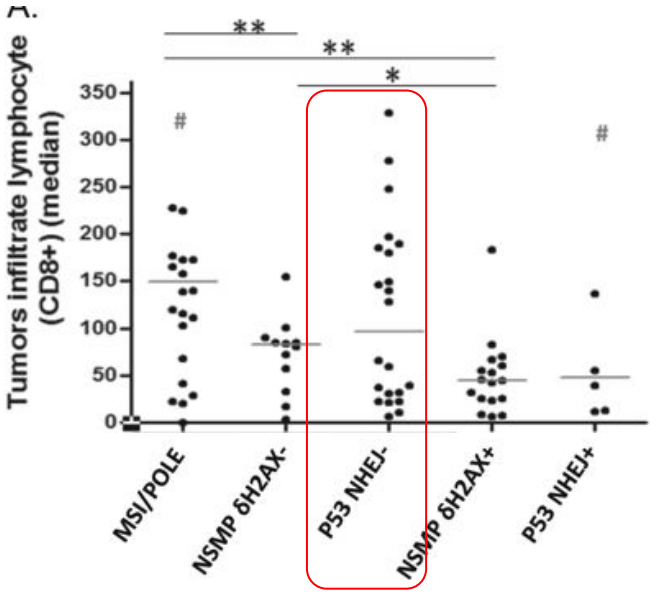
Correlation between DNA repair competency and tumor infiltrating lymphocytes (TILs) and PDL1 expression

High GIS in OC associated with increased TILs and PDL1 expression





DDR biomarkers identifies a further subset of EC beyond MSI and POLE with high TILs and PDL1



ASCO 2017, Modern Path 2018

Impact of NACT on immune microenvironment in high grade OC

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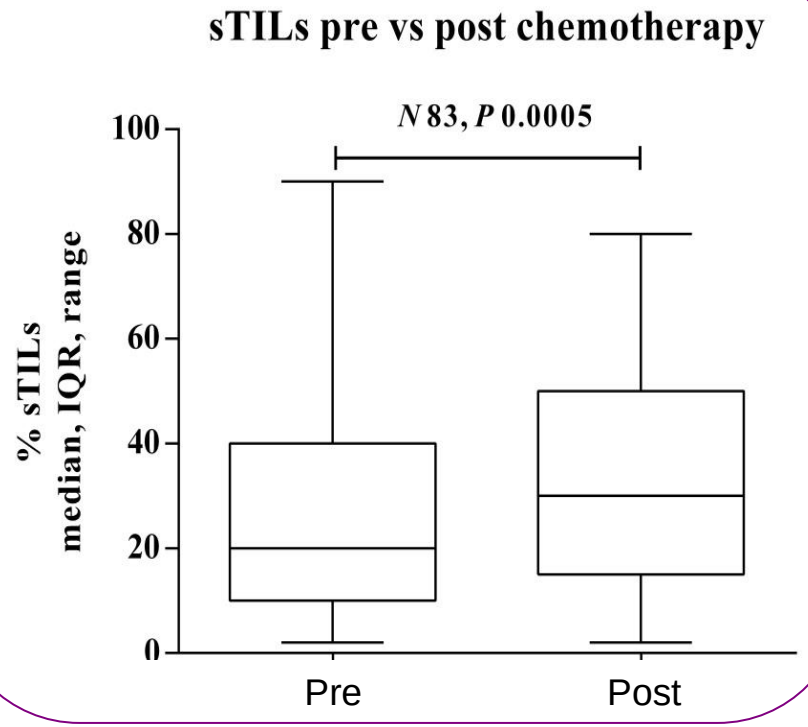
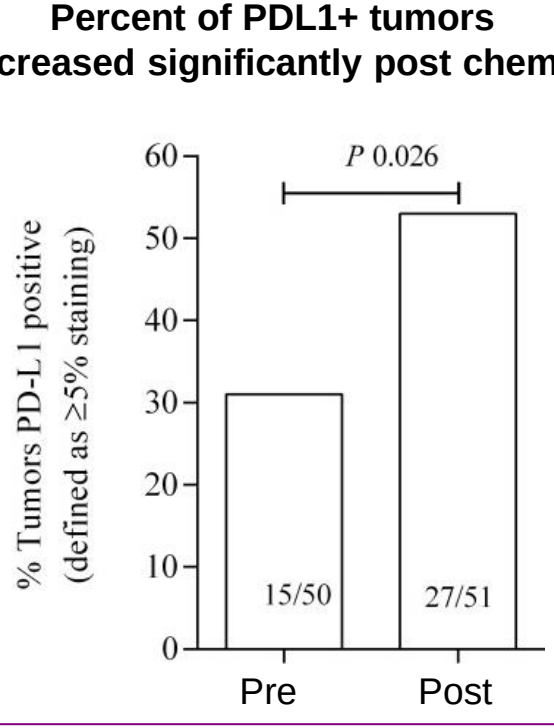
NACT significantly increases TIL infiltration and PDL1 expression in HGOC

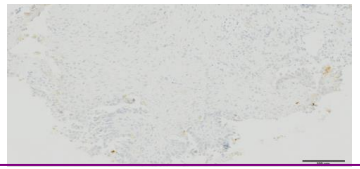




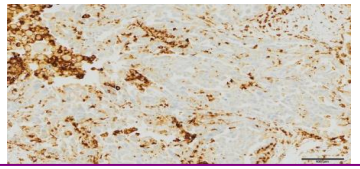
2/3 of **PDL1-negative** tumors at diagnosis became **PDL1-positive** after chemotx

Illustration: PDL1 expression

at Diagnosis



after chemotherapy



Ann Onc 2017

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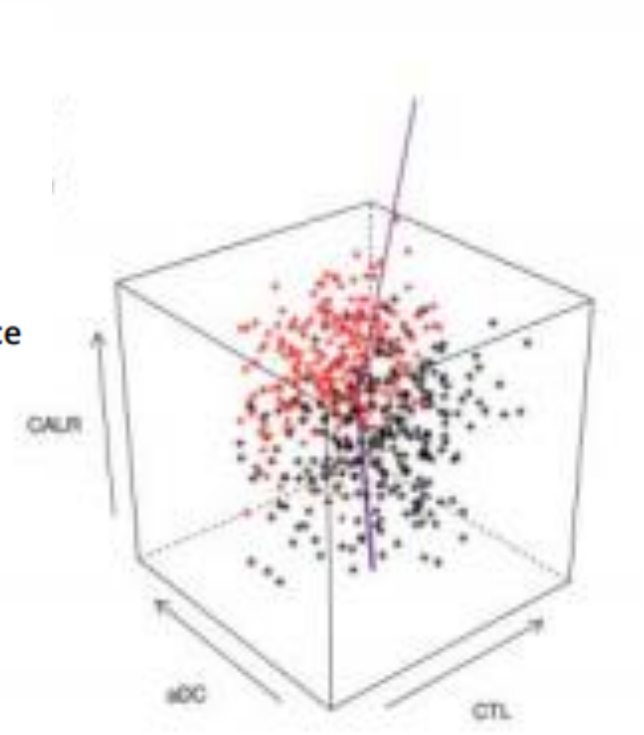
Prognostic implications of Calreticulin expression in OC: loss of an 'eat me' signal

Calreticulin expression: Interaction with the immune infiltrate and impact on survival in patients with ovarian and non-small cell lung cancer

Gautier Stoll, Kristina Iribarren, Judith Michels, Alexandra Leary, Laurence Zitvogel, Isabelle Cremer & Guido Kroemer

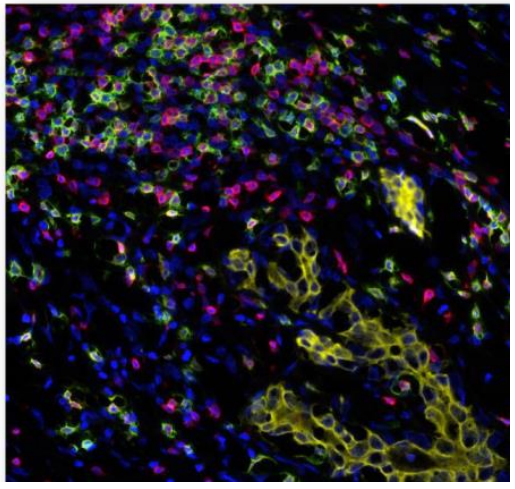


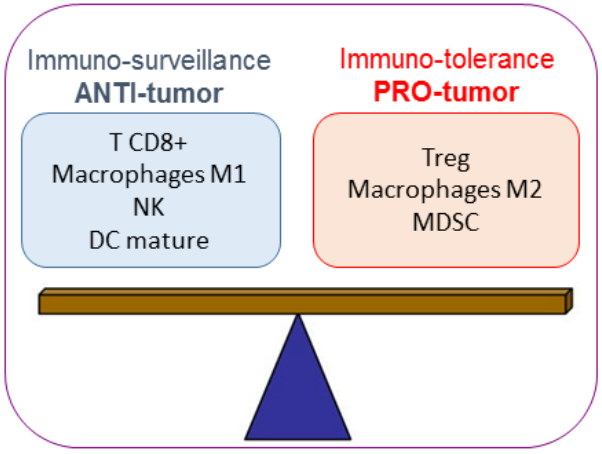
- CALR expression correlated with increased infiltrating T cells (CTLs)
- Loss of CALR promote immune tolerance and negatively impact OS in OC

OncoImmunology 2017

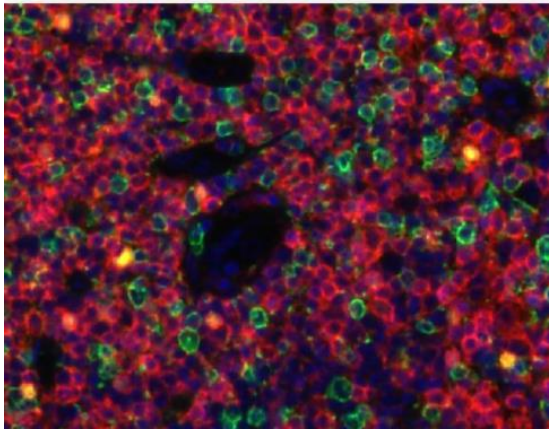
Which immune cells are actually recruited to the tumor bed? Comprehensive multiplexed profiling

CD3/CD8/CK

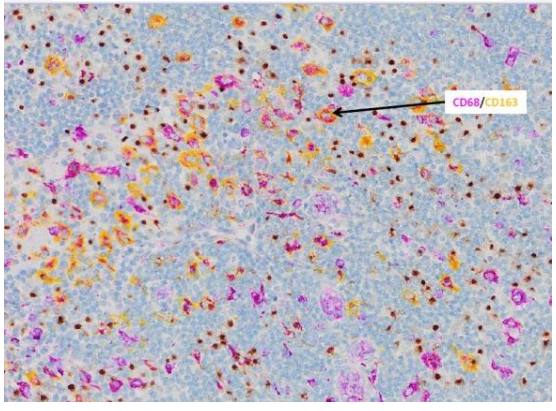




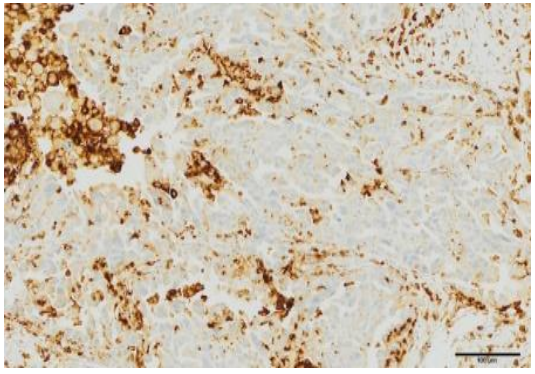
CD4/CD8/FOXP3



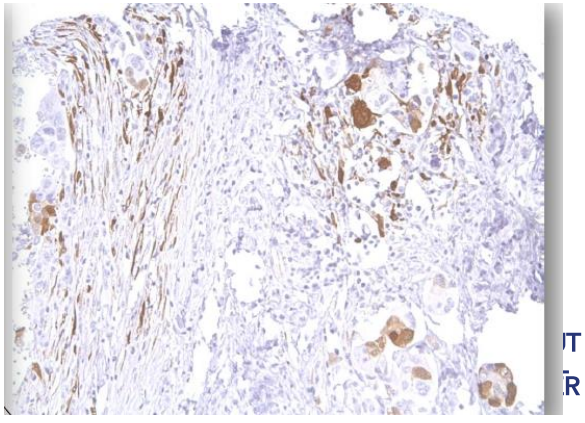
CD68 /CD163/ DC-lamp



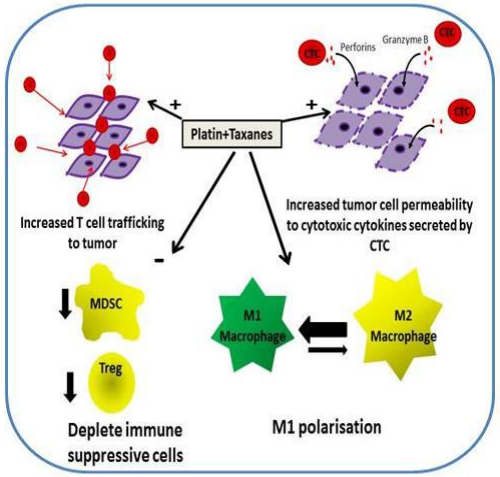
PDL1

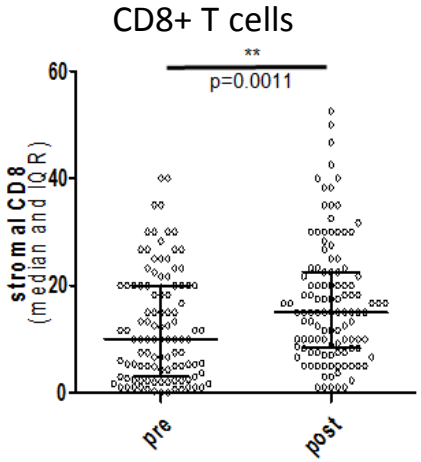


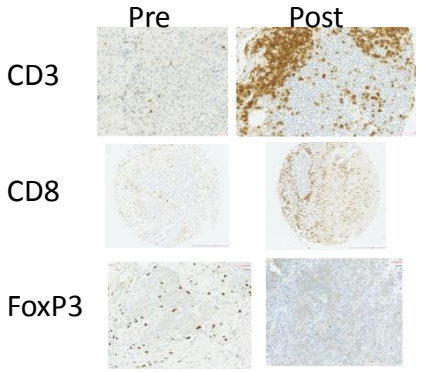
IDO

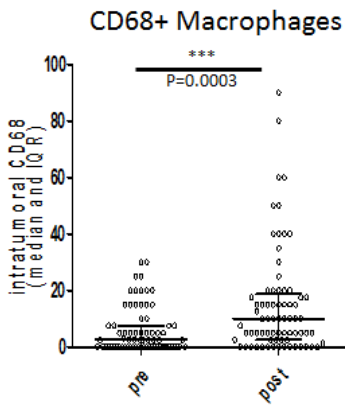


NACT alters the balance of immune-reactive vs immune – tolerant T cells and macrophages in ovarian cancer

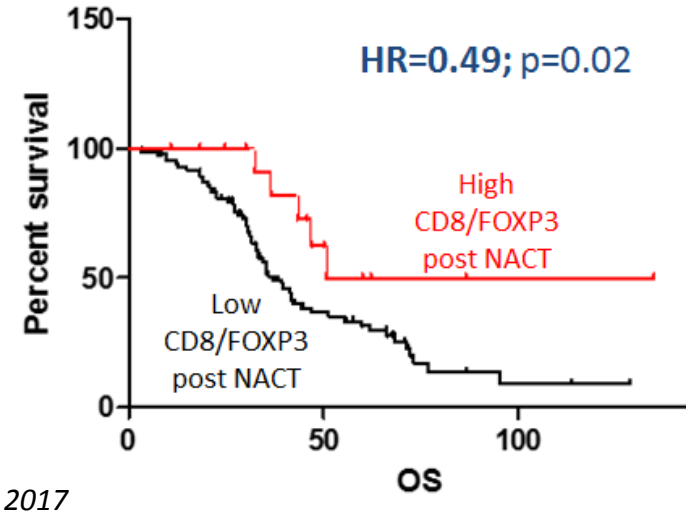


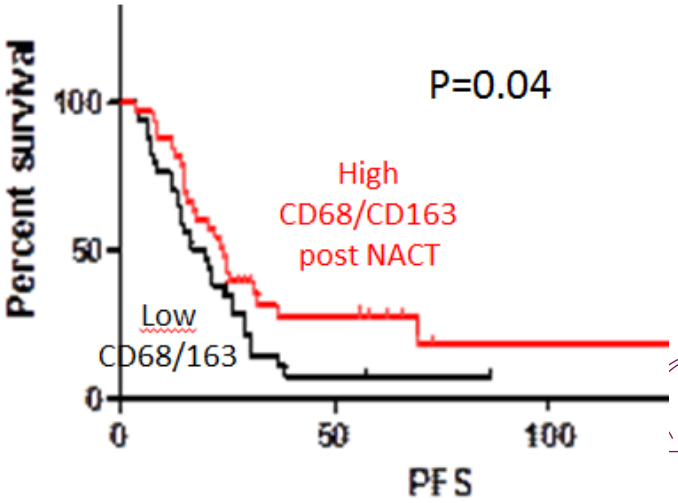






Favorable CD8/FOXP3 and CD68/CD163 ratios <u>after</u> chemotherapy predictive of survival





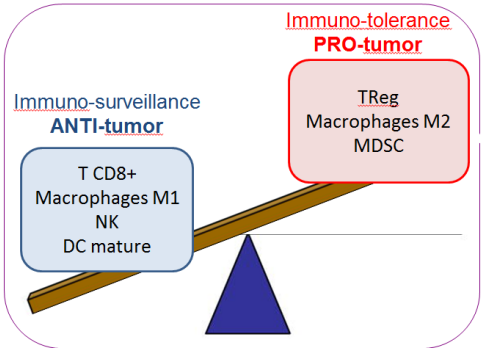
Int J Cancer 2018
ESMO 2017
Annals of Oncology 2017

INSTITUT NATIONAL DU CANCER

Results provided the rationale for clinical trial

Harnessing anti-tumor immunity during neoadjuvant chemotherapy to improve survival in ovarian cancer

ARCAGY - GINECO



IneOV clinical trial:

Neoadjuvant Chemotherapy with an anti-PDL1 alone or in combination with anti-CTLA4 Ab

CI: A Leary

INSTITUT NATIONAL DU CANCER

ctDNA in HGOC:
A prognostic biomarker and
A tool to overcome intra-tumoral heterogeneity

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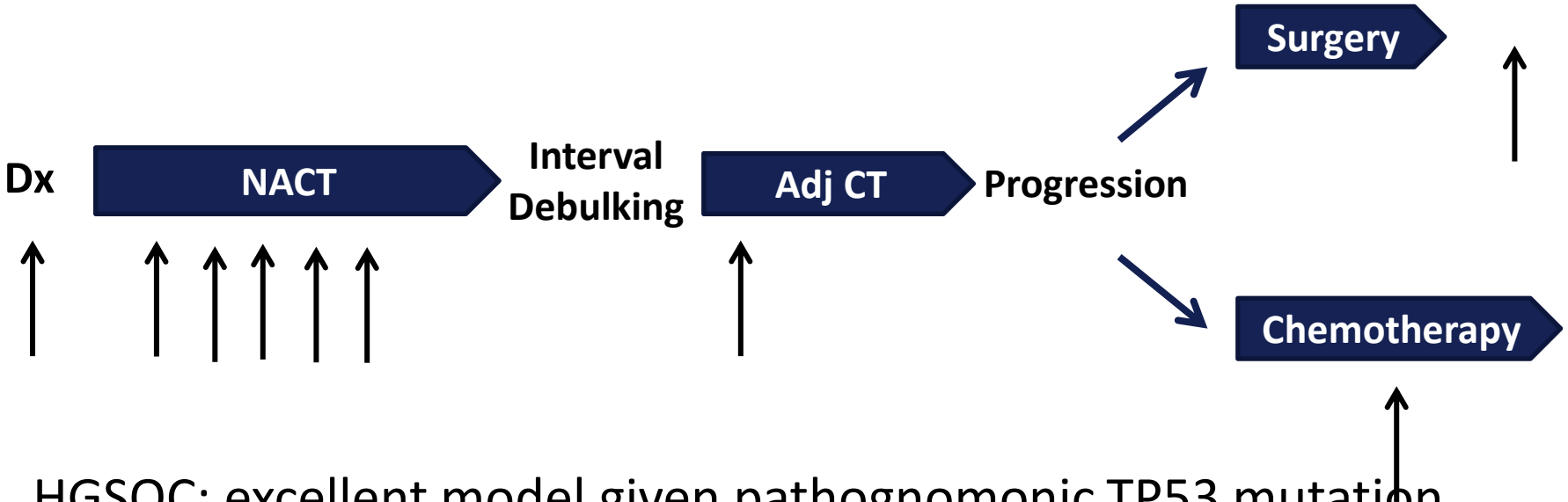
OVBioMARK: ctDNA throughout the disease course



HGSOC: excellent model given pathognomonic TP53 mutation

Good sensitivity

Detected in 100% of samples at baseline and 75% at relapse

In 75% of samples during C1/C2 of neoadjuvant chemotherapy

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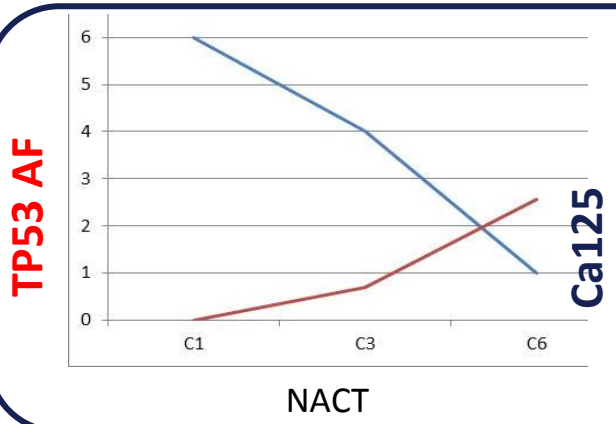
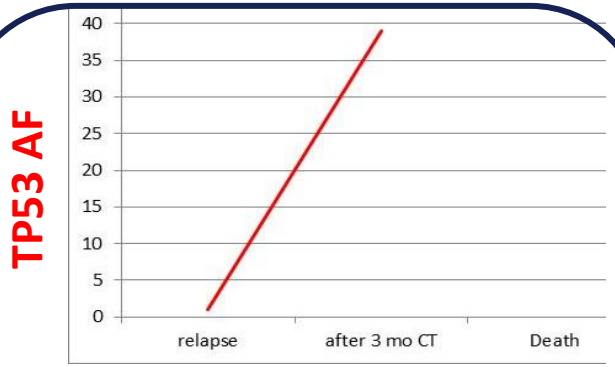
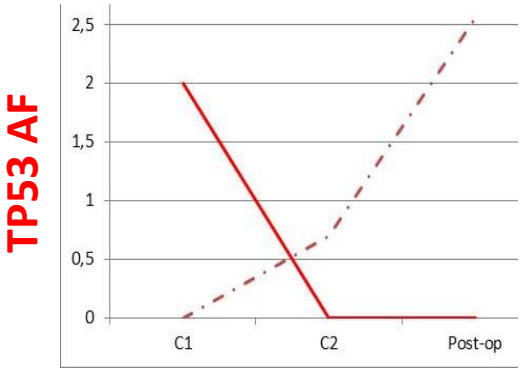
ctDNA : clinical utility: 3 cases



- At baseline ctDNA undetectable
- Rising TP53 ctDNA (R248P) despite decreasing Ca125
- Cannot be operated on and progresses…



- At Dx of relapse ctDNA TP53 AF 1.3
- Starts new treatment
- 3 months later ctDNA up to AF 39
- CT scan show HYPERprogression
- Pt dies one month later



- At baseline ctDNA for TP53 E258 which disappears by C2 neo. Tumor: TP53 Glu258
- New TP53 ctDNA R273H with rising AF during NACT and continues post op

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VALORISATION DU PAIR

Communications original research in international congress

N=12

1 selected for award and oral presentation (ESGO 2017),

2 selected for poster discussion (ASCO 2016, ESMO 2017)

1 Scientific award (ASCO 2016).

Publications

11 articles original research

6 reviews.

(*Annals Onc, Modern Path, Cancer Res , EMBO, Cell Cycle, Oncogene, OncoImmu*…)

Oral communications (educational/scientific symposia)

National congress (SFC, GFCO)

International congress (ESMO, ESO, ICACT, ESGO et ASCO).

Clinical Trials

OVBioMARK: Evolution of Tumor- and blood-based biomarkers throughout the disease course in HGOC

INeOV: Neoadjuvant Ctx with antiPDL1 alone or in combination with anti-CTLA4

VALORISATION DU PAIR

Resulting Grants

TransCAN: 260,000 euros
ARCAGY: 170,000 euros
Maria Bressan Award: 80,000 euros
Taxe d'apprentissage: 50,000 euros
Oakland Med Res Fund: 20,000 euros
Goldman Sacks Fund: 30,000 euros

Resulting Academic Collaborations

1. TransPORTEC Consortium: National CI: *DDR biomarkers in high risk EC*. UK, Holland, Canada, Austr and France

2. TH4R: National CI *Intratumoral Heterogeneity in TNBC and HGSOC*: Italy, Germany, Spain and France

International academic Exchanges

1 ESMO Fellow
1 Australian PhD student
3 European Master students

Industrial Collaborations:

INIVATA
SANOFI
MERUS
FOUNDATION MEDICINE

Acknowledgements

GYNE Transla Res Lab U981 A LEARY




AUGUSTE
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Post-Doctorant

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technicienne

ANNIBAL
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Aya
Master student

KUBELAC
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ESMO fellow

GENESTIE
Catherine
Expert Pathologist

PAIR Partners

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M Castedo

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Genomic Stability and Oncogensis
E Renaud
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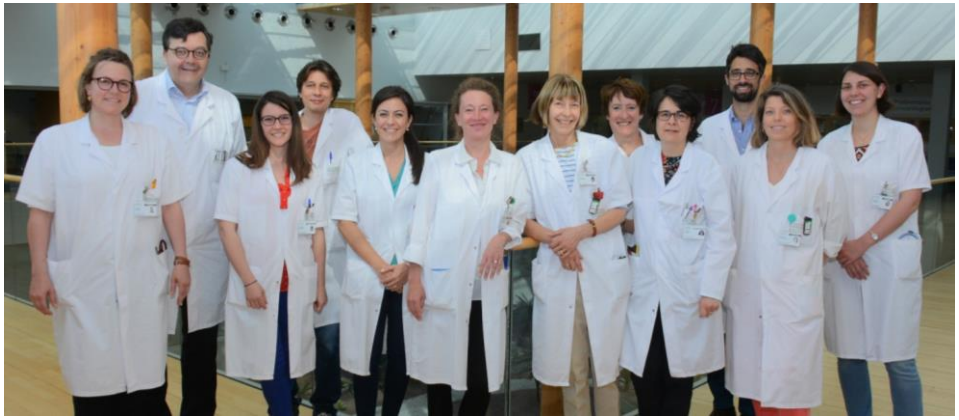
Gustave Roussy Clinicians



Research Plateforms involved in PAIR

Pathology
SCOAZEC Jean Yves
DRUSCH Françoise

Molecular Pathology
ROULEAU Etienne
LACROIX Ludovic
LAPORTE Mélanie
RICHON Catherine
FAUCHER Gladwys

Genomic platform
PATA-MERCI Noémie

Bioinformatic platform BiGR UMS AMMICA INSERM US23 / CNRS UMS3655
MEURICE Guillaume
DELOGER Marc
JOB Bastien
BOURSIN Yannick