Réparation de l’ADN, métabolisme 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%

Excision of all residual disease post-chemo

Complete surgery

Nest of highly proliferative tumor cells

Sterilized by chemotherapy

Opportunity to study minimal residual disease (chemoresistant clones?)

Inform treatment sequence (maintenance)

ASCO 2016 Educational Leary A: Translational value of neoadjuvant chemotherapy in OC
• 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?
METHODS: OPTIMIZING SMALL TUMOR SAMPLES

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

Prospective cohort: OVBioMARK Biological Trial

FFPE bloc PRE-NACT

FFPE bloc POST-NACT

3 cores (1.2mm) → 3 cores (1.2mm) → DNA extraction for genomic biomarkers

CGH, SNPArray, NGS

TMAs

Proteomic IHC/IF

>30 Biomarkers evaluated

2 cores (1.2mm) -20°C

2 cores (1.2mm) -20°C

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

GIS in Pre-NC HGOC

- HR=3.204
- P-value=0.0473
- Med: 35.1 vs 95.3 months,

ASCO 2016
GIS significantly decreases with NACT
→ selection of genomically stable (DNA repair competent?) clones

but significantly increases at platinum sensitive relapse
Comprehensive evaluation of DNA repair proteomic biomarkers

At diagnosis and evolution with treatment

Objective: To 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
BIOMARKERS OF DSB REPAIR: CROSS-TALK AND REDUNDANCY

Types of DNA damage

SSBs

DSBs

Bulky adducts

Mismatches, Indels

Choice of DSB DNA repair pathway

HR
High fidelity
BRCA1/2
RAD51
FANCD2
ATM

NHEJ
Error-prone
TP53BP1
DNApk

Reviewed in Current Opinion Oncology, Leary et al
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, 60% PAR-PARPi resistance
- 59% FANCD2-negative, 23% RAD51-negative, 20% ATM-negative
- 14% TP53BP1-negative, 14% TP53BP1-defect in DNA repair via BER

Defect in DNA repair via BER (PARPi resistance) and HR defect (FANCD2, RAD51, ATM) explain initial chemosensitivity.

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

AACR DNA Repair 2016
SGO, 2018, manuscript in writing
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
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)

HRD and PARPi sensitive?

HRD BUT PARPi resistant?
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
METABOLIC VULNERABILITY OF PLATINUM RESISTANT OC

- Cisplatin-resistant OC clones strong dependence on glutamine
- Glutamine depletion restored cisplatin responses in cisplatin-resistant clones
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®
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
Validating the ‘best’ biomarker of PARPi responsiveness

Ex vivo models of HGSOC with varying sensitivity to PARPi

Candidat Biomarkers of PARPi responsiveness

1. Mutations in HR genes and others involved in DSB repair
2. Copy number alterations in DSB repair genes
3. Genomic instability Score (GIS)
4. Functional evaluation of HR competency

WES performed and analysis ongoing

RAD51 Assay (collab. Val d’Hebron)
DNA Damage Response (DDR) biomarkers in the closely related high grade endometrial cancers

Eggink et al. Onco Imm, 2016
Prognostic and predictive implications of DDR biomarkers in HR-EC

- **Mutation load**
  - **Genomic instability**
  - **Prognosis**

- **TransPORTEC classification**
  - **GOOD**
  - **POOR**

- **DDR biomarkers (DNA repair capacity)**
  - **Ultra-mutated POLE**
  - **Hyper-mutated MSI**
  - **MMR deficiency**
  - **DDR biomarkers of DSB repair: HR, NHEJ**

- **Targeted therapy**
  - **Immunotherapies**

- **Eggink et al. Onco Imm, 2016**
Refinement of endometrial cancer classification using DDR biomarkers

Modern Path 2018, ImmunoOnco 2017
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
Interaction between DNA repair competency and anti-tumor immunity
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
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
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
Which immune cells are actually recruited to the tumor bed? Comprehensive multiplexed profiling
NACT alters the balance of immune-reactive vs immune-tolerant T cells and macrophages in ovarian cancer

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

HR = 0.49; p = 0.02

High CD8/FOXP3 post NACT

Low CD8/FOXP3 post NACT

P = 0.04

High CD68/CD163 post NACT

Low CD68/CD163 post NACT
Harnessing anti-tumor immunity during neoadjuvant chemotherapy to improve survival in ovarian cancer

IneOV clinical trial:

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

CI: A Leary
ctDNA in HGOC: 
A prognostic biomarker and 
A tool to overcome intra-tumoral heterogeneity
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
ctDNA: clinical utility: 3 cases

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

- 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

- 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
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).

Oral communications (educational/scientific symposia)
National congress (SFC, GFCO)
International congress (ESMO, ESO, ICACT, ESGO et ASCO).

Publications
11 articles original research
6 reviews.
(Annals Onc, Modern Path, Cancer Res, EMBO, Cell Cycle, Oncogene, OncolImmu...)

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
## 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, Austria 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

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- INIVATA
- SANOFI
- MERUS
- FOUNDATION MEDICINE
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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
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