DALLA PRECLINICA ALLA CLINICA NEI TUMORI OVARICI

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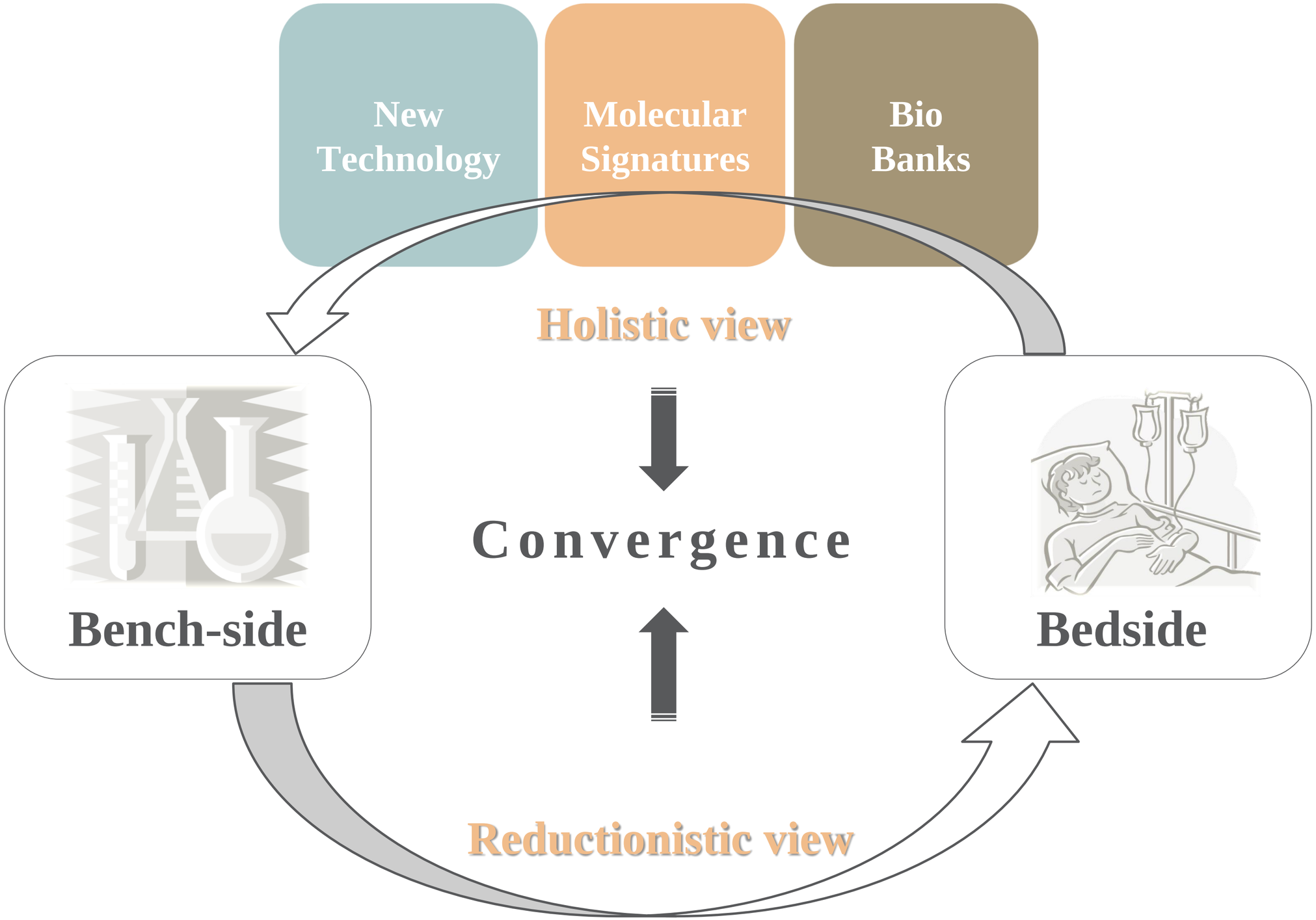
Reggio Emilia, 21 giugno 2019

MaNGO
Mario Negri Gynecologic Oncology

IMN
ISTITUTO DI RICERCHE FARMACOLOGICHE MARIO NEGRI · IRCCS

CANCER RESEARCH



AIMS OF PRECLINICAL STUDIES

1. Investigation of mechanisms of malignancy
2. Pharmacological studies on existing drugs
3. Identification of biomarkers for patient stratification
4. Development of new therapies

AIMS OF PRECLINICAL STUDIES

1. **Investigation of mechanisms of malignancy**

Pharmacological studies on existing drugs

3. Identi

- Identification of **cancer driver genes**
- Identification of mechanisms of **tumor aggressiveness**
- Identification of mechanisms of **drug resistance**

aimed at the discovery **new druggable targets**

AIMS OF PRECLINICAL STUDIES

1. Investigation of mechanisms of malignancy
2. **Pharmacological studies on existing drugs**
3. Identification of biomarkers for patient stratification
4. Develo

- **PK/PD studies**
- Study of the effects on **tumor/normal cells**
- **Comparison of different compounds** of the same class

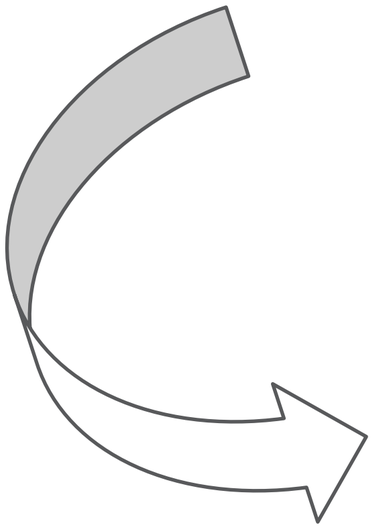
AIMS OF PRECLINICAL STUDIES

1. Investigation of mechanisms of malignancy
2. Pharmacological studies on existing drugs
3. **Identification of biomarkers for patient stratification**
4. Dev

- Discovery of **prognostic/predictive biomarkers**
- Discovery of **biomarkers addressing therapeutic choice**
- Discovery of biomarkers useful to **monitor therapeutic response**

AIMS OF PRECLINICAL STUDIES

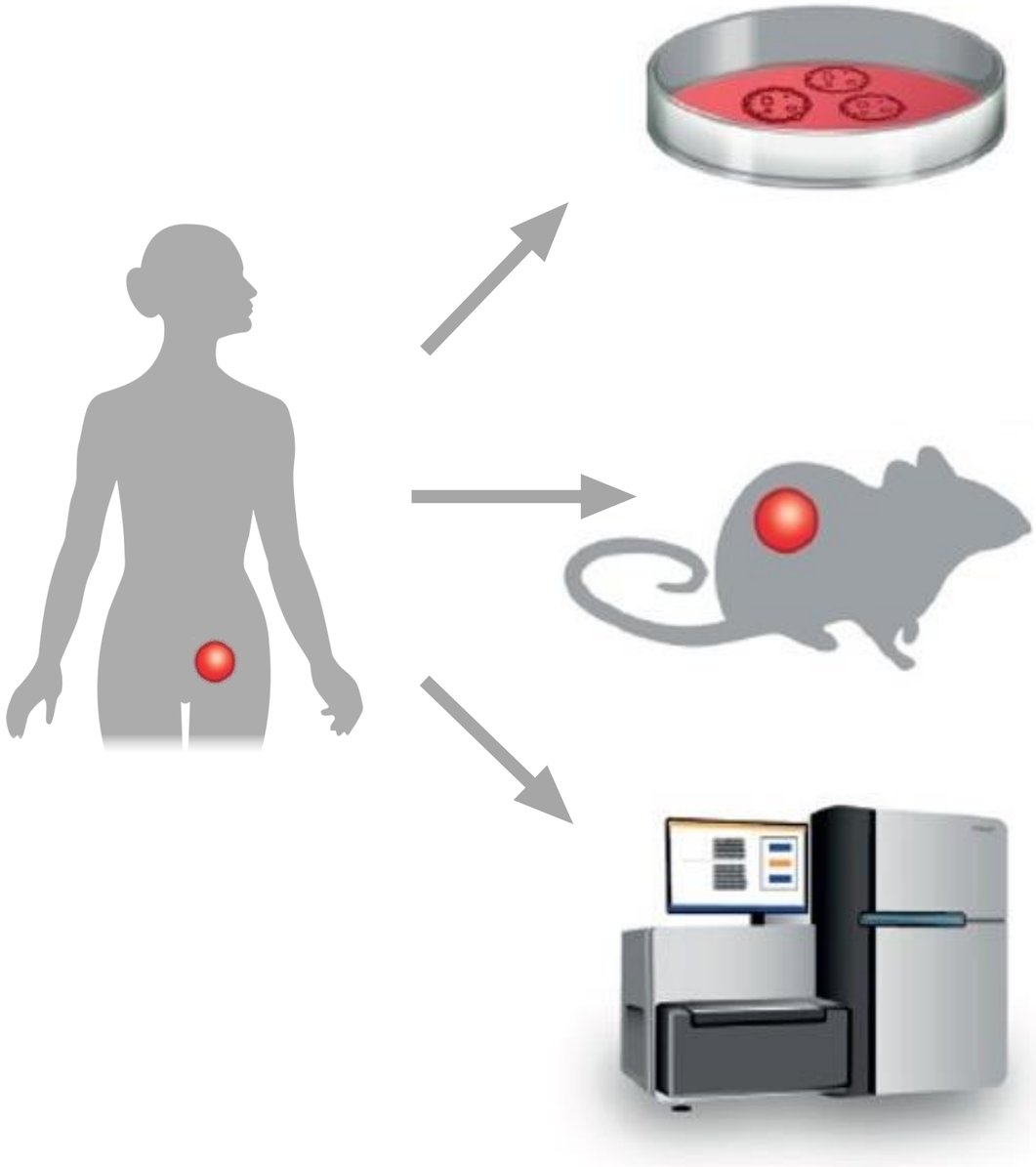
1. Investigation of mechanisms of malignancy
2. Pharmacological studies on existing drugs
3. Identification of biomarkers for patient stratification
4. **Development of new therapies**



Investigation of:

- new effective **compounds**
- new effective **treatment schedules**
- new effective **combinations**

TOOLS OF PRECLINICAL STUDIES



In vitro

- Primary cell cultures
- Immortalized cell lines
- 3D cell cultures

In vivo

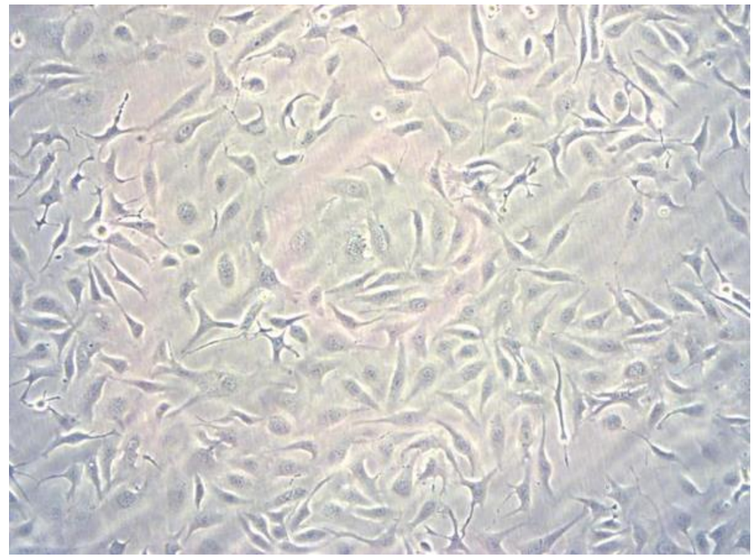
- Cell-derived xenografts
- Patient-derived xenografts
- Genetically modified mice

Ex vivo

- Targeted analysis
- Untargeted –omic analysis

IN VITRO MODELS - CELL CULTURES

- Fast growth, long life-span
- High-throughput capacity
- High reproducibility
- Cost-effectiveness
- Easy genetic manipulation (CRISP-CAS9)
- Reduction of the need for animal experiments (3R policy)



IN VITRO MODELS - CELL CULTURES

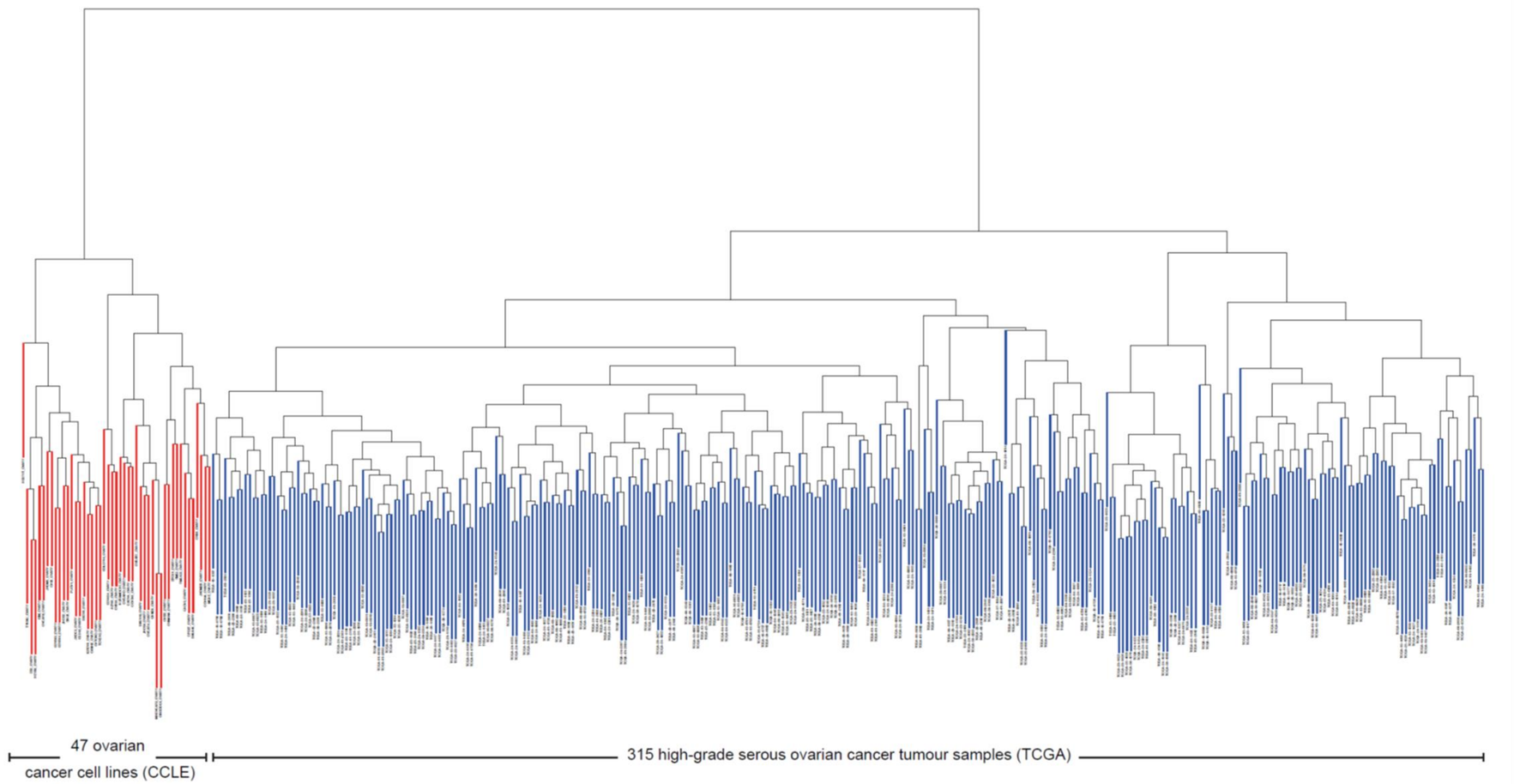
Scarce clinical relevance of commonly used ovarian cancer cell lines

- High frequency of **cross-contamination**
- **Different origin** of cell lines (need of authentication)
- Occurrence of **secondary genomic changes** (e.g. CNVs, transcriptomic drifts)

IN VITRO MODELS - CELL CULTURES

Pronounced differences in molecular profiles between commonly used ovarian cancer cell lines and HGSOC clinical samples in terms of:

- copy-number changes
- mutations
- mRNA expression

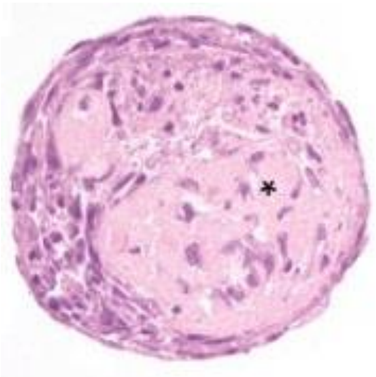


Domcke et al. Nat Commun. 2013

IN VITRO MODELS – 3D CELL CULTURES

Examples:

- Spheroids (cell lines in 3D matrix)
- Organoids (primary cancer cells in 3D matrix)
- Organotypic multicellular spheroids comprising of two or more cell types (e.g. primary stromal cells + ovarian cancer cells + immune cells)
- Microfluidic systems reproducing hydrodynamic forces and release of growth factors or nutrients



Advantages:

- Cell–cell and cell–ECM communication
- Heterogeneity of distribution of oxygen, nutrients and growth factors

RESOURCE
https://doi.org/10.1038/s41591-019-0422-6

nature medicine

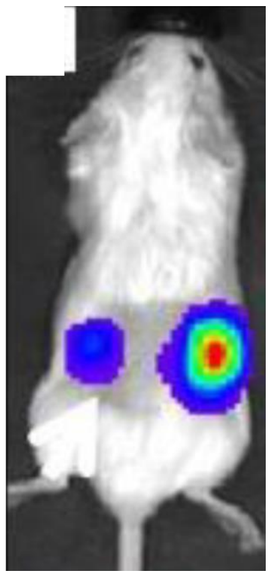
An organoid platform for ovarian cancer captures intra- and interpatient heterogeneity

Oded Kopper[1,2], Chris J. de Witte[3,15], Kadi Lõhmussaar[1,2,15], Jose Espejo Valle-Inclan[3,15], Nizar Hami[2,4], Lennart Kester[1,2], Anjali Vanita Balgobind[1,2], Jeroen Korving[1,2], Natalie Proost[5], Harry Begthel[1,2], Lise M. van Wijk[6], Sonia Aristín Revilla[1,2], Rebecca Theeuwsen[5], Marieke van de Ven[5], Markus J. van Roosmalen[3], Bas Ponsioen[2,4], Victor W. H. Ho[7], Benjamin G. Neel[7,8], Tjalling Bosse[9], Katja N. Gaarenstroom[10], Harry Vrieling[6], Maaike P. G. Vreeswijk[6], Paul J. van Diest[11], Petronella O. Witteveen[12], Trudy Jonges[11], Johannes L. Bos[2,4], Alexander van Oudenaarden[1,2], Ronald P. Zweemer[13], Hugo J. G. Snippert[2,4], Wigard P. Kloosterman[3*] and Hans Clevers[1,2,14*]

NATURE MEDICINE | VOL 25 | MAY 2019 | 838–849

IN VIVO MODELS

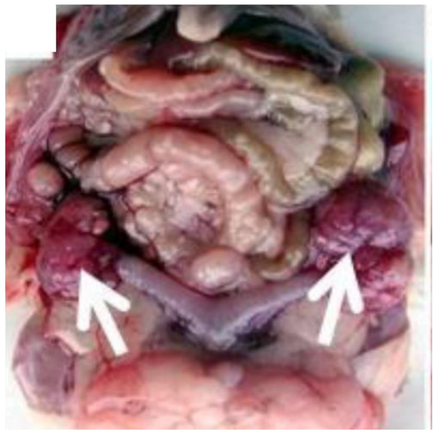
- **Cell-derived xenografts**
- **Patient-derived xenografts**
- **Genetically modified mice**



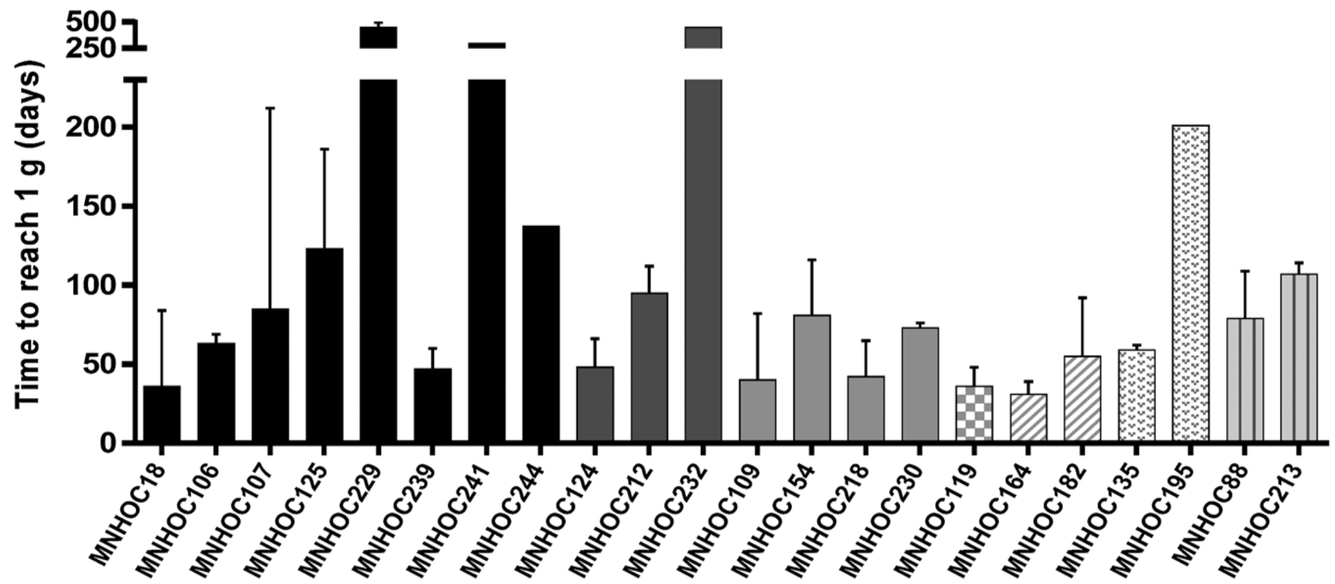
in vivo imaging

Different properties:

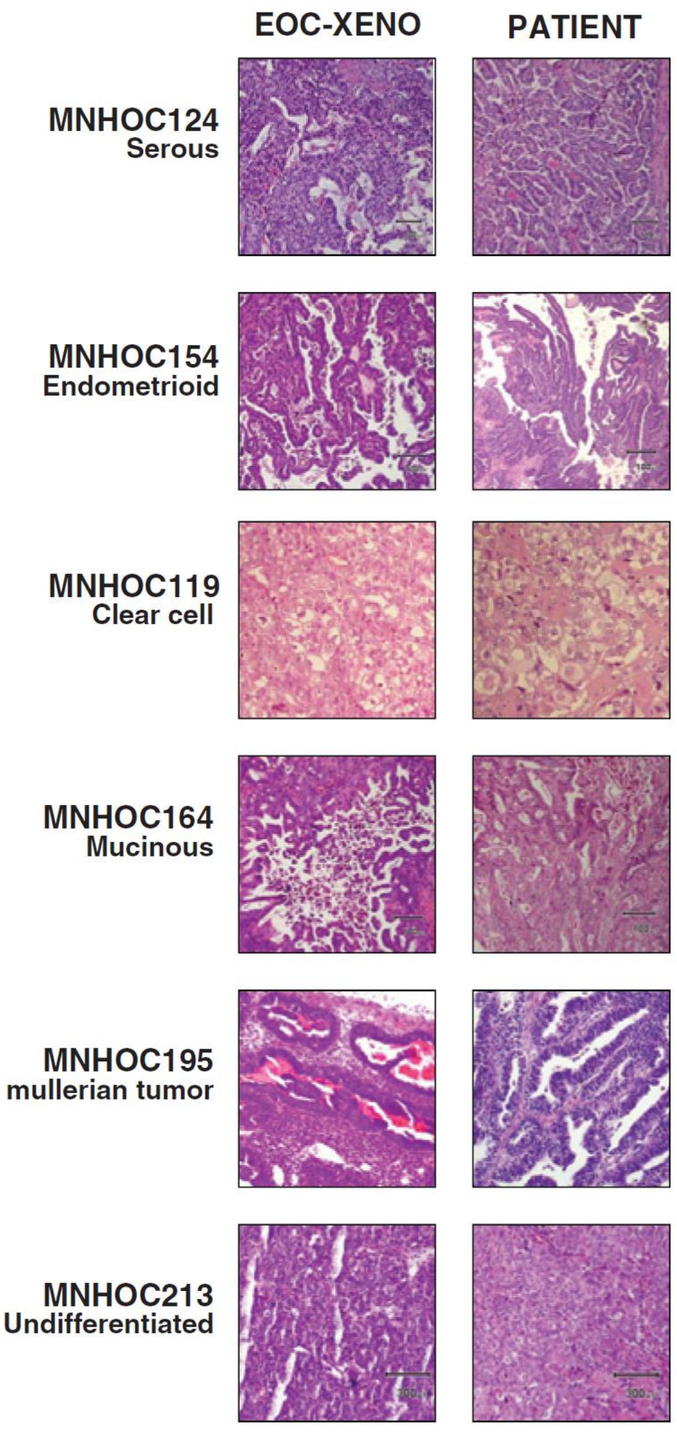
- tumor take, time to tumor formation
- tumor heterogeneity
- metastases development
- dissecting the role of tumor microenvironment (e.g. immune system)
- introducing a reporter gene for functional assays
- generating living biobanks to relate drug sensitivity to tumor genetics



PANEL OF OVARIAN CANCER PATIENT-DERIVED XENOGRAFTS AT IRFMN



	Patient		Xenograft
ID #	Treatment	Response	Response to CDDP
MNH0C8	CBDCA		
MNH0C8Y	CBDCA/EPI/CTX/CDDP		
MNHOC10	CDDP		
MNH0C18	EPI/CBDCA/VP16		
MNHOC125	CBDCA		
MNHOC124	CBDCA/PTX		
MNHOC212	CBDCA/PTX		
MNHOC230	CBDCA/PTX		
MNH0C119	CBDCA		
MNHOC164	CBDCA/PTX		
MNHOC88	CDDP		

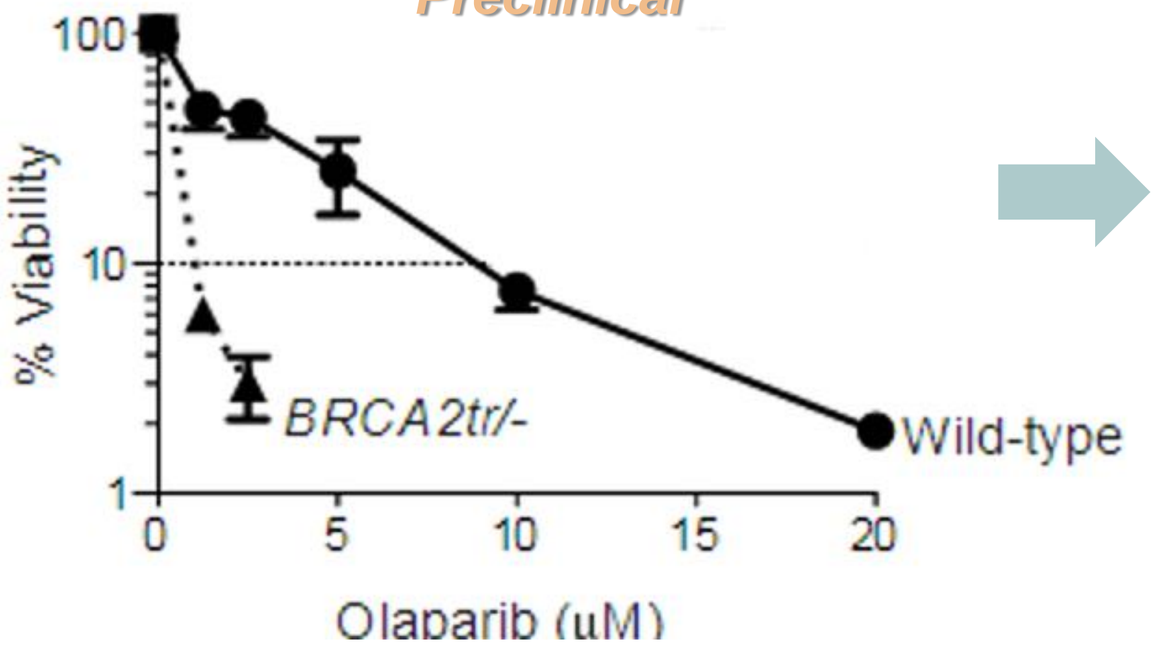


Ricci et al. Cancer Res, 2014

PARP INHIBITORS

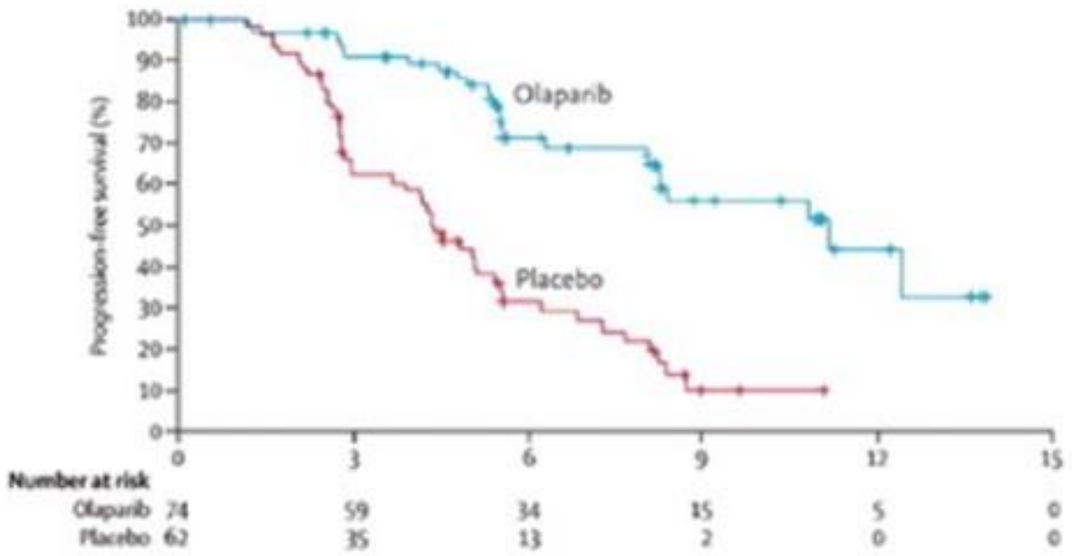
- Original rationale: sensitizing tumor cells to conventional treatments causing DNA damage
- The identification of the synthetic lethal interaction between PARPi and BRCA mutations encouraged further development of this drug class

Preclinical



Murai et al. Mol Cancer Ther. 2014

Clinical



Ledermann et al. *Lancet Oncol*. 2014

PARP INHIBITORS

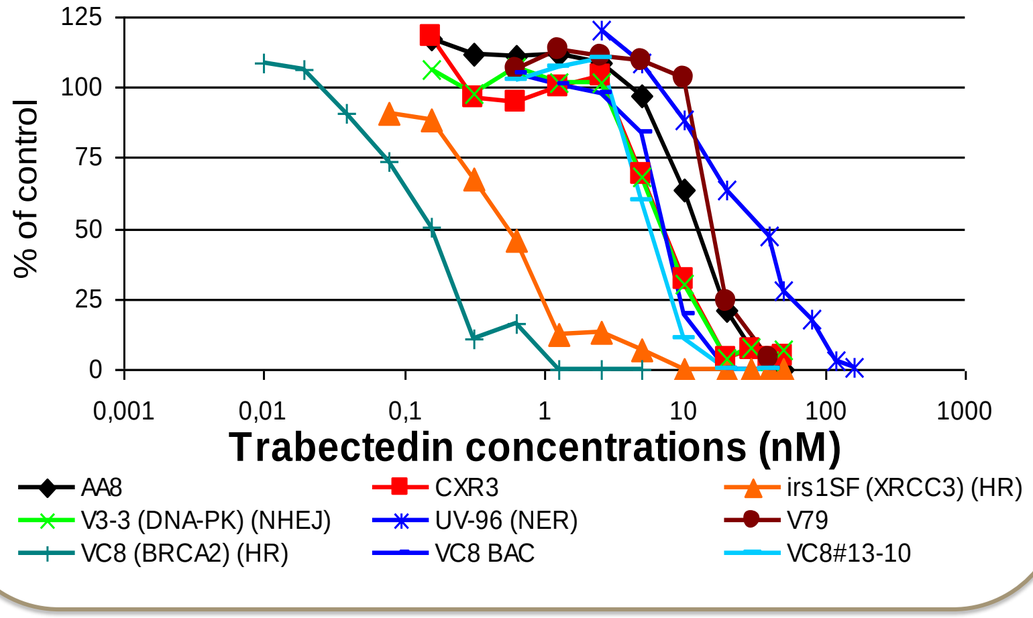
There are still **open questions**:

- **Mechanism of action:**

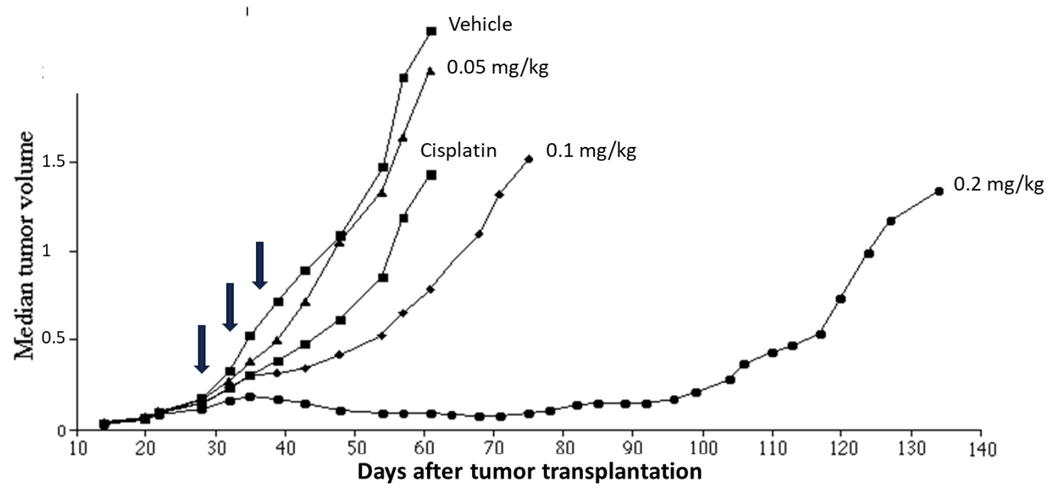
  The prevailing hypothesis is that both **PARylation inhibition** and **PARP trapping** contribute to PARPi cytotoxicity. However, is **replication stress** induced by slowing or acceleration of replicative forks **?**

- How PARPi differently affect components of **tumor microenvironment ?**
- How PARPi influence **gene expression** in different tissues **?**
- What about **long term toxicity** on normal tissues **?**
- What induce the different effects of **compounds belonging to the same class ?** Have olaparib, niraparib, rucaparib, talazoparib different activities on tumor cells relative to BRCA status **?**
- How to **combine PARP inhibitors** with **other drugs** in the most rationale way?

TRABECTEDIN



OC-PDX mice



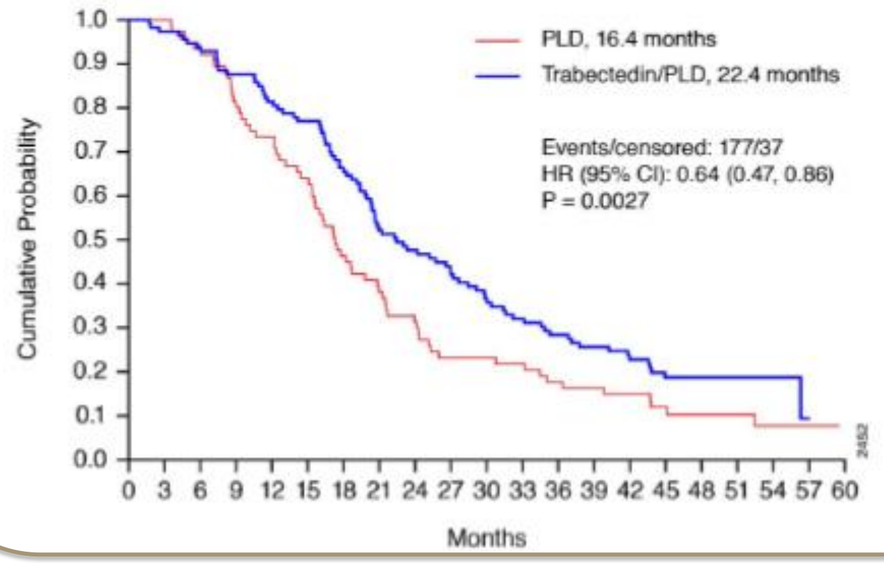
Valoti et al., Clin. Cancer Res, 1998

Patient Category	No. of Patients	CR or PR		SD	
		No.	%	No.	%
y dose					
1,300 $\mu g/m^2$	35	8	23	14	40
> 1,300 $\mu g/m^2$	16	4	25	3	19
y sensitivity to prior treatment					
Platinum-resistant					
PD or SD	11	0		3	27
Responders	17	2	12	5	29
Platinum-sensitive					
6-12 months PFI	11	4	36	5	45
> 12 months PFI	12	6	50	4	33

Sessa et al. J Clin Oncol. 2005

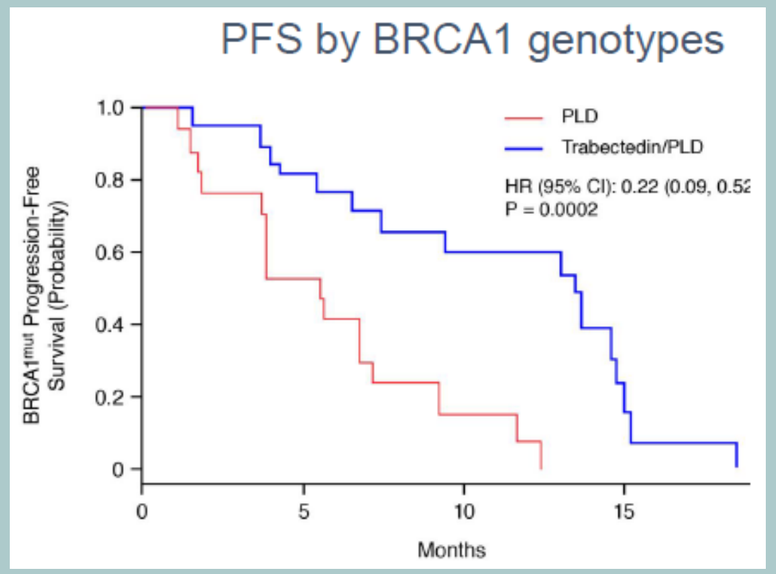
ET743-OVA-301

OS by PFI 6-12 months

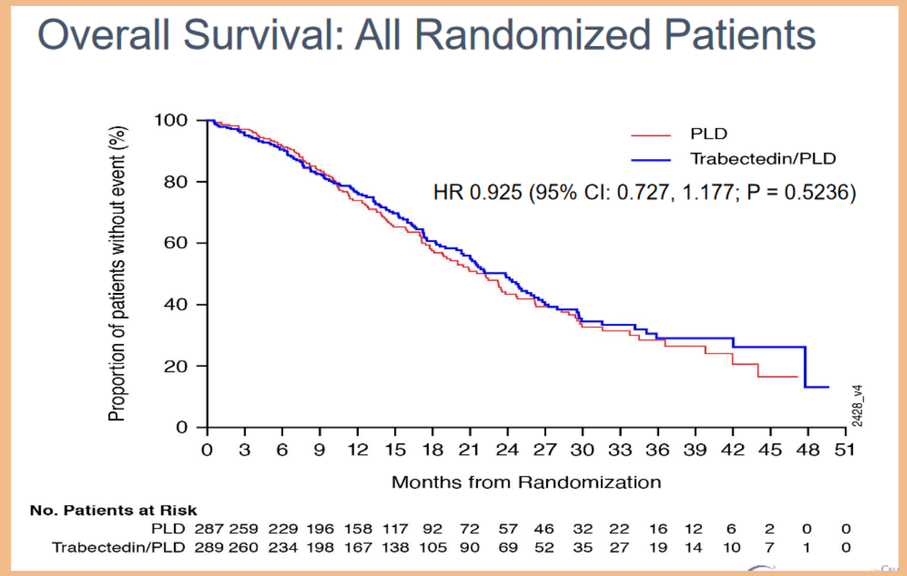


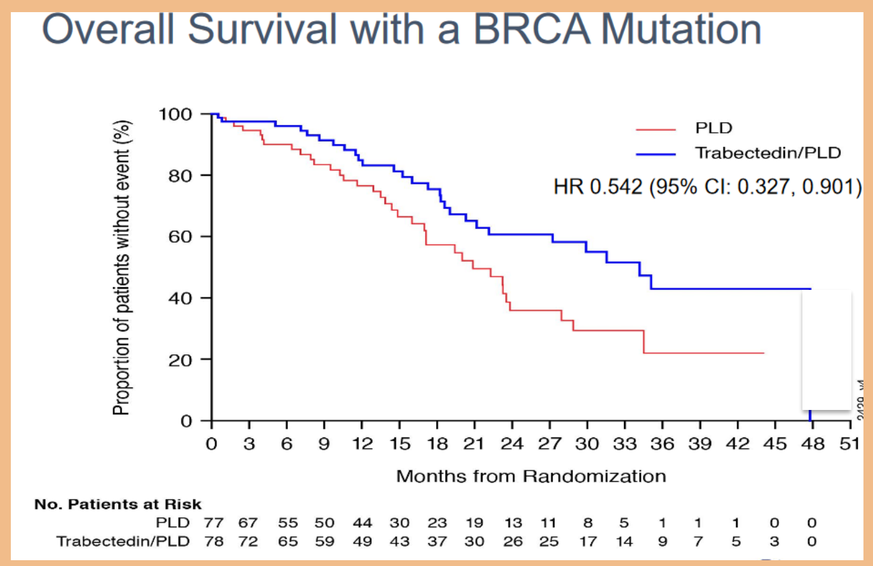
TRABECTEDIN

ET743-OVA-301

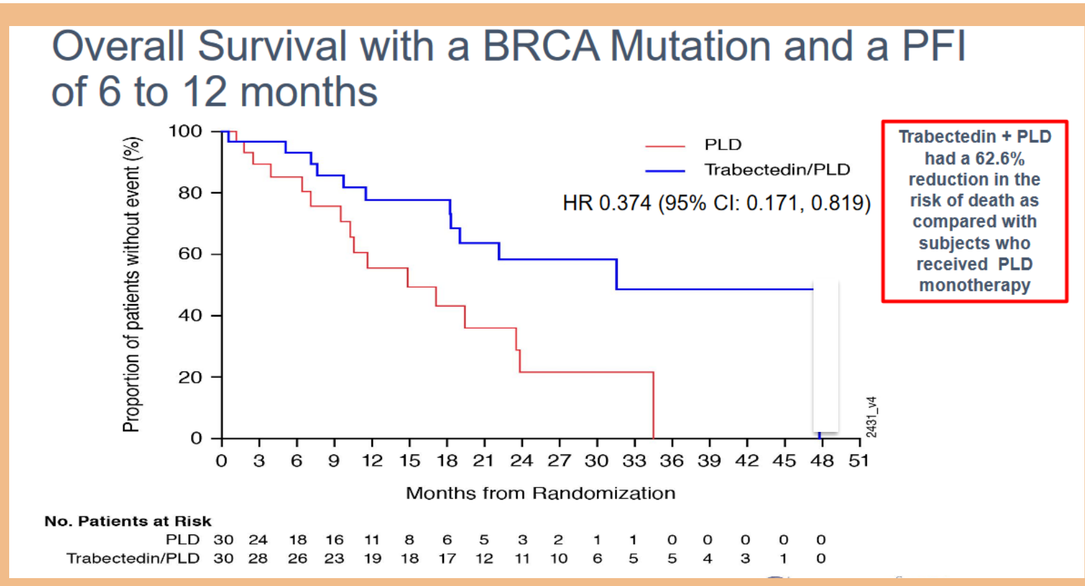


ET743-OVC-3006





Trabectedin+PLD had a 45.8% reduction in the risk of death as compared with subjects who received PLD monotherapy

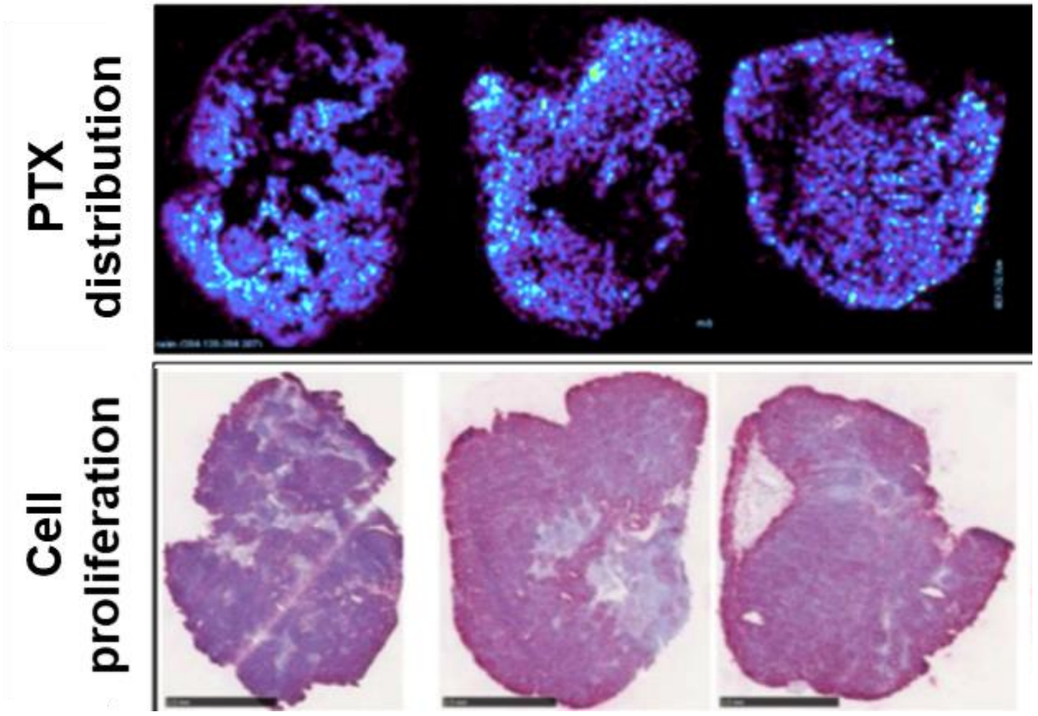


Trabectedin+PLD had a 62.6% reduction in the risk of death as compared with subjects who received PLD monotherapy

PHARMACOKINETIC ANALYSIS IN MOUSE MODELS

PK studies on tumor samples grown in mice allow:

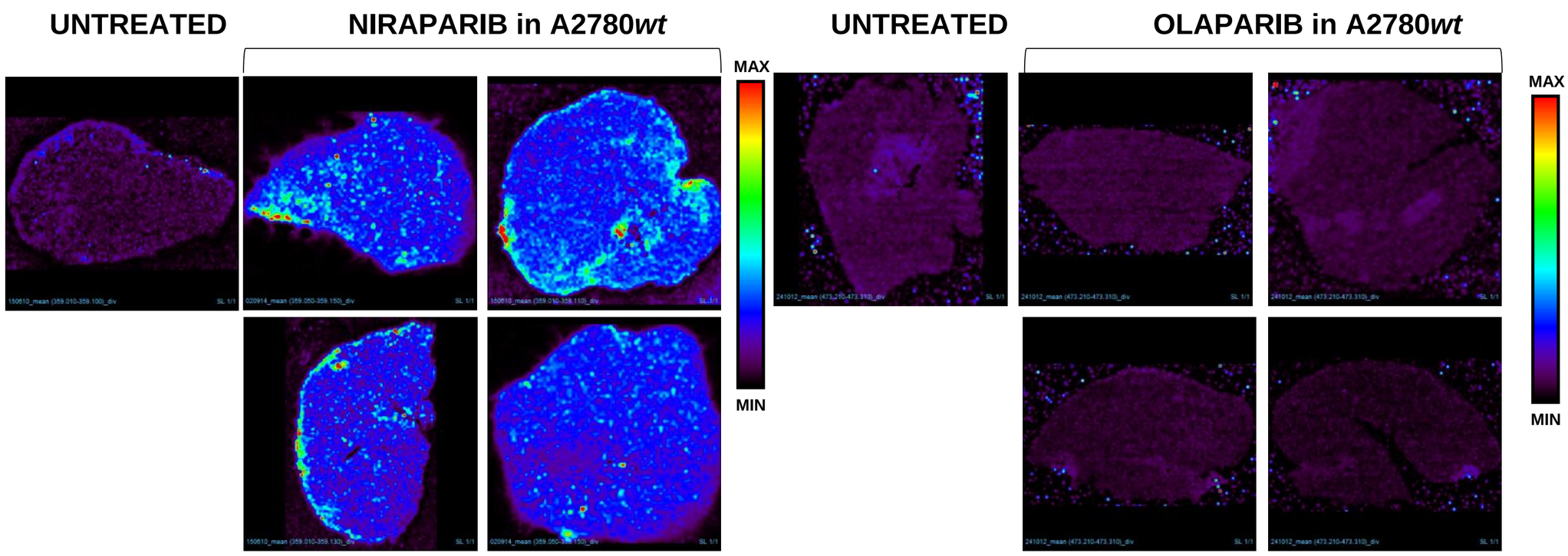
- studies of drug distribution, particularly in tumor, for a first screening in drug development
- PK/PD studies
- visualization of drug distribution within tumor tissue and investigation of different strategies to improve drug penetration



Cesca et al. *Mol Cancer Ther,* 2016

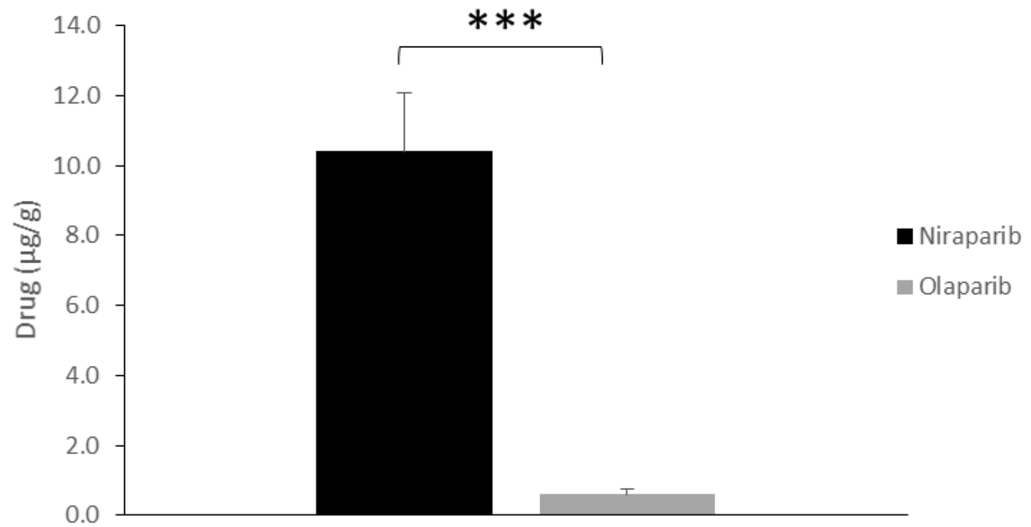
PARPi DISTRIBUTION in ovarian cancer model

MASS SPECTROMETRY IMAGING: drug distribution



LC-MS/MS: drug concentration in tumor (2h after treatment)

TUMOR BEARING MICE WERE TREATED WITH 50 mg/kg NIRAPARIB P.O. OR WITH 67 mg/kg OLAPARIB P.O.

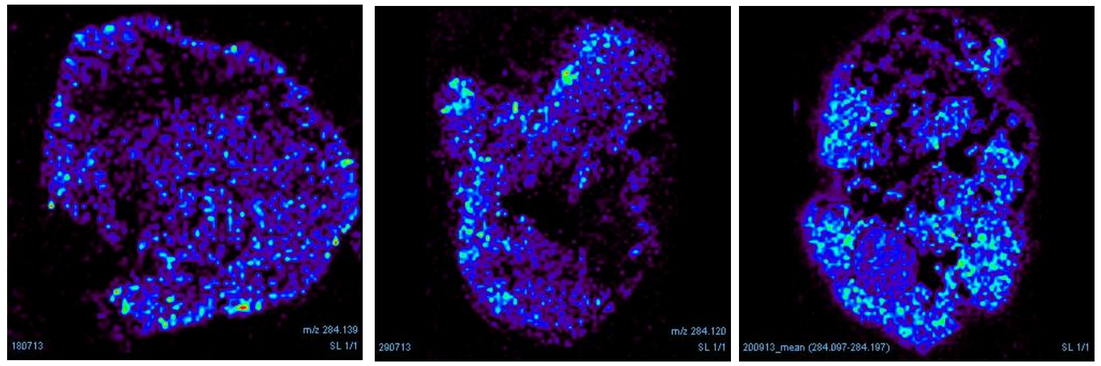


IMPROVE TUMOR DRUG DISTRIBUTION

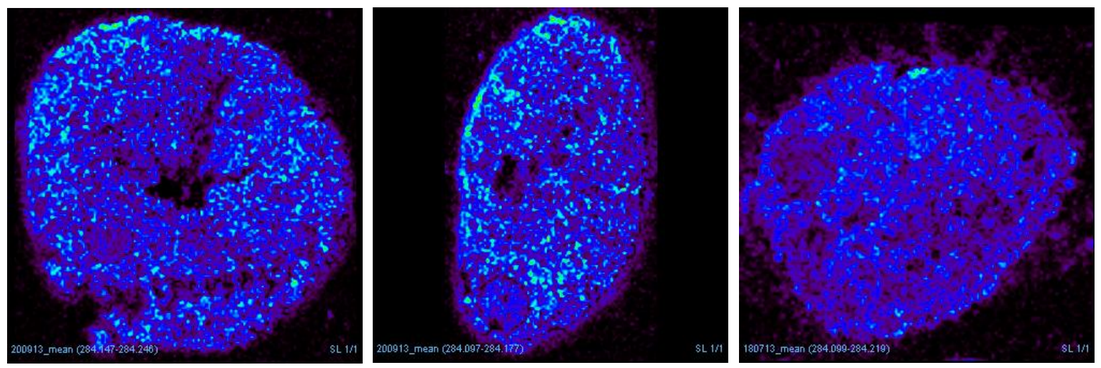
➢ TARGETING THE TUMOUR VASCULATURE

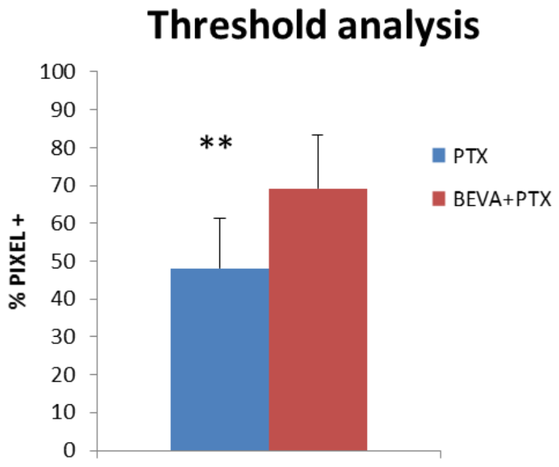
(angiogenesis inhibition)

PTX



BEVACIZUMAB+PTX

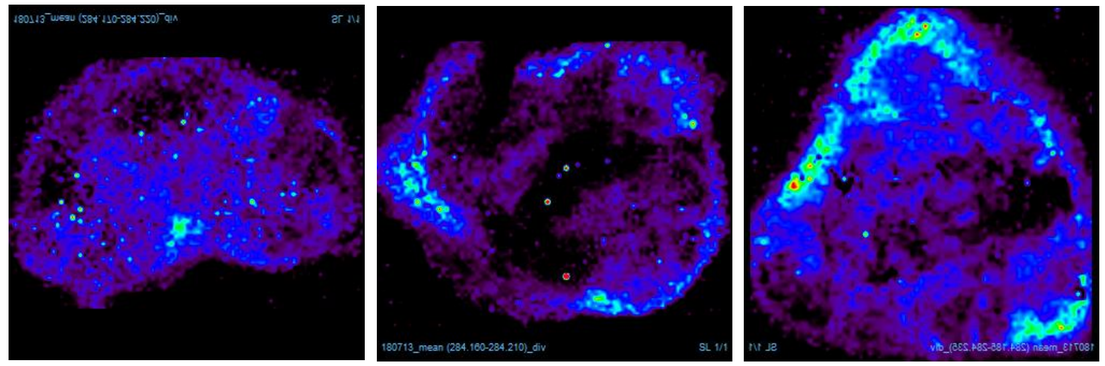




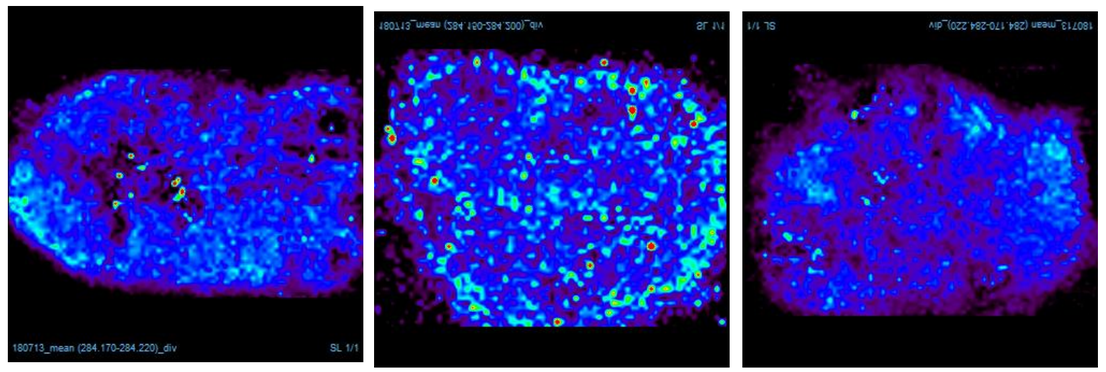
➢ TARGETING THE TUMOUR STROMA

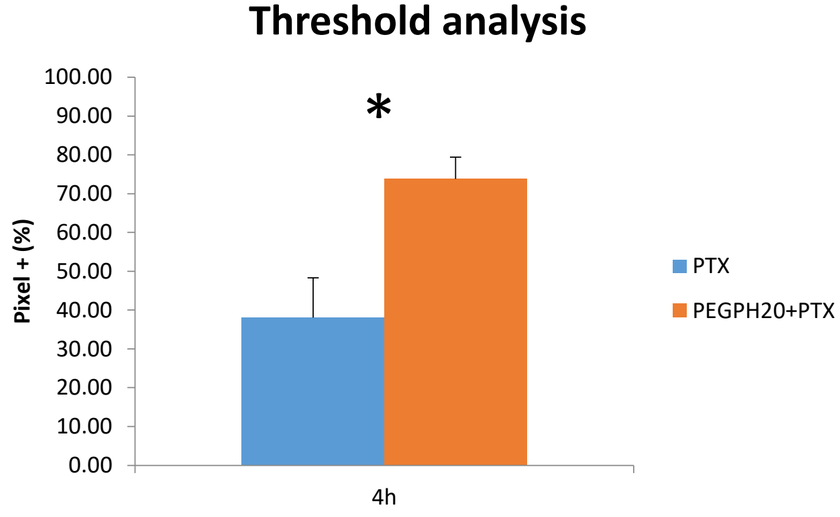
(extracellular matrix degradation)

PTX

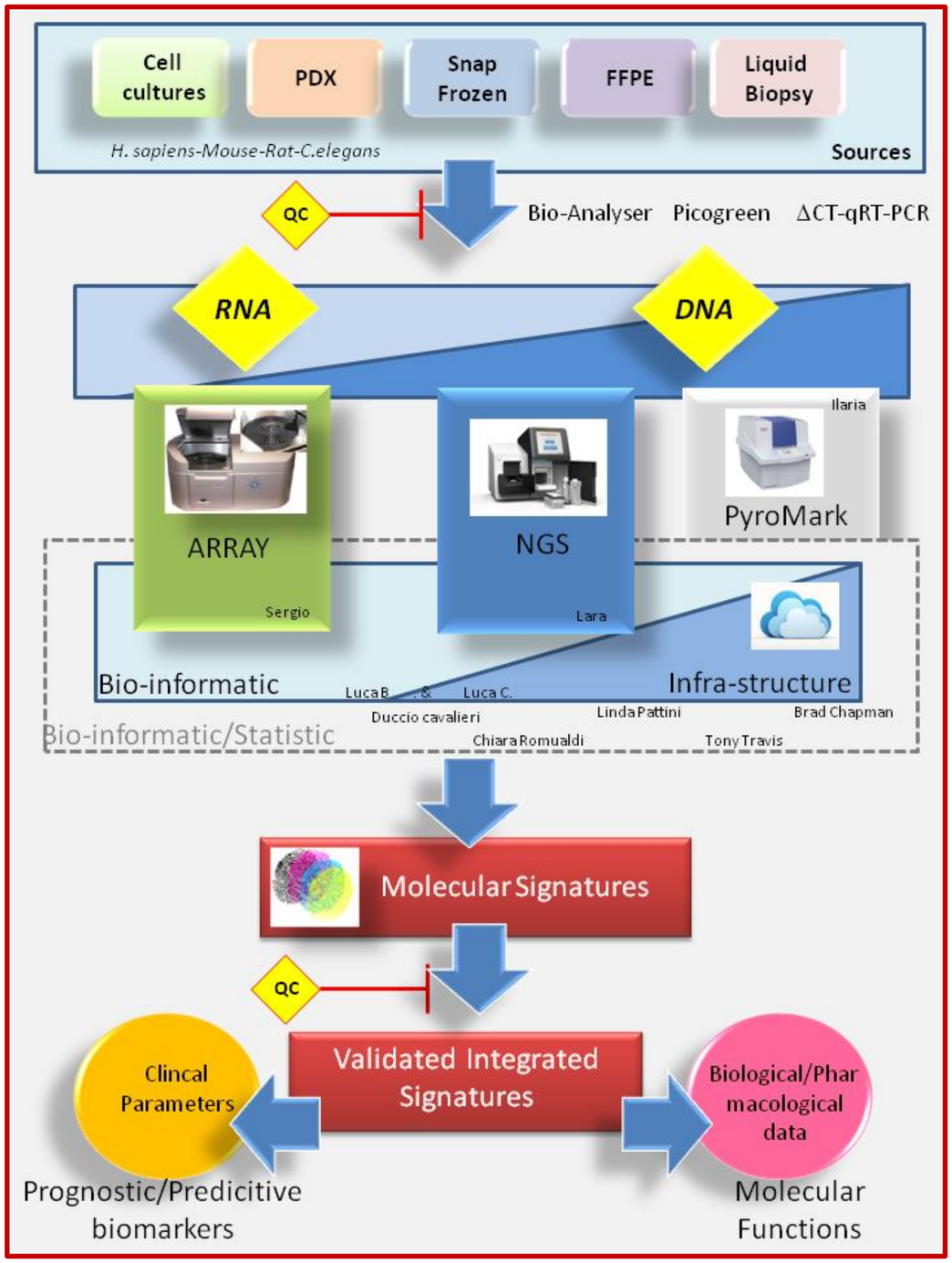


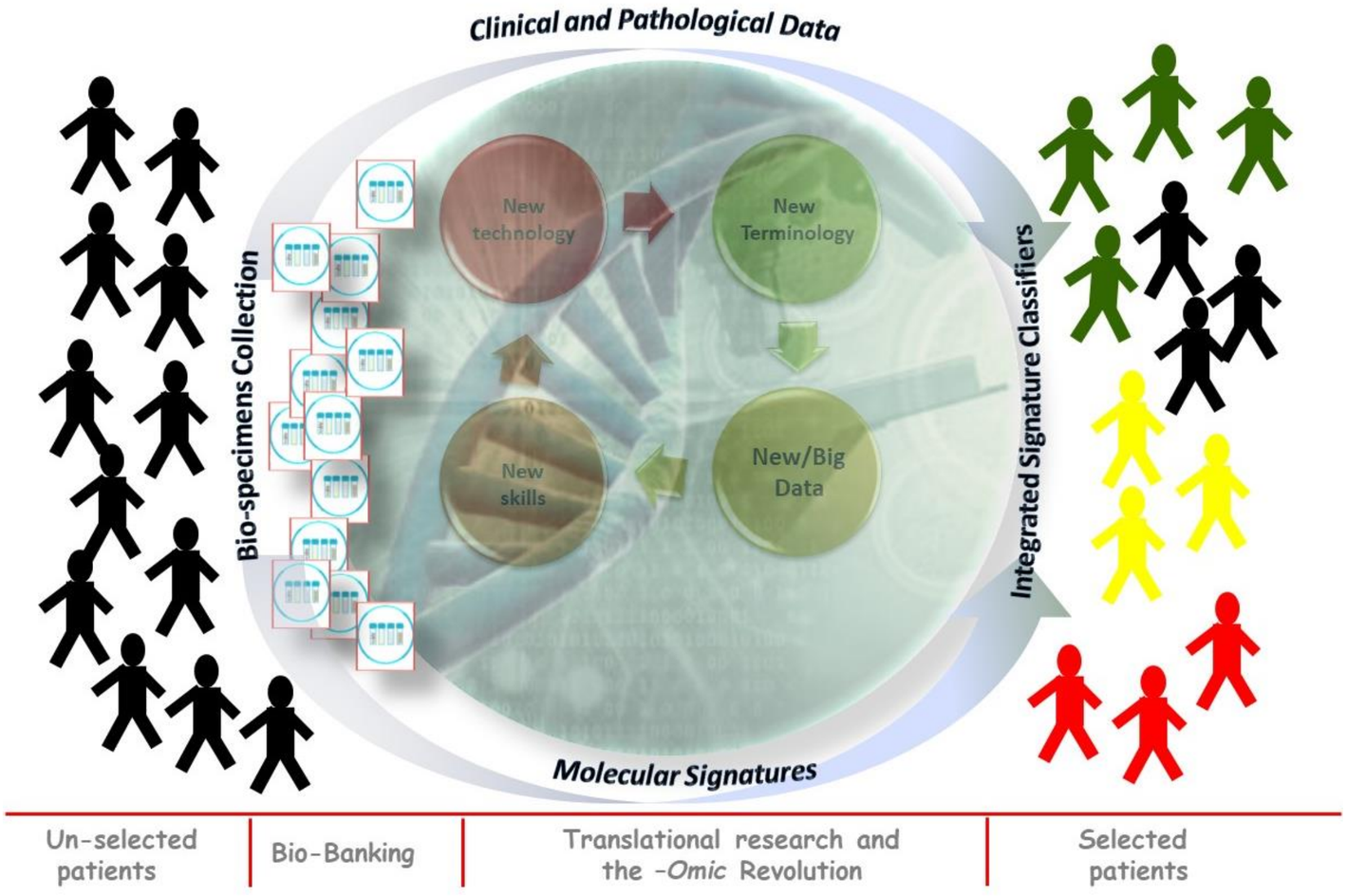
HYALURONIDASE+PTX





STUDIES ON CLINICAL SAMPLES

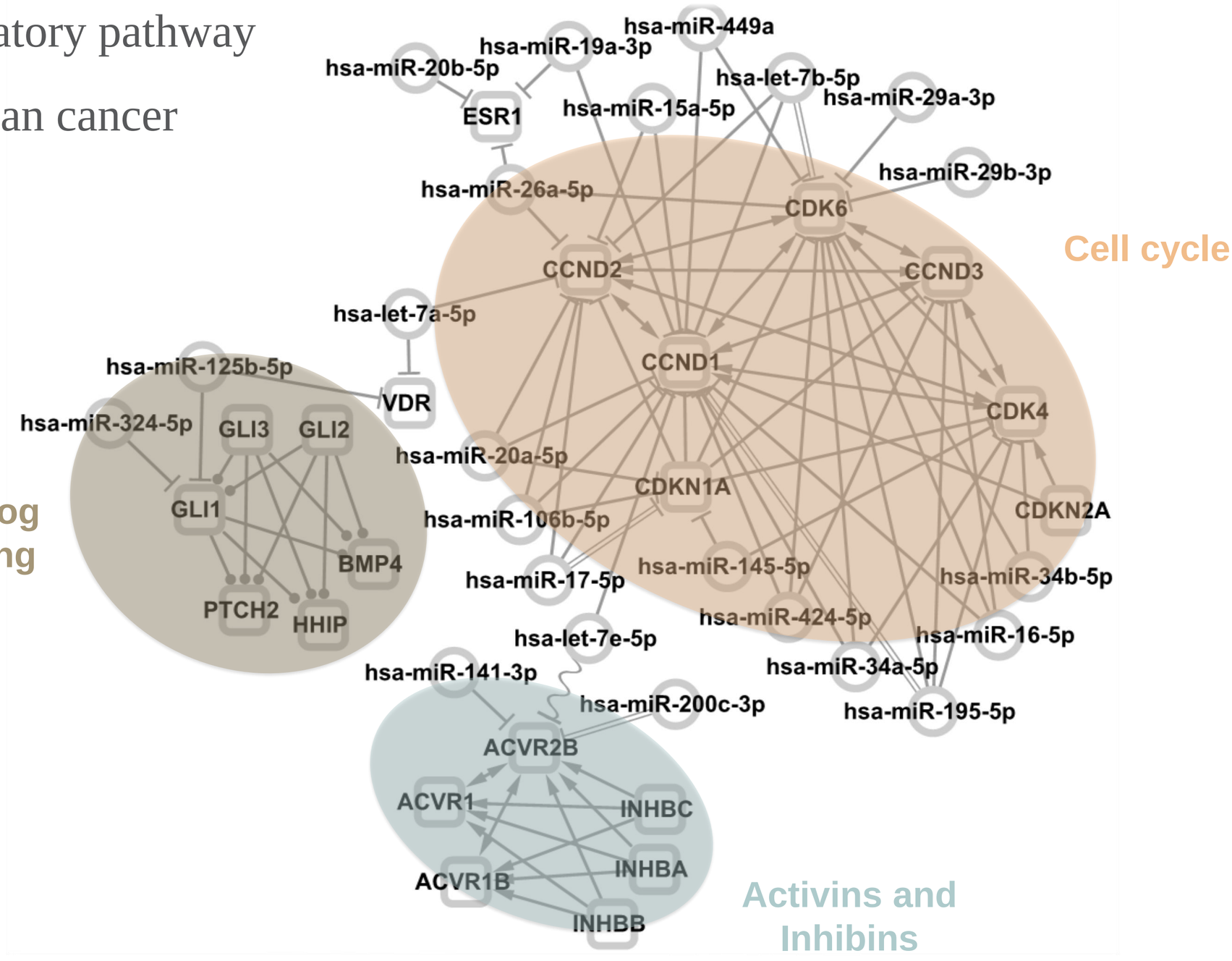




IDENTIFICATION OF BIOMARKERS FOR PATIENT STRATIFICATION

A prognostic regulatory pathway

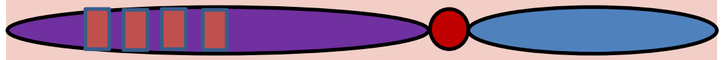
in stage I ovarian cancer



Calura et al. Ann Oncol. 2016

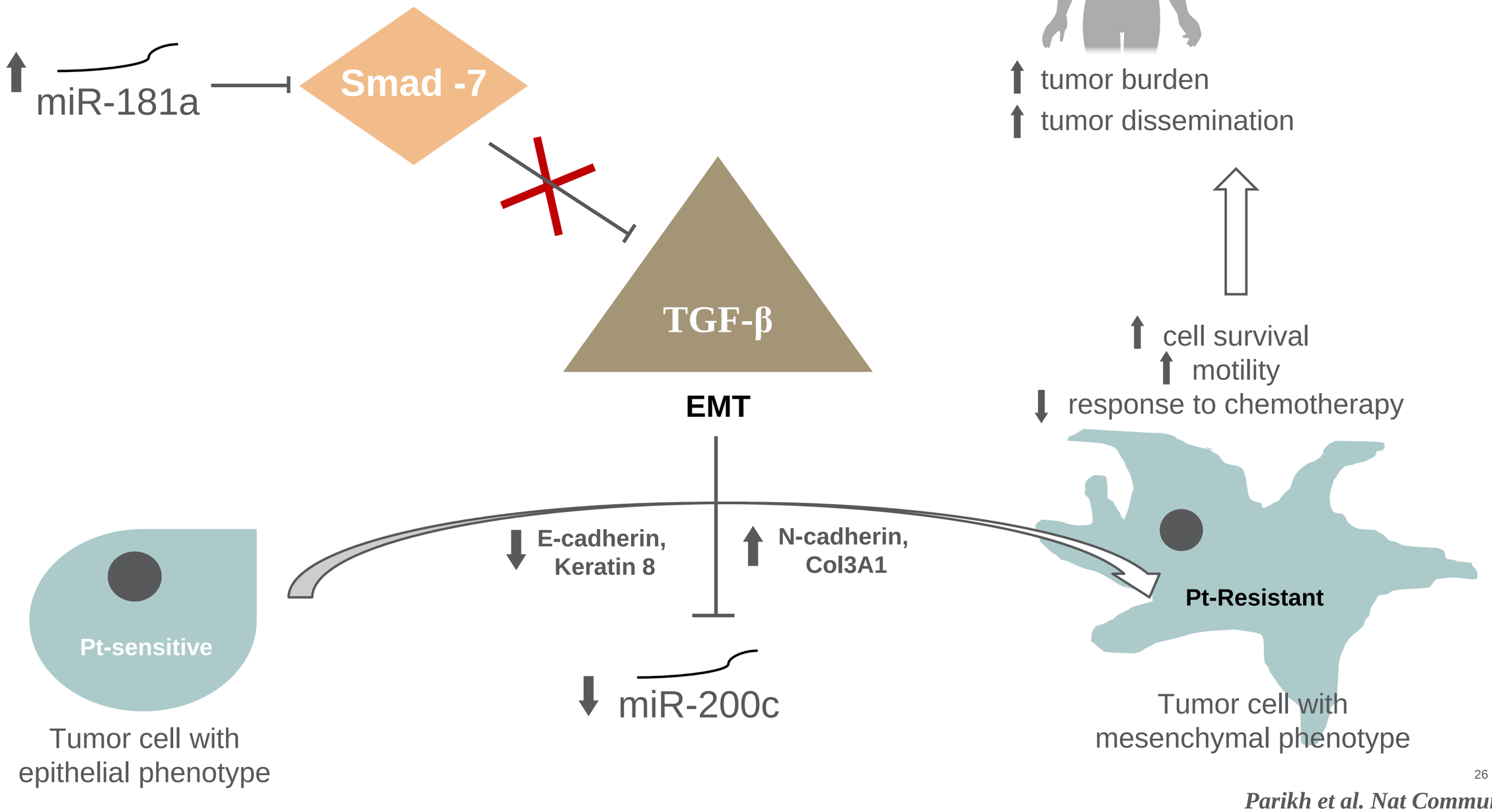
Stage I EOC

Somatic Copy Number Alterations (SCNAs) Profile

STABLE	UNSTABLE	HIGHLY UNSTABLE
0% of genome affected by SCNAs	15% of genome affected by SCNAs	50% of genome affected by SCNAs
NO SCNAs	**LARGE REARRANGEMENTS**	**FOCAL ALTERATIONS**
	Amplification/Deletion of entire chromosome/chromosome arms: 	 Enriched in High Grade Serous Histotype

IDENTIFICATION OF BIOMARKERS FOR PATIENT STRATIFICATION

Identification of miRNAs and pathways associated with survival and drug response in HGSOC patients



Parikh et al. Nat Commun. 2014

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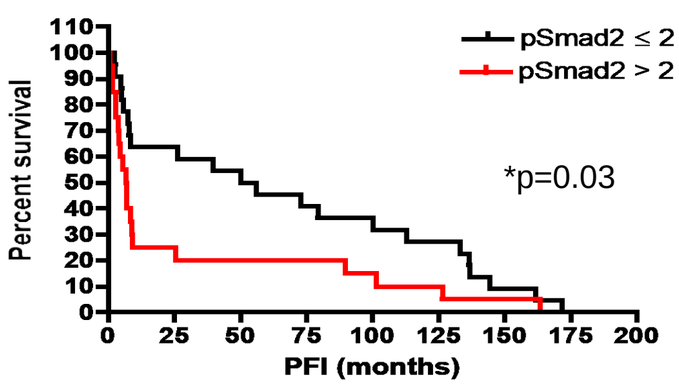
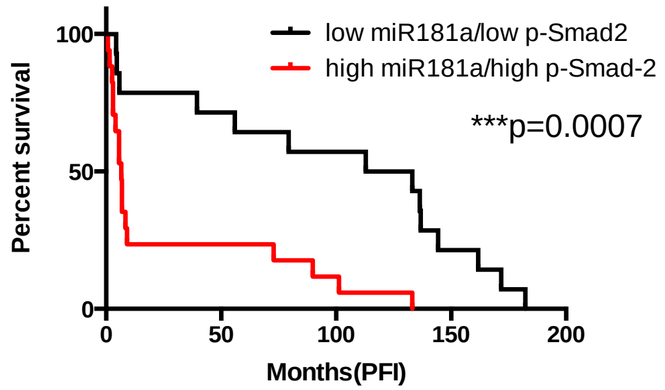
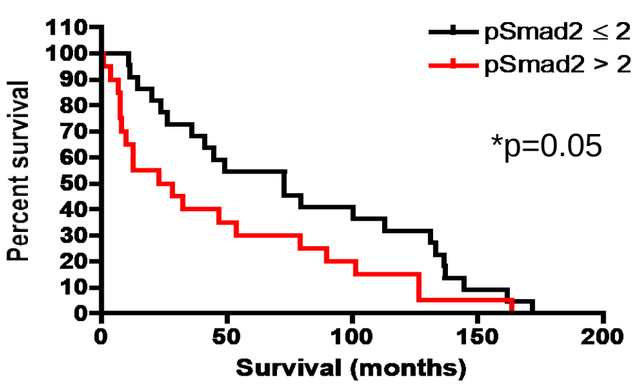
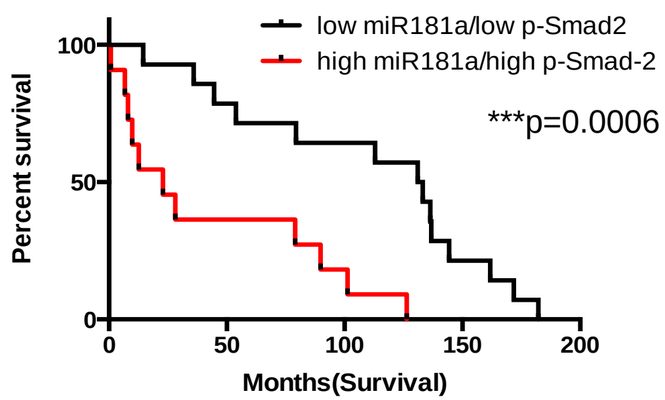
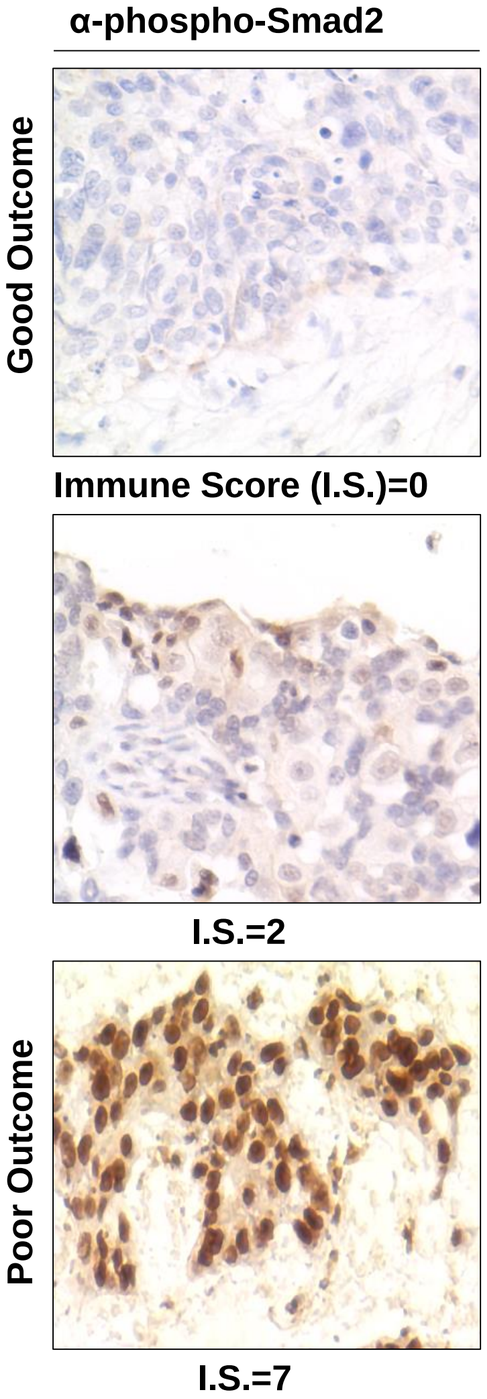






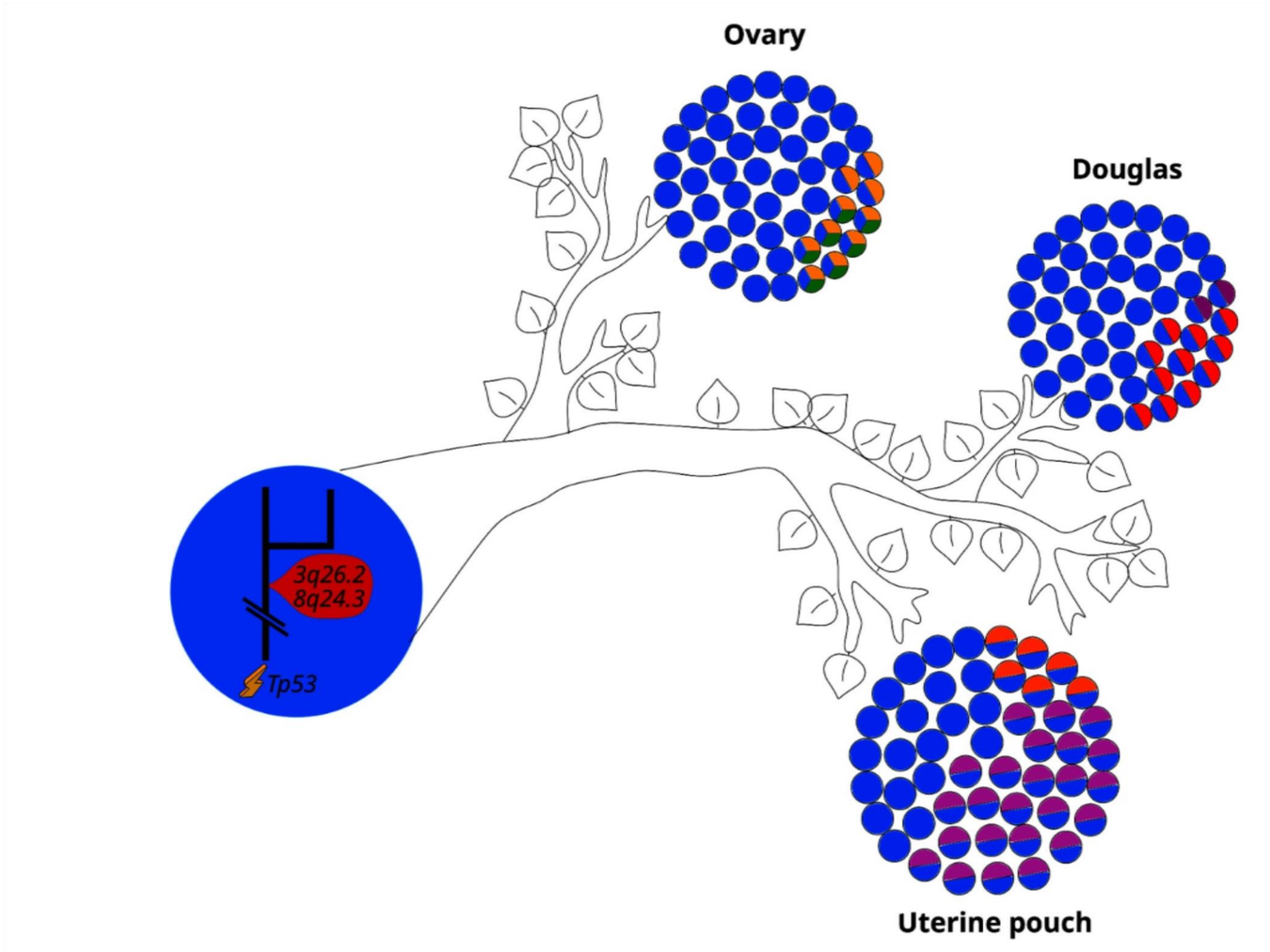




Petrillo et al. Ann Oncol. 2016
Parikh et al. Nat Commun. 2014

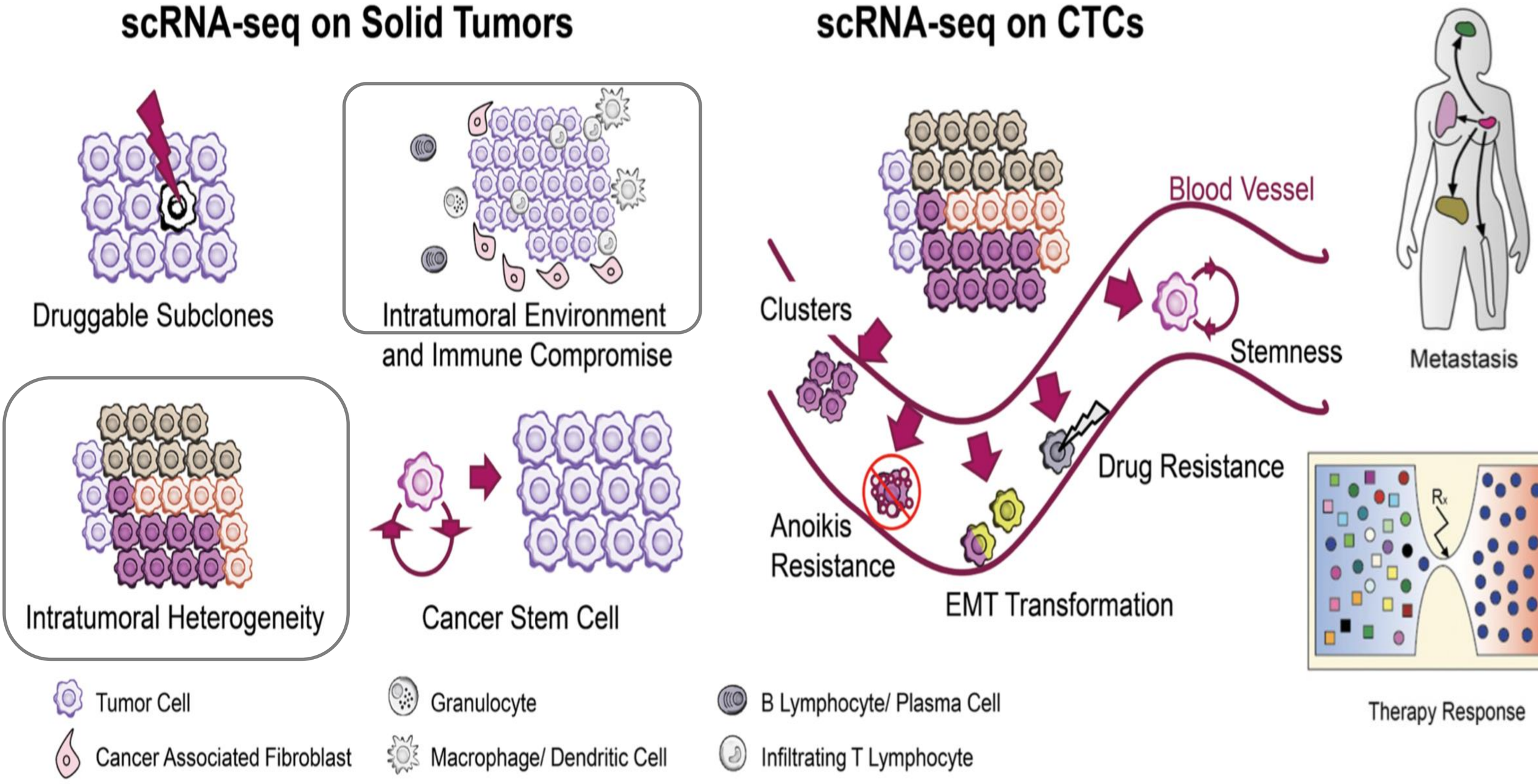
IDENTIFICATION OF NEW TARGETS

Genomic regions of focal and recurrent copy number alteration in 3q26.2 and 8q24.3 in HGSOC



Ballabio S., et al. Int J Cancer 2019

SINGLE-CELL TECHNOLOGY FOR CANCER RESEARCH IN SOLID TUMOR TISSUES AND CIRCULATING TUMOR CELLS



Winterhoff et al., Gynecol Oncol., 2017

McPherson et al., Nat Genet., 2016

STUDIES ON CLINICAL SAMPLES

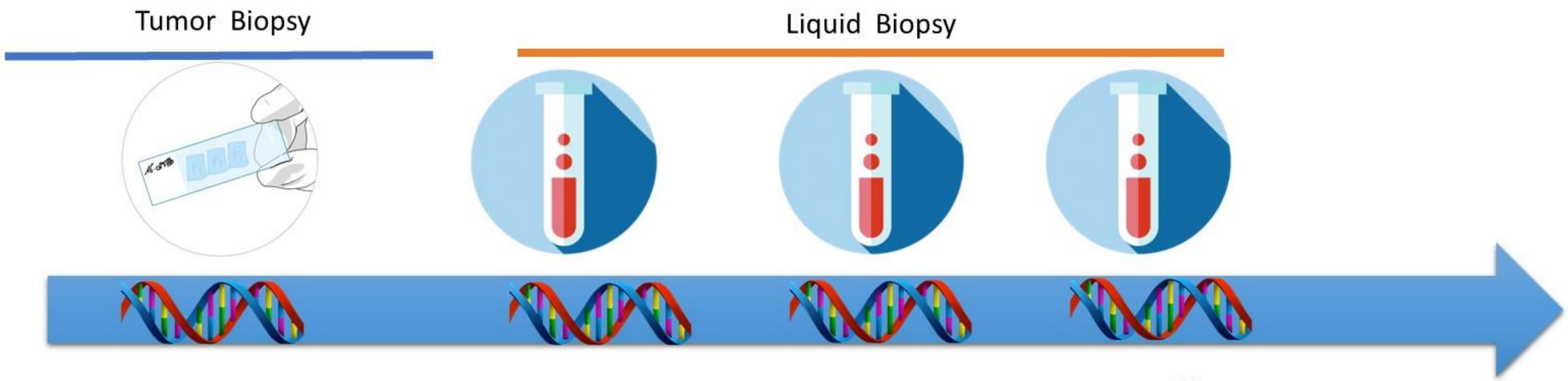
-OMIC ANALYSES ON TUMOR SAMPLES

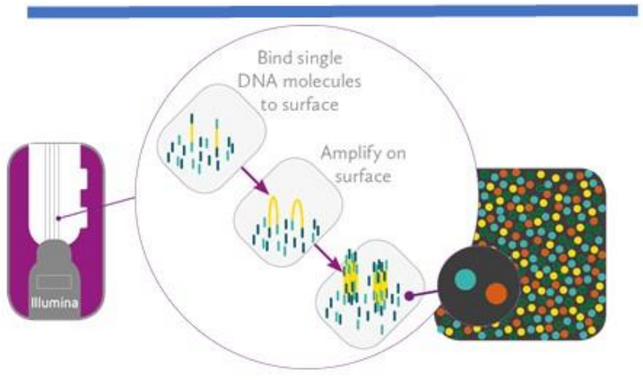
Major limitation

- **Spatial limits**:
  - only the primary tumor is analyzed
  - only a small fraction of tumor tissue is analyzed
- **Temporal limits**:
  - tumor progression and pharmacological therapy modifies the molecular profile of tumor cells

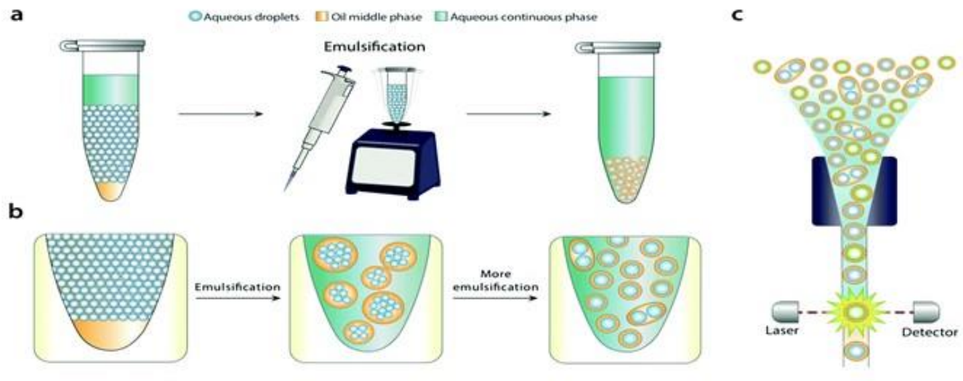
Additional approaches are necessary to complement –omic techniques and guide therapy choice

LIQUID BIOPSY





Full lenght sequencing to identify the trunk mutation in the *TP53* gene by NGS.



ctDNA monitoring by ddPCR of selected Tp53 mutation

CONCLUDING REMARKS

- Much research is still needed to set up preclinical models that mimic the complexity and heterogeneity of ovarian cancer in an adequate fashion. The recent development of organoids requires a validation

- Human ovarian cancer xenografts representative of different histotypes are useful to investigate drugs acting directly on cancer cells, but new syngenic models are needed to investigate immune mechanisms and immunotherapies

CONCLUDING REMARKS

- New powerful technologies are providing potential molecular signatures to drive treatments in a more rational way, even though there is still need of validation of each new biomarker by rigorous statistical approaches

- Even applying the most sophisticated and state-of-the art technologies our knowledge is still very limited and thus our research is necessarily still empirical. Therefore translational research, from the lab to the clinic and from the clinic to the lab is essential to make significant progress

- The complexity and rapidly evolving medical research requires multidisciplinary teams including not only medical doctors (surgeons, oncologists, pathologists), biologists, pharmacologists, but also mathematicians, statisticians, engineers and bioinformaticians

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