



ISTITUTO DI RICOVERO E CURA A CARATTERE SCIENTIFICO

## **IRE – WEIZMANN COLLABORATION PROGRAM A. BAGNATO (IRE) AND Y. YARDEN (WIS)**

### **Targeting Endothelin-1 and Amphiregulin-driven signaling in the ecosystem of ovarian cancer**



מכון ויצמן למדע

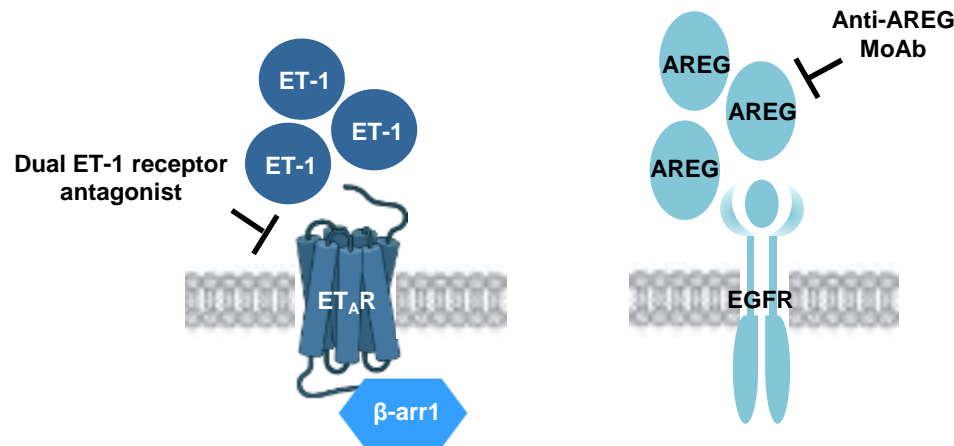
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# Synopsis

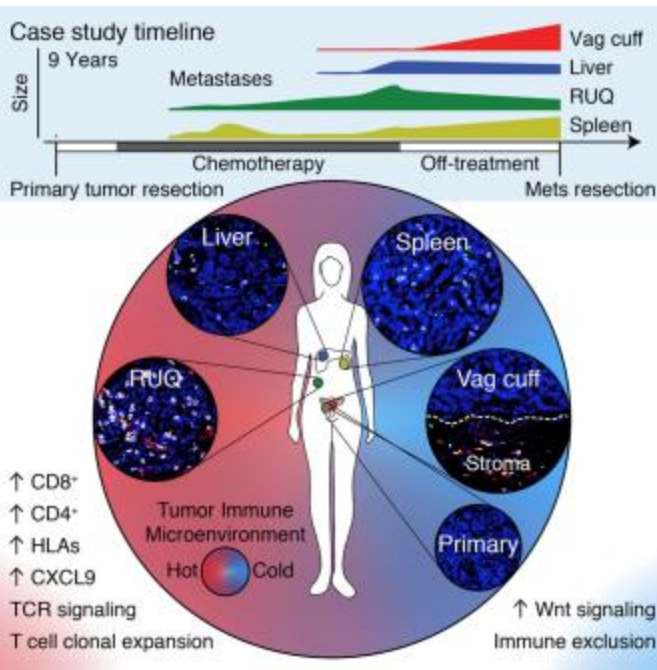
**Background:** The identification of new effective therapies for high-grade serous ovarian cancer (HG-SOC) represents an urgent unmet clinical need. Our previous studies focused on two pathways affecting the growth and progression of HG-SOC: amphiregulin (AREG), a ligand stimulating EGFR, a receptor tyrosine kinase (RTK), and endothelin-1 (ET-1), which activates a G protein coupled receptor (GPCR) called ET-1R. Importantly, each pathway funnels signals into a linear cascade that includes YAP and the tumor suppressor p53.

**Hypothesis:** ET-1R/ $\beta$ -arr1/YAP and AREG/EGFR can act as relevant modulators of the mutant p53-mediated transcriptional network and affect the dynamic interactions between HG-SOC and immune cells, fibroblast and endothelial cells, influencing the response to therapy. Therefore ET-1R and/or AREG interference can represent therapeutic options for HG-SOC.



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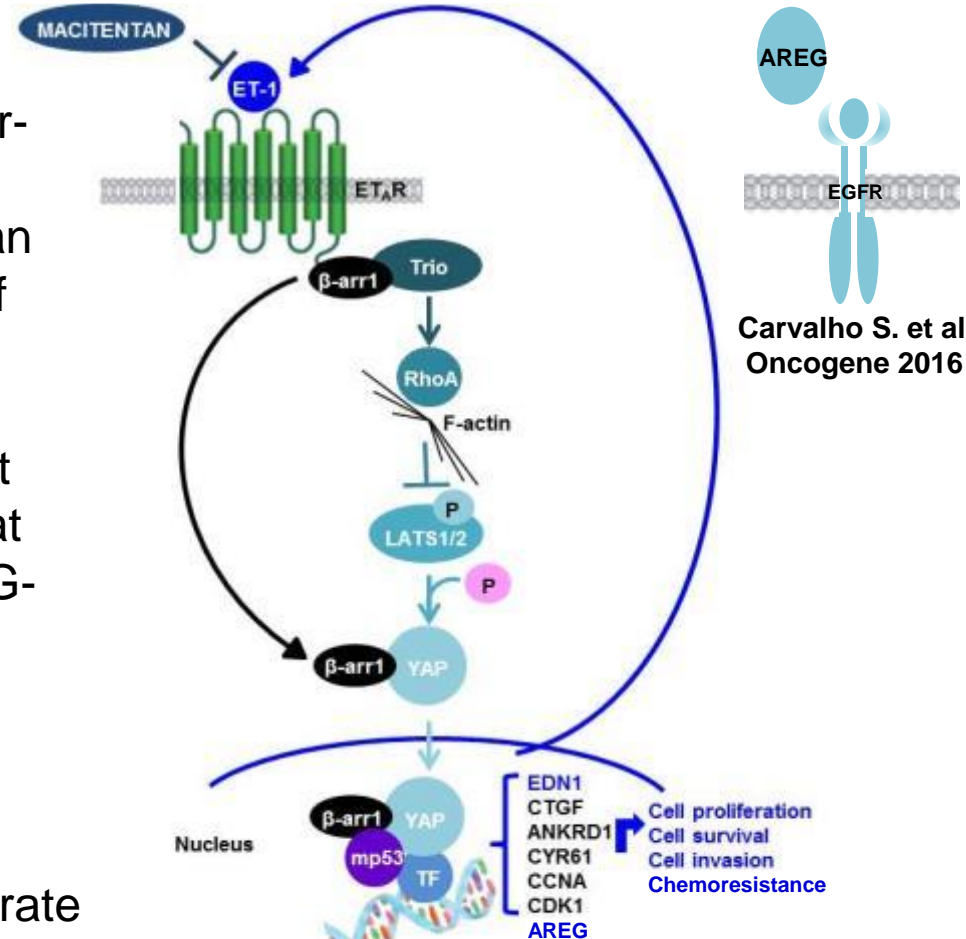


Jiménez-Sánchez A. et al. Cell 2017

Jimenez-Sanchez A. et al. Nature Genetics 2020

## Background

The intra- and inter-patient **tumor heterogeneity** is an intrinsic feature of HG-SOC. Within single individual different TME can coexist at the diagnosis of HG-SOC



AREG  
EGFR  
Carvalho S. et al  
Oncogene 2016

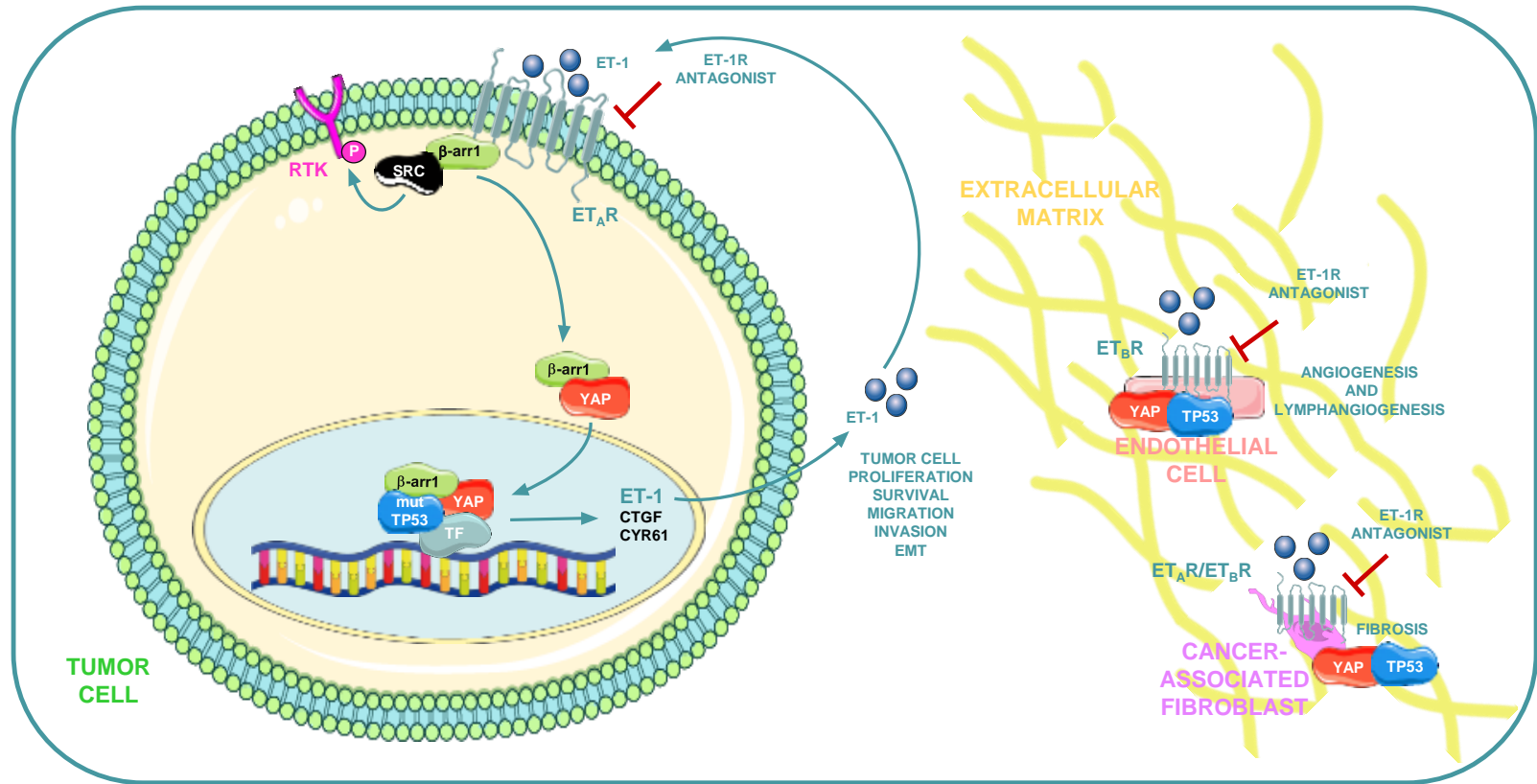
Tocci P. et al. Nat Commun. 2019

Tocci P. et al. Cancer Lett. 2020

In this complex scenario, oncogenes often cooperate with altered signaling nodes (**ET-1** and **AREG**), both implicated in the regulatory dialogue between HG-SOC and TME elements.

# Working hypotheses

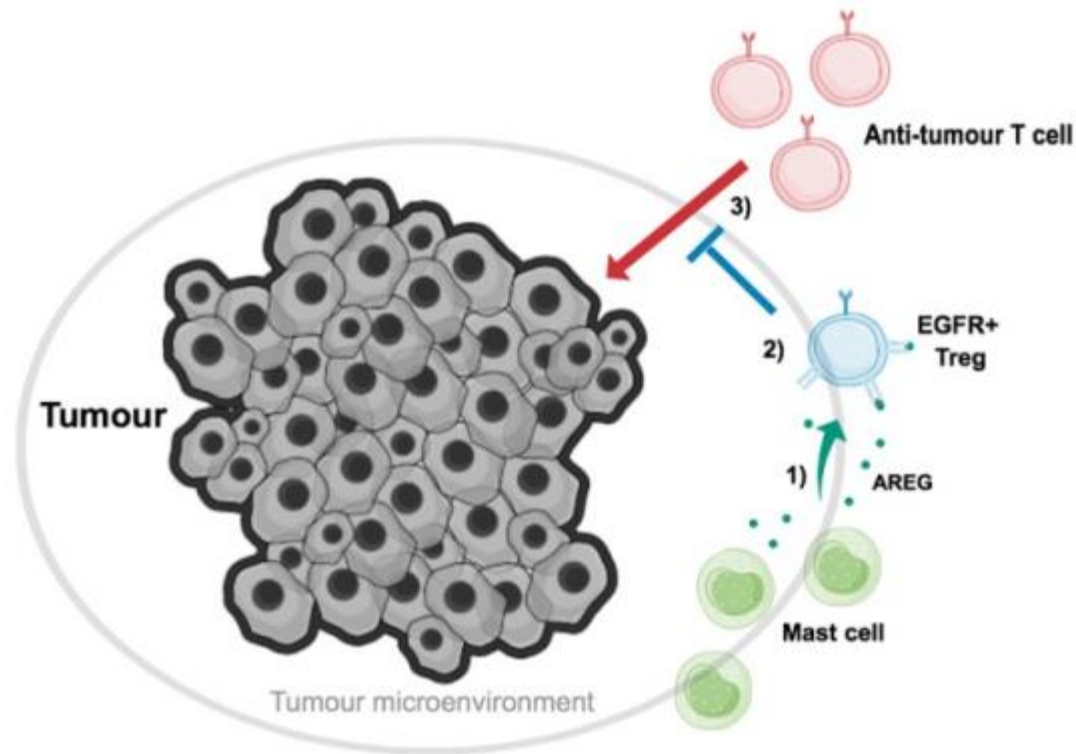
**Hypothesis 1:** Can the ET-1R-driven  $\beta$ -arr1-transcriptional 'interactome', which includes YAP/mutp53, modulate the cross-talk with cellular elements of the TME, to impact HG-SOC plasticity and drug response?



# Working hypotheses

**Hypothesis 2:** Are epithelial ovarian tumors shielded by an immune suppressive microenvironment regulated by amphiregulin?

Tregs employ AREG as a cardinal mediator of their suppressive actions. Therefore, we hypothesize that intercepting AREG in the context of HG-SOC would inhibit tumor progression by blocking autocrine signaling, as well as by preventing local suppression of the immune system.



MacDonald F, Zaiss DMW. Front Pharmacol. 2017.  
Zaiss DMW et al., Immunity, 2013

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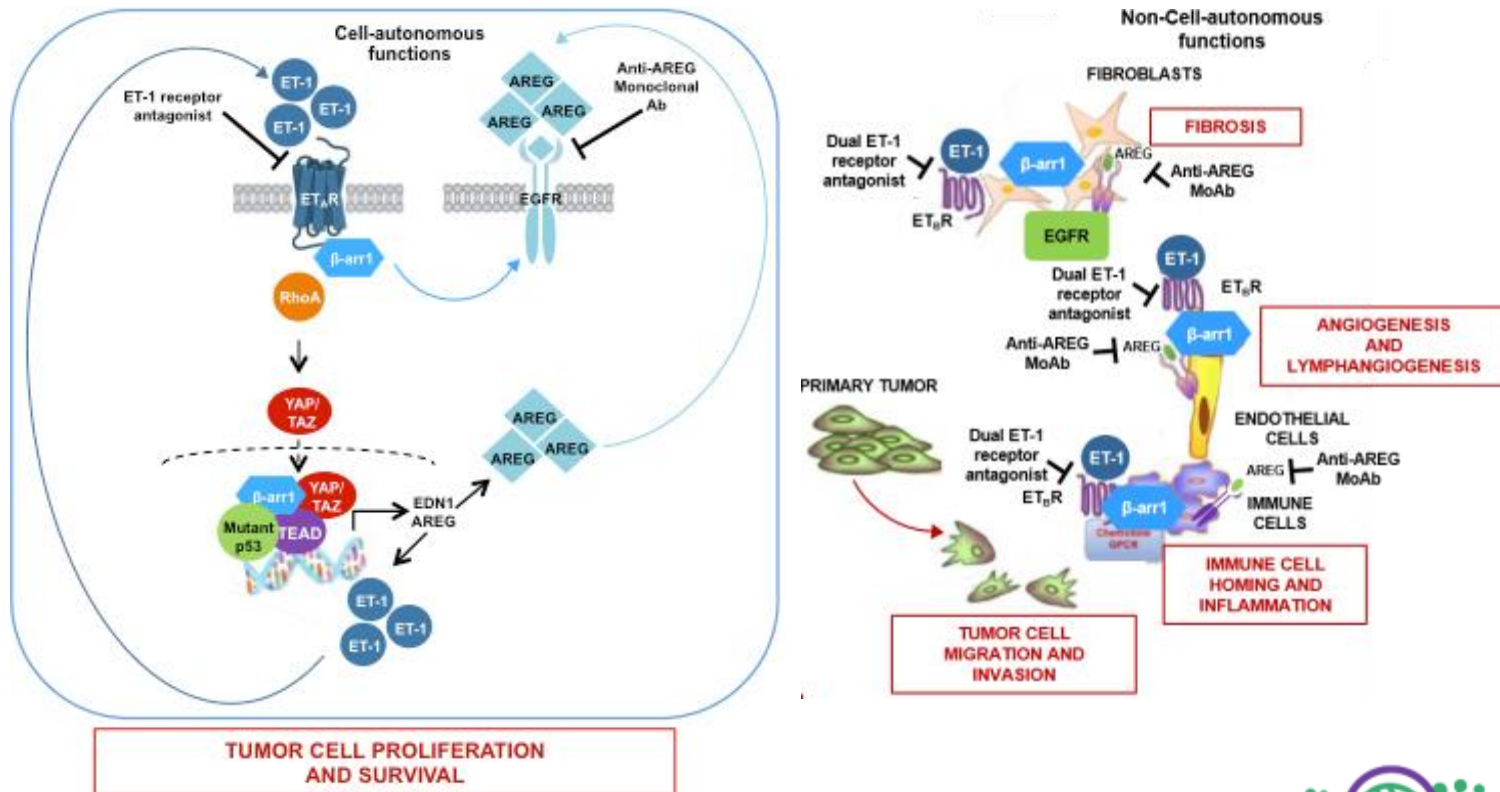
# Specific AIMS

In this proposal, we will investigate tumor-stroma interactions and their therapeutic potential by focusing on two soluble mediators, **ET-1** and **AREG**.

**Specific aim 1:** To dissect the roles played by YAP and p53 in the in ET-1- and AREG- driven signaling pathways in HG-SOC models.

**Specific aim 2:** To resolve involvement of TME cells, such as CAFs, endothelial cells and Tregs, in tumor progression and drug response regulated by the ET-1/ET-1R and AREG/EGFR axes.

**Specific aim 3:** To test several drug combinations in animal models, including chemotherapy, blockers of AREG and ET-1R, along with checkpoint and PARP inhibitors.



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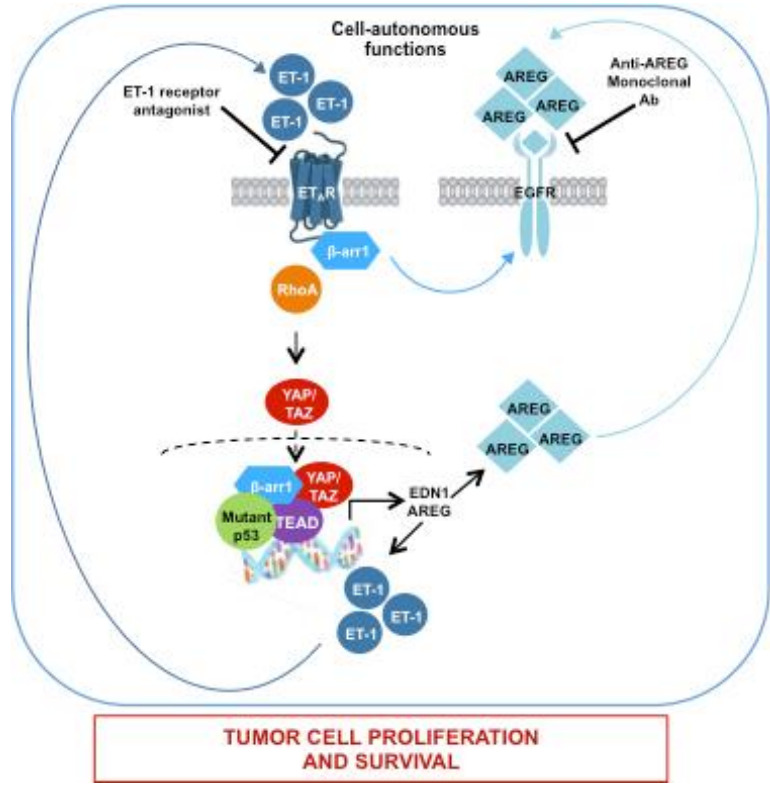
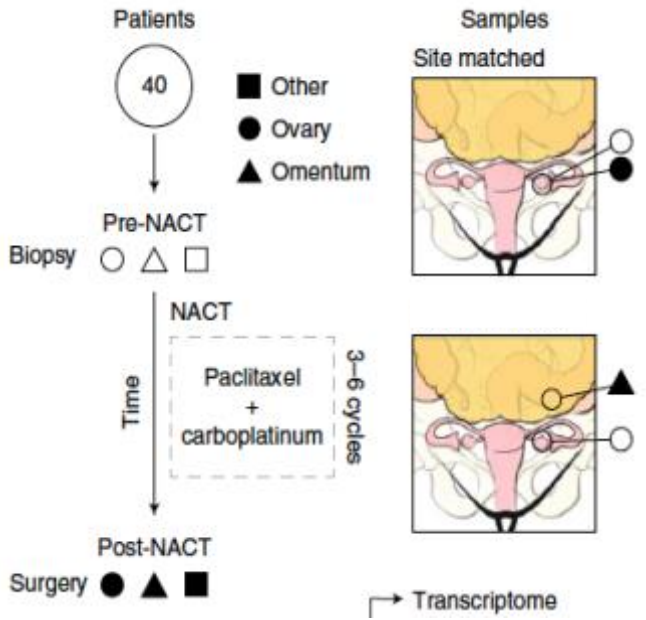


# RESEARCH PLAN:

**Specific AIM1:** To dissect the roles played by YAP and p53 in the in **ET-1** and **AREG**-driven signaling pathways in HG-SOC models

## Accurate sampling

The difficulty in multisampling individual patients as their tumor evolves to perform differential expression analysis between pre- and post-chemotherapy samples with distinct TME



Upon ET-1R activation, YAP was engaged in a complex with  $\beta$ -arr1 in a greater extent in platinum-resistant cells.

**Is this mechanism relevant to the level of AREG expression ?**

To investigate the effect of chemotherapy on the tumor/stroma ecosystem, we will analyze single-cell or bulk gene expression profiles that will allow us to identify the adaptive responses of HG-SOC to different microenvironmental niches.

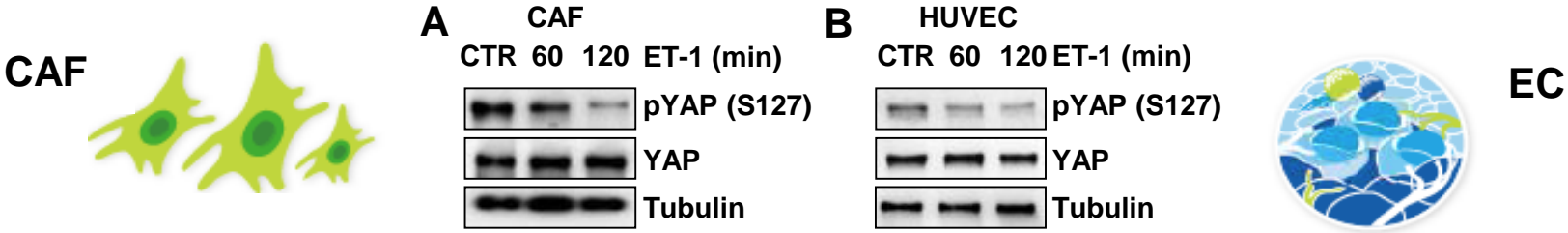
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**Specific AIM 2:** To resolve involvement of TME cells, such as CAF, endothelial cells and Tregs, in tumor progression and drug response regulated by the **ET-1/ET-1R** and **AREG/EGFR** axes

**Cancer-associated fibroblasts (CAF) and Endothelial cells (EC):** To analyze the roles of CAF and EC in tumor progression and drug response, we will test whether **ET-1** or **AREG** can regulate CAF and EC functions via YAP signaling.

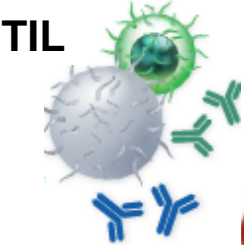
### Preliminary results AIM 2



### Tumor-infiltrating cells

To examine the roles for AREG in the recruitment of **immune cells**, we will make use of AREG-knockout mice and a set of syngeneic models of ovarian cancer.

- o ID8 mouse ovarian cancer cells, including derivatives lacking p53 and BRCA1/2.
- o A DMBA-induced rodent ovarian model.
- o A MISIIR-Tag murine model of epithelial ovarian cancer,
- o GEMM models established from genetically lacking BRCA, p53, and Pten genes.



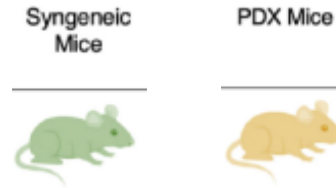


# Specific aim 3: To test several drug combinations in animal models, including blockers of AREG and ET-1R, checkpoint inhibitors and chemotherapy

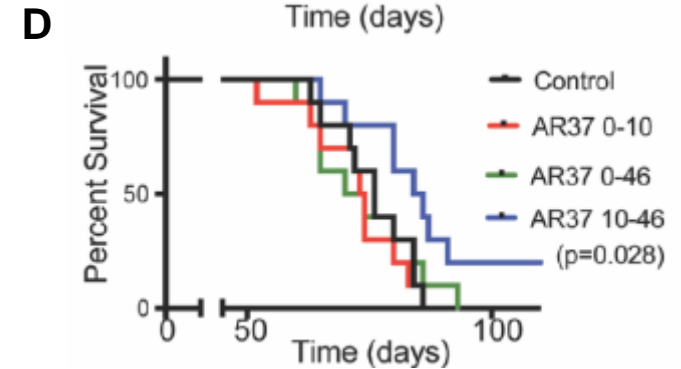
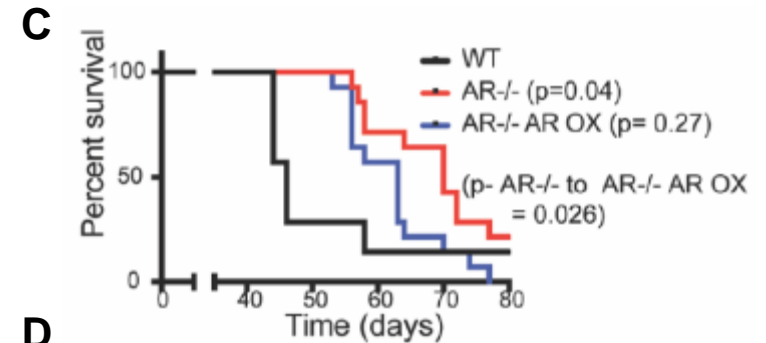
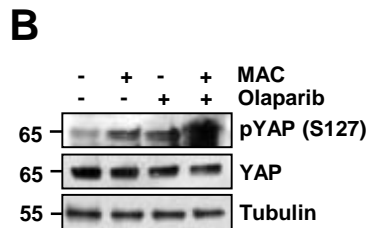
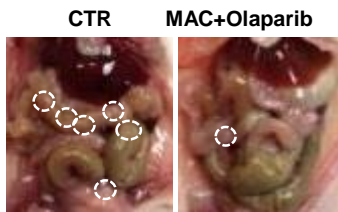
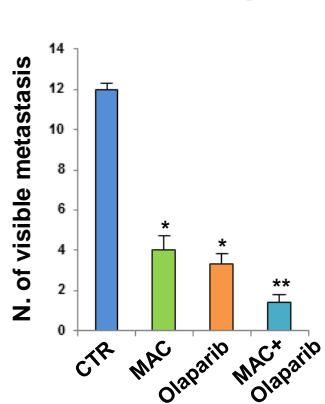
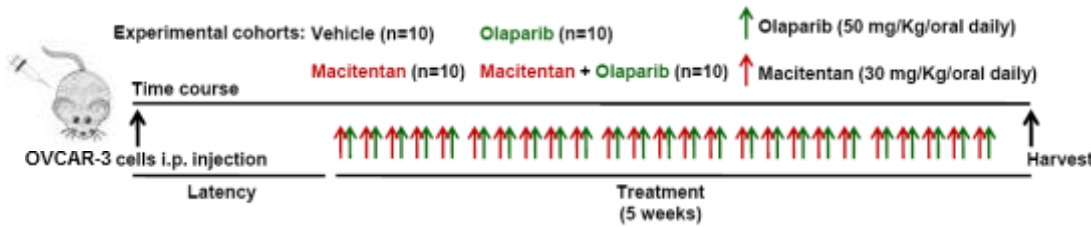
This Aim is designed to assess the feasibility of pharmacological targeting of ET-1R and AREG, either alone or in combination with

- (i) cis-platinum and paclitaxel;
- (ii) PARP inhibitors;
- (iii) anti-PD-1/PD-L1 therapy.

Drug effects will be assessed in terms of tumor size, metastatic nodules, and changes in the TME at early and late time-points following therapy by using GeoMx Digital Spatial Profiler (DSP) technology.



## Preliminary results AIM 3

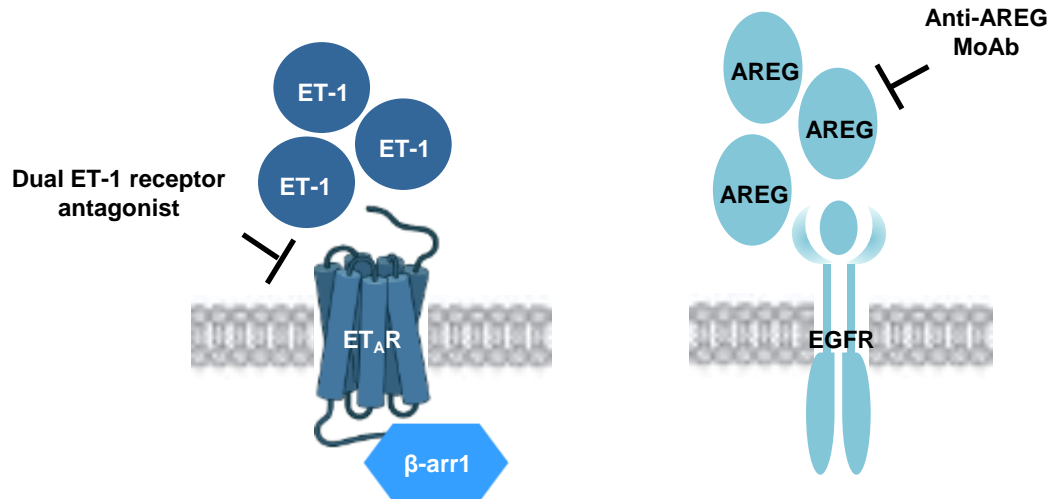


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## Expected outcomes

The results of this project will generate novel concepts to reprogram the TME of aggressive HG-SOC cells, activated by ET-1/ET-1R and AREG/EGFR through YAP/mutp53, using ET-1R antagonist or anti-AREG MoAb, in combinatorial regimens. These experiments would provide pre-clinical evidence suggesting that ET-1R/AREG blockade therapy may be effective for patients with mutp53 and ET-1R/EGFR positive HG-SOC in combination with standard of care chemotherapy or other molecular drugs to support early phase clinical trials for the treatment of HG-SOC.



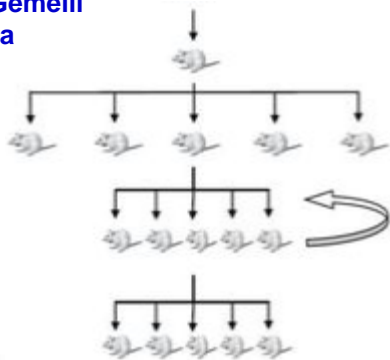
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# Thanks to:

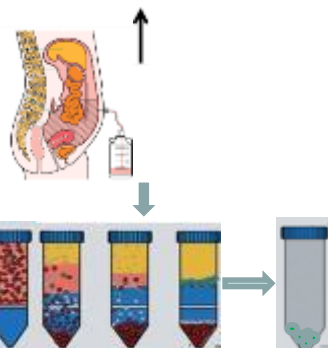


**HIGH-GRADE  
SEROUS OVARIAN  
CANCER (HG-SOC)**  
Gynecological Oncology IRE  
Prof. E. Vizza  
Gynecological Oncology  
Università Cattolica A. Gemelli  
Prof. G. Ferrandina



Development of HG-SOC patient-derived xenografts (PDX)

**HG-SOC Patient**  
Oncology IRE  
Dr. A. Savarese

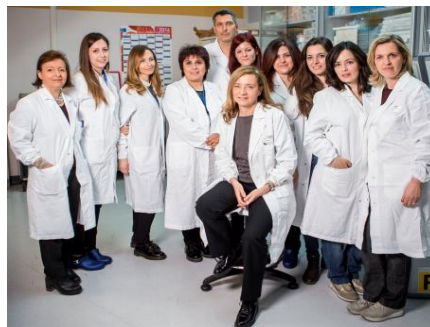


Development of patient-derived models: primary cultures, co-cultures, 3D cultures

Isolation of tumor cells



**IDENTIFICATION OF SPECIFIC TARGETS**  
Oncogenomic and Epigenetic Unit, IRE  
Dr. S. Donzelli  
Dr. A. Sacconi



**Anna Bagnato's Lab.**  
Preclinical Models and  
New Therapeutic Agents Unit

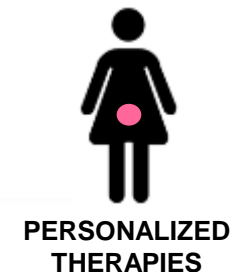


**Yosef Yarden's Lab.**  
Department of Biological Regulation  
Faculty of Biology

New therapeutic approaches tested in patient-derived models (PDX)



Development of new combined therapies  
Actelion Pharmaceuticals, Ltd  
Idorsia Pharmaceuticals, Ltd  
(Allschwil, Switzerland)  
Dr. M. Clozel



**PERSONALIZED  
THERAPIES**

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