TESIS DOCTORAL

Therapeutic implications of Notch signaling in ovarian cancer

Presentada por **IVAN DIAZ PADILLA** para optar al grado de "Doctor con Mención Internacional" por la Universidad de Sevilla

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Organización de la Tesis Doctoral

De acuerdo la normativa reguladora del régimen de tesis doctoral (Acuerdo 9.1/CG 19-4-12) de la Universidad de Sevilla pueden presentarse para su evaluación como tesis doctoral un conjunto de trabajos publicados por el doctorando. La presente Tesis Doctoral se presenta bajo la modalidad de Compendio de Publicaciones.

Las publicaciones incluidas en este compendio son:

1) **A phase Ib combination study of RO4929097, a gamma-secretase inhibitor, and temsirolimus in patients with advanced solid tumors.**

   Authors: Ivan Diaz-Padilla, Hal Hirte, Amit M. Oza, Blaise A. Clarke, Brenda Cohen, Michael Reedjik, Tong Zhang, Suzanne Kamel-Reid, S. Percy Ivy, Sebastien J. Hotte, Albiruni A. R. Razak, Eric X. Chen, Irene Brana, Monika Wizemann, Lisa Wang, Lillian L. Siu, and Philippe L. Bedard

   Reference: Invest New Drugs. 2013 Oct; 31(5):1182-91

2) **A phase II study of single-agent RO4929097, a gamma-secretase inhibitor of Notch signaling, in patients with recurrent platinum-resistant epithelial ovarian cancer: a study of the Princess Margaret Cancer Centre, Chicago and California phase II consortia.**

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Mención Internacional en el título de Doctor

De acuerdo con la normativa reguladora del régimen de tesis doctoral (Acuerdo 9.1/CG 19-4-12) de la Universidad de Sevilla puede solicitarse la mención internacional en el título de Doctor siempre que concurran las circunstancias expuestas en el artículo 15. El doctorando IVAN DIAZ PADILLA solicita la Mención Internacional del título de Doctor dado que las siguientes circunstancias concurren en la presentación de esta tesis:

a) Durante el periodo de investigación el doctorando IVAN DIAZ PADILLA realizo una estancia de 2 años en el centro de investigación Princess Margaret Hospital, en Toronto (Canadá). Se acredita certificado oficial de tal instancia en el apéndice de esta tesis
b) La presente tesis doctoral ha sido redactada íntegramente en inglés.
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Resumen/Abstract

The cancer stem cell (CSC) theory states that only some cells from a heterogeneous tumor are capable of tumorigenesis. These cells have been collectively termed 'CSCs' due to their stem cell-like properties of self-renewal, differentiation and tumorigenesis. It is therefore expected that therapies that target the key signalling pathways involved in CSC biology would ultimately lead to improved outcomes for the treatment of cancer patients. Notch is an evolutionary conserved signalling pathway involved in CSCs and in the development and progression of malignant tumors, like ovarian cancer. Gamma-secretase inhibitors (GSIs) are small molecules able to inhibit the Notch signalling pathway and exert antitumor effects in preclinical models. RO4929097 is one the first GSIs that entered clinical development in solid tumors based on promising early clinical efficacy data. As a result, its clinical evaluation has been conducted both in combination with chemotherapy and other targeted agents (e.g. temsirolimus) and as a single-agent in selected malignancies (e.g. ovarian cancer). The results presented herein have demonstrated that RO4929097 has a tolerable safety profile both in combination with temsirolimus in patients with solid tumors and as a single agent in women with advanced ovarian cancer. However, based on its unfavourable pharmacokinetic profile and its limited antitumor efficacy further clinical development will not be pursued. A better understanding of the biology of CSCs and their role in tumor progression, coupled with increased knowledge about the Notch signalling pathway will likely facilitate the development of other Notch-targeted agents aimed at having a clinical meaningful impact in outcomes of cancer patients.

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1. INTRODUCCION

En esta sección se indican los antecedentes que motivaron el estudio de este tema, las hipótesis que surgieron a partir de su investigación y los principales objetivos de este trabajo.

1.1. Background

Patients with advanced solid malignancies often have limited therapeutic options beyond institutional standard of care. For patients whose cancer has become refractory to all available treatments, there is a significant unmet therapeutic need. The recent development of novel molecularly targeted treatments has changed the management and the prognosis of many cancers. This improvement can be attributed to a better understanding of intracellular signaling pathways which have been abnormally activated or inhibited during tumorigenesis.

1.1.1. Cancer biology

Malignant tumors are composed of a heterogeneous group of cells, demonstrated by the fact that some tumor cell fractions can support new growth in xenograft models, whereas other cell fractions do not. Traditionally, two models have been proposed to explain tumor cell heterogeneity: the stochastic model and the hierarchy model.[1] The stochastic model states that tumors arise as a biologically homogeneous group of cells, with functional heterogeneity arising through random (that is, stochastic) events. In this model, tumor initiation may occur in any cell as a result of an accumulation of DNA mutations, epigenetic regulation, and a permissive micro environment. The stochastic model suggests that all tumor cells have the potential to become cancer stem cells (CSCs), given the appropriate conditions. Alternatively, the CSC (or hierarchy) model suggests that tumors are composed of a heterogeneous group of cells that have arisen from stem-like precursors. As these tumor cells differentiate, they form a mixture of cells with different biological and phenotypic characteristics, forming a cellular hierarchy. At the apex of this hierarchy are CSCs, which serve as the source of newly formed

tumor cells. A third model, which blends characteristics of the stochastic and CSC models, may provide a mechanism for the formation of both primary and metastatic tumors.4 Chromosomal instability within the CSC population, together with extrinsic environmental factors, may lead to the appearance of CSC heterogeneity. Self-renewing CSCs, comprising a small minority of tumor cells, initiated tumor growth and formed new progenitor and bulk tumor cells in severe combined immunodeficient mice.[2] CSCs use a variety of signaling pathways to undergo self-renewal and differentiation, including Wnt, Notch, and Hedgehog. The slow growth rate and chemo-resistant characteristics suggest that CSCs may survive routine chemotherapy, only to reinitiate tumor growth at a later point in time.

1.1.2. Cancer stem cells

During development and tissue remodeling, pluripotent stem cells serve as the source of differentiating cells, giving rise to non-proliferating specialized cell types. The fate of these cells appears to depend on primordial regulatory pathways that are active during development. Deregulation of these pathways is linked to the rapid and uncontrolled proliferation of tumors.

The concept that a rare population of cells with embryonic stem cell–like properties is the source of tumors or of post-therapeutic tumor recurrence was proposed a century ago. The finding that only certain cells are capable of initiating, maintaining, and promoting the development of tumors strongly supports the CSC hypothesis. The modern revival paradigm of the CSC hypothesis was suggested by Spangrude and colleagues who isolated multipotent hematopoietic progenitor cells and demonstrated that not all human cancer cells xenografted into immunocompromised animals had the same ability to initiate tumors.[3] At present, CSC properties, as postulated by the American Association for Cancer Research, include tumorigenic or self-renewal capacity, the potential for multi-lineage differentiation, the ability to participate in serial passage, and expression of a unique repertoire of surface markers that allows for their reliable identification and purification. The origin of the CSC has long been

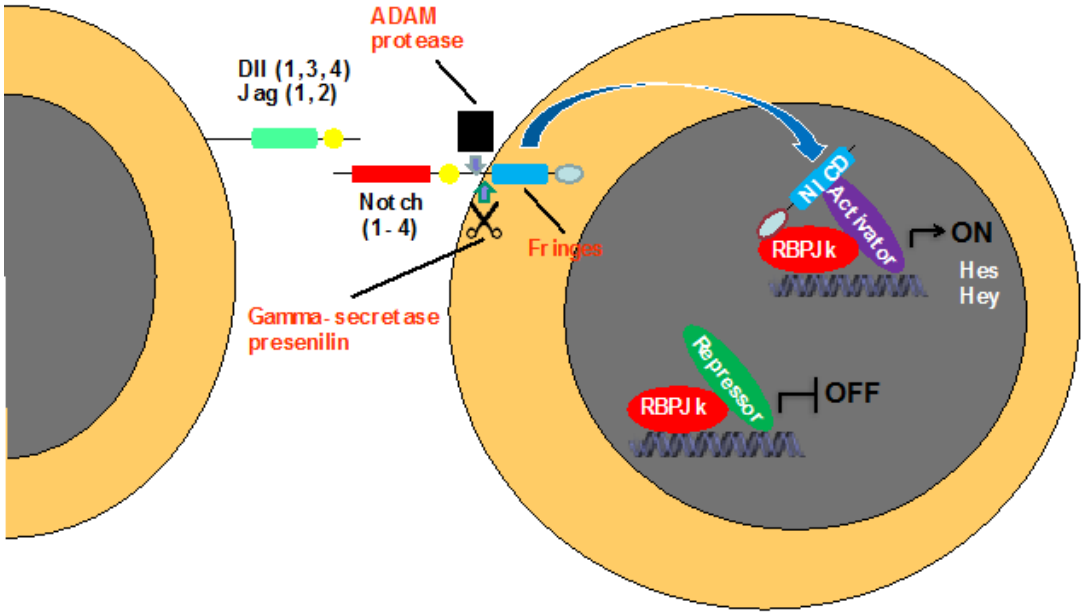
debated, particularly whether CSCs are somatic stem cells that have undergone malignant change or whether they are more differentiated cells that return to “stem” status as part of malignant transformation. Normal stem cells are the likely targets of mutagenesis, leading to the formation of CSCs, because they already possess active self-renewal pathways, whereas induction of self-renewal genes is required to transform differentiated cells. Furthermore, normal stem cells are the only cells with a life span long enough to accumulate the genetic mutations that lead to tumorigenesis. However, experimental cancer models have clearly determined that CSCs are not necessarily derived from normal stem cells. Growing evidence suggests that there are two potential pathways: loss of growth regulation in a normal stem cell and a mature somatic cell (non–stem cell) acquiring the properties of self-renewal.

1.1.3. Notch signaling pathway and cancer

The Notch signaling pathway is important in regulating cell differentiation, proliferation, apoptosis and cell-cell communication and several studies highlight the association between Notch signaling and tumorigenesis. [4-6]

Notch signaling is primarily oncogenic but has been shown to have the potential to also act as a tumor suppressor.[7, 8] In mammals, two structurally distinct families of Notch ligands (delta-like (DLL) 1, 3, 4 and Jagged 1, 2), have been described. These interact with four Notch transmembrane receptors (Notch 1-4) causing a conformational change which allows proteolytic cleavage by metalloprotease and gamma-secretase.[4] This releases the Notch intracellular domain (NICD) that subsequently translocates to the nucleus, where it binds a transcriptional repressor known as C promoter-binding factor (CBF-1), or CSL (CBF-1/Suppresor of Hairless/Lag1), thus activating the Notch target genes, Myc, p21, and Hes (hairy/enhancer of split) family members (**Figure 1**).

Figure 1. Notch signaling pathway



1.1.3.1. RO4929079

The formation of the NICD is an important step in the Notch pathway. Gamma-secretase inhibitors (GSIs) prevent the final cleavage step consequently decreasing the level of NICD. To date, over 100 GSIs have been synthesized with more than 20 clinical trials investigating their role.[9] These novel therapeutic agents are being investigated as monotherapy or in combination with targeted or chemotherapy in a variety of solid tumors.[9] RO4929097 is selective oral small molecule inhibitor of gamma-secretase that produces a less transformed, slower-growing phenotype in a variety of cancer cell lines. [9, 10] In *in vivo* applications, RO4929097 shows antitumor activity in three out of four sensitive animal models tested. RO4929097 is active when dosed orally using an intermittent or continuous daily dosing schedule. Efficacy is maintained for up to 90 days post-dosing with histological analysis showing a phenotype indicative of Notch signaling inhibition. Preclinical toxicology studies

have indicated that the target organs in the rat and dog include the gastrointestinal tract, lymphoid system, peripheral blood leukocytes, and ovaries. In addition, RO4929097 administration caused changes in hair and skin pigmentation in the dog. Based on the preclinical studies it is anticipated that in the clinic RO4929097 treatment could result in gastrointestinal toxicities, granulocytosis, lymphopenia, and hepatotoxicity.

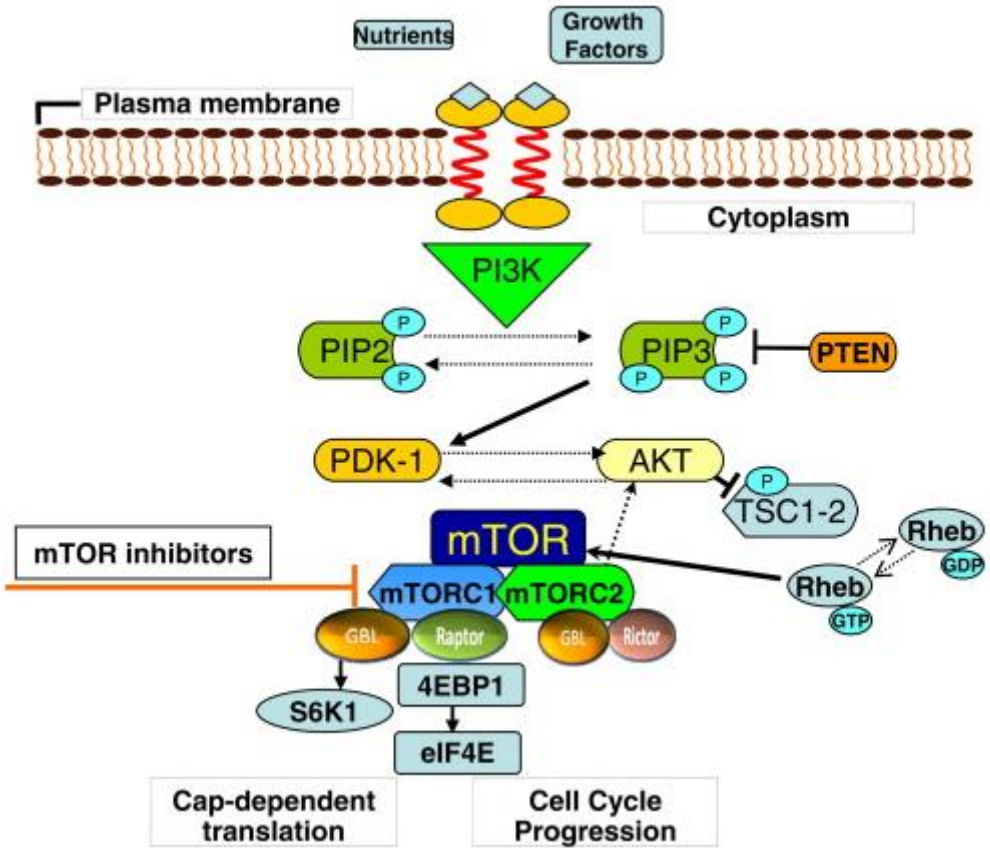
In a phase I study, RO4929097 was well tolerated, with the most common reported toxicities being nausea, vomiting, diarrhea, fatigue, hypophosphatemia and skin rash.[11] Preliminary evidence of activity was observed, with prolonged (≥ 3 months) stable disease (SD) seen in three of nine patients with recurrent ovarian cancer.[11] In this phase I study a dose-dependent decrease in drug exposure at doses above 24 mg occurred, consistent with auto-induction of CYP3A4.[11] Furthermore, a weak-to-moderate pharmacokinetic-pharmacodynamic association was demonstrated between several putative markers of Notch inhibition including Aβ-40 protein and vascular endothelial growth factor receptor 2 protein in plasma, and Hes1 mRNA expression in hair follicles, but these did not appear predictive of response.[11, 12]

1.1.4. mTOR/PI3K signaling pathway

mTOR is a member of the PI3K-related protein kinase family. Growth factor receptor stimulation leads to activation of PI3K, which phosphorylates phosphatidylinositol-4,5-bis-phosphate (PIP2) to generate phosphatidylinositol-3,4,5-triphosphate (PIP3). The accumulation of PIP3 activates a signaling cascade starting with the phosphorylation (activation) of the protein serine–threonine kinase AKT by PDK1 (**Figure 2**). The phosphatase and tensin homolog PTEN protein can dephosphorylate PIP3, reversing the action of PI3K, thereby modulating phosphorylated AKT (pAKT). AKT phosphorylates and inhibits the tuberous sclerosis complex (TSC), removing its inhibitory effect on Ras-related small GTPase Rheb (Ras-homolog-enriched-in-brain), which acts as a positive upstream regulator of mTOR. Activation of mTOR in complex

with other proteins, in particular with the mTOR complex 1 (mTORC1), associated with the regulatory associated protein of mTOR (raptor), leads to phosphorylation of eukaryotic translation initiation factor 4E binding protein (4E-BP1) and ribosomal protein S6 kinase 1 (S6K1). In cancer cells, 4E-BP1 phosphorylation results in the initiation of translation. The activation of mTORC1 downstream targets leads to protein synthesis, such as cell cycle regulating proteins, vascular endothelial growth factor (VEGF), or c-Myc. Rapalogs bind intracellularly to the immunophilin FKBP-12 (FK 506 binding protein) creating a complex that inhibits the protein kinase activity of mTORC1.25 mTOR complex 2 (mTORC2) couples with the rapamycin-insensitive companion of mTOR (rictor), and is functionally distinct from mTORC1. The mechanisms regulating mTORC2 complex are still not fully elucidated. Its activation promotes cell survival and actin cytoskeleton organization.26 Rapamycin and rapalogs, like temsirolimus, are primarily inhibitors of mTORC1, preventing phosphorylation of 4E-BP1, S6K1, and other proteins involved in cell cycle control, leading to growth arrest in the G1 phase of the cell cycle, and reduced angiogenesis.

Figure 2. mTOR/AKT/PI3K pathway



1.1.4.1. Temsirolimus

Temsirolimus (CCI-779, sirolimus 42-ester with 2,2-bis(hydroxymethyl) propionic-acid), an ester of the macrocyclic immunosuppressive agent sirolimus (rapamycin, Rapamune™), is a cytostatic cell cycle inhibitor with antitumor properties. The agent specifically inhibits the mammalian target of rapamycin (mTOR), a Ser/Thr kinase involved in the initiation of mRNA translation. Temsirolimus has been developed as a cytostatic agent to delay the time to tumor recurrence or progression or to increase survival in patients with various malignancies. Key features of this agent include its good tolerability, unique mechanism of action, ability to arrest cells in the G_1 phase, and ability to induce apoptosis. The observed antitumor and immunosuppressive properties of rapamycin analogs, like temsirolimus, are due to their ability to disrupt the mTOR-dependent signaling pathway.

Temsirolimus safety, pharmacokinetics, and preliminary antitumor effects were evaluated in a phase 1 dose-escalation study with doses of 7.5-220 mg/m2 given as a weekly intravenous infusion to 24 patients with advanced malignancies.[13] Data from in vitro studies of A498 human renal cell lines indicated that temsirolimus had a median growth inhibitory concentration (IC50) of 5 ng/mL. Predicted modeling of IC50 (humans receiving doses as low as 10 mg) suggests that whole blood concentrations would be above the range of 1 ng/mL throughout the entire 1-week dose interval and above 5 ng/mL for the majority of this time period. It is expected that mTOR inhibition would be attained with a 25 mg dose. Clinical pharmacokinetic data are available in patients with cancer receiving temsirolimus both intravenously daily for 5 days every 2 weeks, once weekly schedules, and orally daily for 5 days every 2 weeks. These data indicate that there is no appreciable drug accumulation between cycles and that distribution is extensive. With increasing dose, exposure (AUC) increases in a less than proportional fashion. The mean volume of distribution at steady state is large (57 L after 2 mg IV dose; 900 L following a 250 mg IV dose) and increases with dose. Exposure to the hydrolytic product sirolimus is substantial with mean values of approximately 1.5-2.3-fold greater than those seen with temsirolimus following IV administration. Clearance (CL) of temsirolimus from whole blood increases with increasing dose from approximately 5.2L/h after a 2 mg dose to 100L/h after a 250 mg dose. Inter-subject variability in CL at a given dose was modest and ranged from 16-27%. The terminal half-life (t1/2) following temsirolimus doses of 25 to 250 mg is approximately 15 hours.

1.1.5. Cross-talk between the Notch and the mTOR/PI3K pathways

Crosstalk between signaling pathways has the potential to profoundly add to the complexity of cellular responses to external stimuli. A link between murine mTOR and Notch signaling pathways shows that cells with constitutively activated mTOR also express elevated levels of NICD and Hes1, suggesting that Notch is upregulated by mTOR.[14] Treatment with the mTOR inhibitor rapamycin led to decreased signaling of both mTOR and notch. Up-regulation of

Notch by mTOR acts through the stat3/p63/Jagged pathway. Conversely, down-regulation of Notch signaling inhibited mTOR, AKT, and nuclear factor kappa B signaling.

The Notch pathway regulates activation of both phosphatase and tensine homologue (PTEN) and PI3K/AKT signaling components in normal cells; however, the aberrant activation of Notch signaling pathway induces the direct stimulation of PI3K/mTOR pathway leading to tumor cell growth [8]. *In vitro* studies in pancreatic cancer cell lines have shown a synergistic antitumor effect mediated through enhanced AKT suppression when rapamycin and a γ-secretase inhibitor were administrated [16]. A recent *in vitro* study in T-cell acute lymphoblastic leukemia model has shown that PI3K/mTOR inhibition leads to up-regulation of Notch-myc pathway. However, dual blockade of both PI3K/mTOR and Notch produces enhanced cell-cycle arrest and cell death, providing a rationale for evaluating mTOR inhibitors in combination with Notch inhibitors.

1.1.5.1. Rationale for combining RO4929079 and Temsirolimus

It is a rational approach to combine RO4929097 with temsirolimus as many studies suggest cross talks between Notch and mTOR signaling pathways. Notch pathway seems to regulate activation of both PTEN and PI3K/AKT signaling components in normal cells [15]. However the aberrant activation of Notch signaling pathway induces the direct stimulation of PI3K/mTOR pathway and thus led to tumor cell growth [15-19]. This cross activation between both pathways was confirmed *in vivo* in rats whose brains were exposed to ischemia injury [16]. On the other hand, although the tumor cell multiplication induced by over-activation of Notch signaling pathway is reversed by GSIs, T-cell lymphoblastic leukemia cell lines with constitutive Notch1 activity can be resistant to these drugs [15]. Palomero et al. demonstrated that these cells all harbored mutations of PTEN and that activation of PI3K/AKT components led to resistance to Notch 1 inhibition. They showed resistance to γ-secretase inhibitors could be reversed by inhibitors of PI3K/AKT signaling pathway in drosophila [15]. As well, in an in vitro

study, cell chemoresistance linked to Notch-1 activation was reversed by rapamycin [17]. Of note, another in vitro study suggested that downstream Notch signaling pathway could be activated by other signaling pathways such as epidermal growth factor (EGFR) which also stimulates PI3K pathway [20]. Thus it is postulated that simultaneous inhibition of both PI3K/AKT/mTOR and Notch pathways could have synergistic antitumor activities and abrogate GSI resistance.

1.1.6. Ovarian Cancer

Epithelial ovarian cancer (EOC) is the leading cause of death from gynecologic cancer in the United States and Europe. Worldwide, ovarian cancer is the sixth most common form of cancer in women.[21] In general, the highest incidence rates are found in European and North American population groups with the lowest rates in Asian population groups. Approximately 75% to 85% of patients with EOC are diagnosed at the time when their disease has spread throughout the peritoneal cavity. There are still no validated methods for screening or early diagnosis in healthy or high-risk populations.

Ovarian cancer consists of many subtypes. The surface epithelial tumors are the most frequently encountered form of ovarian tumors, and they account for more than 90% of all ovarian cancer in adults. The vast majority of fatal ovarian cancers are high-grade serous carcinomas. These tumors are believed to arise *de novo* from the surface epithelium of the ovary, or perhaps sometimes from the mucosa of the fallopian tube, and progress rapidly. Ovarian cancer is recognized as a heterogeneous disease, and in the last few years a dualistic model for the pathogenesis of this disease has emerged which divides epithelial tumors into type 1 and type 2 ovarian carcinomas. Type 1 cancers tend to be low-grade and indolent tumors and include low-grade serous, endometrioid, mucinous, clear-cell and malignant Brenner tumors. These tumors are characterized by mutations of KRAS, BRAF, ERBB2, PTEN, PIK3CA and ARID1A and are relatively genetically stable. These mutations occur early in the

evolution of type 1 ovarian tumors and are also observed in borderline tumors and endometriosis. A stepwise sequence of tumor development is now well recognized from benign precursor lesions (e.g. borderline tumor) to malignant lesions in type 1 cancers. Conversely, there is no clear precursor lesion for type 2 cancers. These are high-grade, aggressive tumors comprising high-grade serous, high-grade endometrioid, malignant mixed mesodermal tumors and undifferentiated tumors. Type 2 tumors are very frequently associated with TP53 mutations, and one landmark study found that 97% of high-grade serous cancers were associated with a TP53 mutation.

1.1.6.1. Diagnosis and Staging

Patients with ovarian cancer confined to the ovary may have few or no symptoms, making clinical diagnosis of early ovarian cancer more difficult. Symptoms are most commonly seen with advanced disease. Recognized symptoms of all stages include abdominal or pelvic pain, constipation, diarrhea, urinary frequency, vaginal bleeding, abdominal distension and fatigue. In advanced ovarian cancer, ascites and abdominal masses lead to increased abdominal girth, bloating, nausea, anorexia, dyspepsia and early satiety. Extension of disease across the diaphragm to the pleural cavities can produce pleural effusions and the development of respiratory symptoms. Patients may become aware of an abdominal or nodal mass either in the inguinal region, axillae or the supraclavicular fossa. [22]

Following a full clinical assessment, measurement of serum CA-125 is routinely used to aid diagnosis. However, its utility to detect early disease is questionable as it is elevated only in about 50% of patients with the International Federation of Gynecology and Obstetrics (FIGO) stage I disease. In advanced disease, CA-125 is elevated in about 85% of patients. It is not specific for ovarian cancer and raised CA-125 levels may be found in non-gynecological malignancies (e.g. breast, lung, colon and pancreatic cancer) and benign disease (e.g. endometriosis, pelvic inflammatory disease and ovarian cysts). Serum carcinoembryonic

antigen (CEA) and CA 19–9 levels are sometimes measured in situations where it is unclear whether an ovarian mass is of gastrointestinal origin, or a primary mucinous ovarian tumor. Similarly, in these situations, colonoscopy and/or gastroscopy are sometimes considered, particularly when CA-125/CEA ratio is $\leq$25. Ultrasonography of the abdomen and pelvis is usually the first imaging investigation recommended for women in whom ovarian cancer is suspected. Trans-vaginal ultrasonography has improved the visualization of ovarian structures, thus improving the differentiation of malignant versus benign conditions. Computed tomography (CT) scans are routinely used to determine the extent of disease and to aid in surgical planning. Imaging of the chest with CT or chest X-ray should be done to look for pleural effusions and disease above the diaphragm. FIGO staging remains the most powerful indicator of prognosis (**Figure 3**). Although surgically defined, preoperative assessment with cross-sectional imaging (CT or MRI) is essential as it guides surgery and the pathway of intervention. Given the variation in histological subtypes and evolving different patterns of care, reliance on a cytological diagnosis should be avoided and a histological diagnosis should be obtained if at all possible. Primary surgery remains the most common and preferred approach, but where this is deemed not feasible, an image-guided or laparoscopic biopsy should be carried out.

Figure 3. FIGO staging system for ovarian cancer

Stage I	Growth limited to the ovaries
IA	Growth limited to one ovary; no ascites present containing malignant cells. No tumour on the external surface; capsule intact
IB	Growth limited to both ovaries; no ascites present containing malignant cells. No tumour on the external surfaces; capsules intact
IC[a]	Tumour either stage IA or IB, but with tumour on surface of one or both ovaries, or with capsule ruptured, or with ascites present containing malignant cells, or with positive peritoneal washings
Stage II	Growth involving one or both ovaries with pelvic extension
IIA	Extension and/or metastases to the uterus and/or tubes
IIB	Extension to other pelvic tissues
IIC[a]	Tumour either stage IIA or IIB, but with tumour on surface of one or both ovaries, or with capsule(s) ruptured, or with ascites present containing malignant cells, or with positive peritoneal washings
Stage III	Tumour involving one or both ovaries with histologically confirmed peritoneal implants outside the pelvis and/or positive regional lymph nodes. Superficial liver metastases equal stage III. Tumour is limited to the true pelvis, but with histologically proven malignant extension to small bowel or omentum
IIIA	Tumour grossly limited to the true pelvis, with negative nodes, but with histologically confirmed microscopic seeding of abdominal peritoneal surfaces, or histologically proven extension to small bowel or mesentery
IIIB	Tumour of one or both ovaries with histologically confirmed implants, peritoneal metastasis of abdominal peritoneal surfaces, none exceeding 2 cm in diameter; nodes are negative
IIIC	Peritoneal metastasis beyond the pelvis >2 cm in diameter and/or positive regional lymph nodes
Stage IV	Growth involving one or both ovaries with distant metastases. If pleural effusion is present, there must be positive cytology to allot a case to stage IV. Parenchymal liver metastasis equals stage IV

1.1.6.2. Treatment

Early-stage disease (Stage I)

The aim of surgery for early ovarian cancer is to resect the tumor and to undertake adequate staging. This will provide prognostic information and will define whether chemotherapy is needed. The diagnosis may be made preoperatively, but sometimes a tumor is an incidental finding. Accurate surgical staging is important as it may unmask occult advanced disease. Depending on the histological grade and subtype, up to 30% of the patients with apparently early epithelial ovarian cancer will be upstaged after comprehensive surgical staging. When young women are affected, fertility-sparing surgery could be considered in early-stage disease, but always after thoroughly informing the patient about the potential risks. Patients with stage

IA or stage IC with unilateral ovarian involvement and favorable histology, (i.e. mucinous, serous, endometrioid or mixed histology) and grade 1 or 2, would be amenable to organ-preserving surgery, but only in combination with complete surgical staging. This would include a lymphadenectomy to exclude more advanced disease. A recent Cochrane meta-analyses of five large prospective clinical trials (4 of 10 with platinum-based chemotherapy) showed that chemotherapy is more beneficial than observation in patients with early-stage ovarian cancer

Advanced-stage disease (Stage II-IV)

In advanced epithelial ovarian cancer, the aim is complete cytoreduction of all macroscopic visible disease, since this has been shown to be associated with a significantly increased overall survival (OS) and progression-free survival (PFS). In order to achieve this, a maximal surgical effort is required, including intestinal resection, peritoneal stripping, diaphragmatic resection, removal of bulky para-aortic lymph nodes and splenectomy. There is an increasing body of evidence that suggests specialist training and surgical expertise results in improvements in the rate of cytoreduction, with no increase in morbidity as a result of this process.[23] Thus, women with advanced disease are advised to undergo surgery in specialized centres with adequate infrastructure and training. Optimal cytoreduction is defined as total macroscopic tumor clearance with no residual visible disease. A recent meta-analysis evaluating the surgical outcome of more than 3120 patients showed that residual tumor is a more powerful prognostic determinant than FIGO stage; patients with suboptimally debulked stage IIB–IIIB tumors had a worse outcome that those with completely debulked stage IIIC tumors.[24]

The risks of recurrence for disease spread beyond the ovary are significant, and chemotherapy is recommended for all patients with FIGO stage II–IV disease post-surgery. Standard chemotherapy consists of a combination of paclitaxel 175 mg/m^2 and carboplatin AUC 6-5, both administered intravenously every 3 weeks. Angiogenesis is an important component driving the growth of ovarian cancer. Two large randomized clinical trials (GOG-218 and ICON-

7) have assessed the addition of bevacizumab to the combination of paclitaxel and carboplatin in front-line therapy. Bevacizumab is a monoclonal antibody targeting vascular endothelial growth factor. In both trials patients in the experimental arm received bevacizumab intravenously every 3 weeks during the chemotherapy phase, followed by a limited period of maintenance with the same schedule of bevacizumab. GOG-218 included a second experimental arm of bevacizumab with chemotherapy, followed by maintenance with a placebo. There were significant differences in both trials in terms of dose (7.5 mg/kg in the ICON-7 versus 15 mg/kg in the GOG-218), duration (12 months in the ICON-7 versus 15 months in the GOG-218) and patient characteristics (GOG-218 included only patients with stage III–IV and macroscopic residual disease after surgery, but ICON-7 included patients also with high-risk early stage, and patients in a more advanced stage but without macroscopic residual disease after surgery). Both trials met their primary end point, which was PFS for the two bevacizumab maintenance arms. Therefore, the addition of bevacizumab is recommended for patients with advanced ovarian cancer with poor prognostic features such as stage IV or suboptimal debulking as defined in the ICON-7 trial. Bevacizumab should be given with paclitaxel or carboplatin with treatment duration of one year.

1.1.6.3. Recurrent ovarian cancer

Despite optimal upfront surgery and the administration of front-line paclitaxel–carboplatin chemotherapy, approximately 70% of patients will relapse in the first 3 years. The prognosis and probability of response to second-line therapy and subsequent lines depends in great part on the progression-free interval after the last dose of the preceding line of chemotherapy. These categories are based on the response to a re-challenge with platinum-based drugs but probably apply to non-platinum therapies as well. A recent categorization defines ‘platinum-refractory’ as patients progressing during therapy or within 4 weeks after the last dose; ‘platinum-resistant’ patients progressing within 6 months of platinum-based therapy; ‘partially platinum-sensitive, patients progressing between 6 and 12 months; and ‘platinum-sensitive’

patients progressing with an interval of more than 12 months. It should be noted that these categories are based on observational studies and that the categorization is probabilistic, with the likelihood of response being a continuous variable. Furthermore, the category of 'platinum-resistant/ refractory' comprises patients whose disease recurs after one or several lines of treatment. The biological behavior of the tumor in these groups may be very variable, with differing growth rates and distribution of symptoms requiring different approaches to treatment. Treatment of patients with 'platinum-resistant or refractory' disease should be focused on quality of life and control of symptoms. Traditionally, this is a poor prognosis population with a short expected OS, usually <12 months. Four different agents, weekly or 3-weekly paclitaxel, topotecan, liposomal doxorubicin and gemcitabine, have been shown to have some activity in phase III trials, with overall response rates no >15% and a median PFS of 3–4 months. Occasionally, platinum drugs continue to be used in the 'platinum-resistant' population with, for example, a dose-dense regimen. However, as no agent has proven to be superior to another, the selection of therapy should be based on toxicity, clinical situation of the patient and convenience of administration. Randomized trials of combination chemotherapy have shown no advantage in this population; it compounds toxicity. Given this situation, there is a clear unmet medical need for better treatments that potentially improve clinical outcomes, and molecularly-targeted therapies could represent one of those treatment options.

1.1.6.4. Notch pathway in ovarian cancer

Many advocate that ovarian cancer, particularly, high-grade serous carcinoma, adheres to the cancer stem cell model with tumor initiation, treatment failure and relapse reflecting intrinsically resistant CSCs and our relative inability to effectively target them. Many CSCs stay in G0 phase of the cell cycle and as such are not susceptible to cell cycle specific chemotherapeutic agents. Increasing evidence suggests that elucidating the genetic mutations and pathways that specifically target CSCs is critical. [25] Deregulation of key signaling

pathways (e.g. Notch, WNT, Hedgehog and PTEN) involved in the functionality of such CSCs has been theorized to be associated with tumor recurrence and the development of platinum-resistance in ovarian cancer.[26-28] Notch is a conserved pathway that has been implicated in the maintenance of tissue homeostasis by regulation of self-renewal and cell-fate determination in normal stem cells and early progenitors. In EOC alterations in this pathway have been frequently described and have been associated with poor outcomes.[29, 30] The Notch-3 receptor appears of particular interest with amplification and up-regulation associated with worse outcome.[4, 29, 30] Targeting Notch-3 has been associated with growth inhibition and induction of apoptosis.[29]Furthermore, a comprehensive genomic analysis of a prospectively annotated sample set of high-grade serous ovarian cancers identified that Notch signaling was altered in approximately 20% of cases,[31] thus making Notch a rational therapeutic target. Preclinical studies have shown that inhibition of Notch signaling blocks the growth of both ovarian cancer cell lines *in vitro* and cell line-derived xenografts *in vivo*.

1.1.6.5. Rationale for evaluating RO4929097 in ovarian cancer

RO4929097 is a potent and selective inhibitor of GSI, involved in the Notch pathway, with preclinical evidence of notch signaling inhibition in tumor cells and early signs of clinical activity. There is an unmet medical need in platinum-resistant ovarian cancer, and new and more effective treatments are needed.

Based on published evidence, Notch is allegedly a rational therapeutic target in ovarian cancer, in light of the clonal nature of this disease and the preclinical evidence supporting the over-expression of both the Notch receptor and ligand in ovarian cancer specimens. Both *in vitro* tumor growth inhibition and Notch target gene transcription are inhibited by exposure to a gamma secretase inhibitor. [32] It was therefore proposed to conduct a proof-of-concept single-arm phase II study of RO4929097 in recurrent platinum-resistant ovarian cancer. A complementary correlative science component was embedded into the study design.

1.2. Hypothesis

Based on the above data, the two main hypotheses of this doctoral thesis are as follows:

- The combination of a GSI like RO4929097 with an mTOR inhibitor (i.e. temsirolimus) is feasible and exerts antitumor activity in patients with advanced solid tumors.
- Single agent RO4929097 is an effective treatment option in patients with advanced, platinum-resistant ovarian cancer

1.3. Objectives and Endpoints

- **Primary Objectives**:
    - To determine the recommended phase II dose (RP2D) and safety profile of temsirolimus in combination with RO4929097 (**Study #1**)
        - Primary Endpoint: Incidence of Dose Limiting Toxicities (DLTs) during the first cycle of treatment
        - Primary variable: Frequency and severity of adverse events (AEs)
    - To investigate the anticancer activity of RO4929097, as a single agent, in patients with advanced, platinum-resistant EOC, fallopian tube carcinoma, or primary peritoneal cancer (**Study #2**)
        - Primary Endpoint: 4-cycle PFS rate. Four-cycle PFS (symptomatic, RECIST v1.0, or CA-125 progression) rate is defined as the proportion of the study population that has not had tumor progression or died at the completion of the four cycles.
- **Secondary Objectives**:
    - To assess the antitumor activity of RO4929097 in combination with temsirolimus in patients with advanced solid tumors, or as a single agent in patients with advanced platinum-resistant ovarian cancer (**Study #1**)
        - Secondary Endpoint: objective response rate (RECIST v1.1.)
    - To evaluate the pharmacokinetics (PK) and pharmacodynamic effects of the combination (**Study #1**):
        - Secondary PK Endpoints:
            - Compartment-dependent PK profiles of RO4929097 and temsirolimus

- Estimation of fixed effect parameters (clearance, volumes of distribution)
- Estimation of random effect parameters (inter and intra-individual variability)
- Impact of individual covariates on unexplained inter-individual variability of fixed effect parameters.

Exploratory Objective:

- To determine the expression of Notch biomarkers in tumor specimens and evaluate the presence biomarkers with clinical outcome to RO4929097 and/or Temsirolimus (**Study #1 and Study #2**)

Assessment of tumor response

For the purposes of these study, patients were be re-evaluated for response every 6 (**study #1**) or 8 weeks (**study #2**). In addition to a baseline scan, confirmatory scans were obtained 4-6 weeks following initial documentation of objective response. Response and progression was evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

2. METODOLOGIA

En esta segunda sección se detallan los materiales y métodos empleados en los artículos de este compendio de Tesis Doctoral. Se aclaran las características de las muestras, la definición de las variables empleadas, las evaluaciones de seguridad y de eficacia, los métodos estadísticos utilizados, y los procedimientos de laboratorio llevados a cabo para los estudios de biología molecular.

2.1. Patients

2.1.1. Clinical Study #1

The study was conducted at two institutions, the Princess Margaret Cancer Centre (Toronto, Ontario, Canada) and the Juravinski Cancer Centre (Hamilton, Ontario, Canada). The trial was approved by the Ontario Cancer Research Ethics Board. The study was registered at clinicaltrials.gov (NCT 01198184), sponsored by the Princess Margaret Hospital Phase I Consortium, and supported by the National Cancer Institute (NCI) Contract No. U01-CA132123.

Patients were required to have a histologically confirmed advanced, incurable solid malignancy that was refractory to conventional therapy or for which no standard therapy existed, age ≥18 years, life expectancy ≥12 weeks, an Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) ≤1, with adequate hematological, hepatic, and renal function. Prior therapy with a γ-secretase inhibitor, or any inhibitor of the PI3K/AKT/mTOR pathway was prohibited. Key exclusion criteria included: a) concomitant use of medications that were strong inducers/inhibitors or substrates of CYP3A4; b) patients with malabsorption syndrome or other condition that would interfere with intestinal absorption; c) uncontrolled hypocalcemia, hypomagnesemia, hyponatremia, hypophosphatemia or hypokalemia despite adequate electrolyte supplementation; d) a QTc interval ≥470 mSec as measured by Bazett's formula; e) pre-existing significant pulmonary infiltrates of unknown origin.

2.1.2. Clinical Study #2

This study was conducted by the Princess Margaret Hospital, Chicago and California Phase II consortia. Patients provided written consent consistent with local institutional requirements. The protocol was approved by the institutional review board at each of the participating institutions.

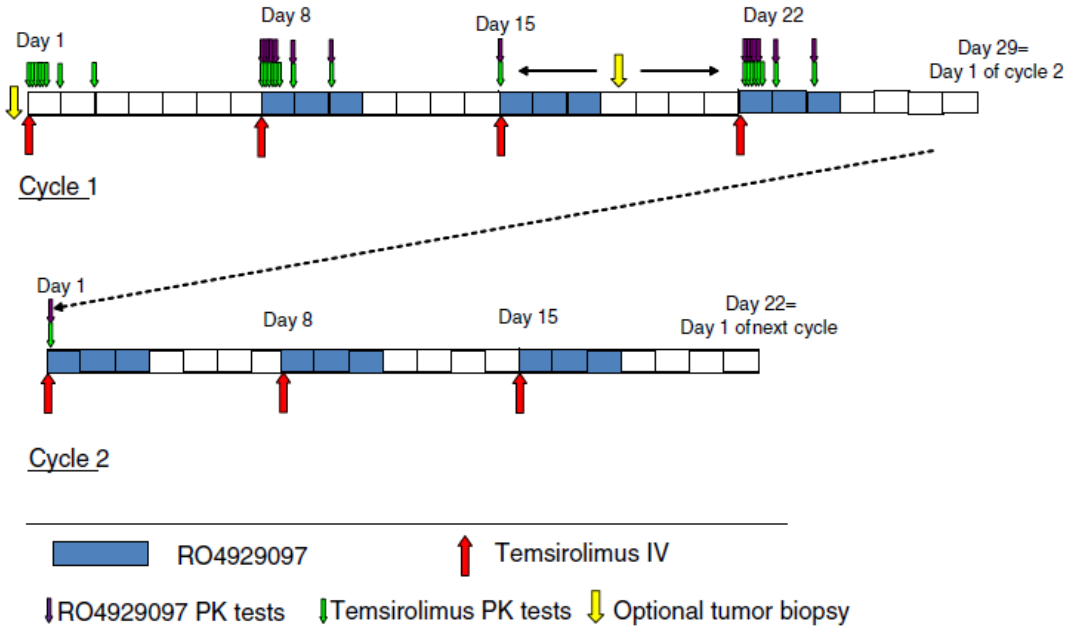
Women (age ≥18) with histologically or cytologically confirmed recurrent or metastatic, platinum-resistant EOC, fallopian tube carcinoma, or primary peritoneal carcinoma were eligible for this trial. Central pathology review was undertaken by a pathologist blinded to outcome results. Cases were classified according to the two-tier grading system.[33] Platinum-resistant disease was defined as a treatment-free interval of less than six months since the completion of the last platinum-based chemotherapy. A maximum of two prior chemotherapy lines for recurrent disease was allowed. Prior hormonal treatments and/or biological agents were allowed. Patients had to have measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1,[34] or evidence of progression based on an elevated CA-125 (defined as a value of >2 times the upper limit of normal documented on two separate determinations made >2 weeks apart, the most recent being completed within 7 days prior to study treatment).[35] Other key eligibility criteria were an Eastern Cooperative Oncology Group Performance Status ≤2, and adequate hematologic, hepatic, and renal function. Key exclusion criteria included uncontrolled electrolyte abnormalities, and QTc on baseline electrocardiogram (ECG) > 470 msec.

2.2. Study Design

2.2.1. Clinical Study #1

This was an open-label, dose-escalation phase Ib trial. RO4929097 was orally administered on an empty stomach on a three days-on four days-off schedule, weekly; and temsirolimus was given over 30 min intravenously (i.v.) every week (**Figure 3**).

Figure 3. Study Design. Phase Ib clinical trial of RO4029097 and Temsirolimus



A standard 3+3 design was used. Dose cohorts initially included 3 patients. The first dose level was level 1. If 1 patient out of 3 experienced a dose limiting toxicity (DLT), 3 additional patients were recruited at that dose level. If only 1 out of 6 patients in that dose level experienced a DLT, escalation to the next level was mandatory. If 2 out of 3 or 2 out of 6 patients experienced DLT, no further dose escalation occurred. The RP2D (equivalent to Maximum Tolerated Dose (MTD)) is defined as the dose level at which ≤ 1/6 patients experienced DLT. No intra-patient escalation was allowed. Patients who achieved complete or partial response or who had stable disease may continue on study treatment until progression.

Due to the observed auto-induction of RO4929097 metabolism at doses above 20 mg in the monotherapy trial, dose escalation of R04929097 above 20 mg was not planned. DL3 included 37.5 mg temsirolimus in order to achieve blood levels equivalent to the standard 25 mg weekly dose in the event of a potential drug-drug interaction with RO49092907 mediated by CYP3A4.

Definition of Dose-Limiting Toxicity

Dose-limiting toxicity (DLT) is defined based on adverse events observed in cycle 1 (28 days) that are possibly, probably or definitively related to study drugs. Patients who do not complete cycle 1 due to reasons besides DLT, for example early withdrawal of consent, intercurrent illness unrelated to study drugs, symptomatic disease progression, etc. will be replaced as they will not be considered evaluable for DLT.. All patients will receive any amount of study drug(s) will remain evaluable for toxicity on study.

DLT was defined as follows:

Hematologic DLTs

- Absolute granulocyte count (AGC) < 0.5 x 10^9/L for 7 or more consecutive days
- Febrile neutropenia (ANC < 1.0 x 10^9/L, fever > 38.5^{o}C)
- Platelets < 25 x 10^9/L or thrombocytopenic bleeding (i.e. platelets < 50 x 10^9/L and associated with clinically significant bleeding)

Non-hematologic DLTs

- Diarrhea ≥ Grade 3 despite adequate management.
- Other ≥ Grade 3 toxicity thought to be treatment related, despite adequate medical intervention as judged by the investigator, including any ≥ grade 3 electrolyte

abnormality including hypophosphatemia, hypocalcemia, or hypomagnesemia that has not resolved in 72 hours with appropriate therapy or is associated with new ECG changes. Excluding toxicities that do not pose a safety risk (e.g., alopecia).

- Treatment-related toxicities that result in failure to receive at least 75% of the planned doses of RO4929097 (i.e. at least 7 of 9 doses) and of temsirolimus (i.e. at least 3 of 4 doses) despite maximal (as judged by the investigator) supportive care measures.

- Inability to resume dosing for cycle 2 at the current dose level within 14 days (i.e. by cycle 1 day 42) due to treatment-related toxicity.

2.2.2. Clinical Study #2

This was an open-label, single-arm, multicenter phase II clinical trial. A Simon's two-stage design with a target sample size of 37 patients was utilized. A clinically interesting 4-cycle PFS rate (p1) was defined as 40%, with the minimal 4-cycle PFS rate set at 20% (p0). The significance level of this design was $\alpha=0.1$ with a power of $1-\beta=0.9$. A first-stage interim analysis was planned after enrolment of 17 evaluable patients. If ≤3 instances of 4-cycle PFS were observed, the study was terminated (this resulted in a 55% probability of ending the study during stage I), whereas if ≥4 instances of 4-cycle PFS were observed, 20 more patients were to be enrolled in the second stage. If 11 or more instances of 4-cycle PFS were observed among the 37 evaluable patients, further investigation of RO4929097 in EOC was warranted. A 4-cycle PFS was chosen in preference to the standard 6 month PFS given the platinum-resistant patient population where the medium PFS is often less than this.

2.3. Pharmacokinetic analysis

Pharmacokinetic (PK) analysis Blood samples for RO4929097 and temsirolimus were collected serially for PK analysis during cycle 1 and 2. The unbound RO4929097 fractions were obtained by filtrating plasma samples using AmicoCentrifree® Micropartition devices (Millipore Corp., Bedford, MA, USA). Plasma temsirolimus, total and unbound RO4929097 concentrations were determined using validated HPLC-tandem mass spectrometry methods. Pharmacokinetic parameters were calculated by nonparametric methods using WinNonlin (Version 5.3, Pharsight Corp., Sunnyvale, CA).

2.4. Biomarker analysis

Archival paraffin-embedded tumors specimens were requested on all subjects for immunohistochemical analysis of components of the Notch pathway: Jagged-1, (NICD) and Notch-3. Immunohistochemistry was performed using standard techniques. Briefly, 4 µm paraffin-embedded tumor sections were preheated to 57° for 15 min, cooled to room temperature, dewaxed in xylene and rehydrated in graded alcohols. Endogenous peroxidase activity was blocked in 0.3 % hydrogen peroxide in PBS for 30 min. Heat induced epitope retrieval was accomplished using 10 mM citrate buffer (pH 6) in Biocare Digital DecloakingChamber (using factory recommended settings for IHC). Anti-NICD (Cell Signaling #2421; 1/50) and anti-Notch-3 (Santa Cruz Biotechnologies #sc-5593; 1/150) staining was performed using VECTASTAIN ABC Kit (Rabbit IgG) (Vector Labs #PK-4001) with the following modifications: permeablization with 0.3 % Triton X100 in PBS for 2×10 min before block; block for 1 h room temperature in PBS 3 % BSA, 20 mM MgCl2, 5 % FBS, 0.3 % Tween20, plus Vector blocking sera); incubation with primary antibody (diluted in blocking buffer plus vector blocking sera) overnight at 4°; secondary antibody diluted 1/100 in PBS 5 % BSA plus Vector blocking sera; all washes 2×5 min PBS, followed by 2×5 min PBS 0.3 % Triton X100. Anti Jagged 1 (R&D Systems #AF1277; 1/50) staining was performed using Cell and Tissue Staining kit (Goat

IgG) (R&D Systems #CTS008) with a 4° overnight incubation with primary antibody. All immunohistochemistry was performed using Shan don Sequenza immunostaining coverplates (Fisher #7219950) and Peroxidase Substrate Kit, DAB (Vector Labs #SK- 4100). Slides were counterstained with hematoxylin, dehydrated in graded alcohols to xylene, and mounted using Permount. The stained slides were scored centrally (the pathologist, was blinded to the clinical outcome) for the presence of Jagged-1, NICD, and Notch-3. The positive antibody reaction was scored into four grades, according to the intensity of the staining: 0, 1+, 2+ and 3+. The percentage of positive cells was also scored into four categories: 0 (0 %), 1 (1–33 %), 2 (34–66 %), and 3 (67–100 %). The product of the intensity and the percentage scores was used as the final.

Mutational analysis: In patients with available archival paraffin-embedded tumor specimens and sufficient DNA, genotyping was performed with either Sequenom MassARRAY (Sequenom) PMH v1.0 customized panel for solid tumors that includes 280 mutations in 23 genes or the MiSeq (Illumina) TruSeq Amplicon Cancer Panel (TSACP) panel that includes 212 amplicons in 48 genes in the CLIA-certified University Health Network Advanced Molecular Diagnostics Laboratory.

Circulating angiogenic factors: Soluble markers of angiogenesis were measured before treatment and post-treatment (on cycle 2, day 1): SDF-1alpha, basic fibroblast growth factor (bFGF), interleukin-6 (IL-6), IL-8 and vascular endothelial growth factor (VEGF) A, C, and D. Venous blood samples were collected, centrifuged at 3000 r.p.m. for 10 min and stored at -70ºC until analysis. Analytes were measured by enzyme-linked immuno-absorbent (ELISA) assay (ELISA kit from Kamiya Biomedical Company, Thousand Oaks, CA). A standard concentration curve was produced for each ELISA plate with the manufacturer's control solution and used to calculate plasma concentrations. Serial samples were assessed on the same ELISA plate to reduce inter-experimental variability. Baseline levels and changes at cycle 2 were correlated with PFS.

2.5. Statistical methods

Standard descriptive statistics were used to summarize the patient sample. Kaplan-Meier plots were used to estimate all time-to-events functions. Statistical analysis was performed using SAS 9.2 software for both studies.

PFS was defined as time from start of treatment until disease progression or death as a result of any cause. The association between clinical characteristics, pathologic factors and immunohistochemical markers was evaluated using the chi-square test or Fisher's exact test. Univariate cox proportional hazard analysis was performed to assess the association between protein expression and PFS. All tests were two-sided, with $p<0.05$ considered significant.

Wilcoxon test was performed for comparison of Notch biomarker protein expression between patient subgroups (clinical study #1). Logarithmic transformation to a normal distribution was performed to analyze baseline levels of SDF-1alpha, bFGF, IL-6, IL-8 and VEGF. The paired t-test was used to assess changes in circulating angiogenic factors (CAFs) from baseline to cycle 2. Univariate analysis using Cox-ph model was carried out to identify the association between baseline expression of the biomarkers of interest (SDF-1alpha, bFGF IL-6, IL-8 and VEGF A, C and D), changes in expression of these markers (%) and PFS (clinical study #2).

A fixed-effect ANOVA test was used to compare temsirolimus PK parameters at three different time points during cycle 1 (day 1, day 8 and day 15).

3. RESULTADOS

3.1. Results from Study # 1

A phase Ib combination study of RO4929097, a gamma-secretase inhibitor, and temsirolimus in patients with advanced solid tumors.

Patient demographics: Between August 2010 and March 2012, 17 patients were enrolled (7 males; 10 females). Median age was 62 (range 28–84); most patients were ECOG PS 1 and had been treated with a median of 3 lines of chemotherapy (**Table 1**). Most common tumor types were sarcoma (n=6, 35 %), neuroendocrine (n=2, 12 %) and squamous cell carcinoma of the head and neck (n=2, 12 %).

Dose escalation and RP2D: Eight, three, and six patients were enrolled in dose levels (DL) 1, 2, and 3, respectively. Treatment is summarized in **Table 2**. Two patients in DL1 (RO4929097 10 mg, Temsirolimus 25 mg) were non-evaluable for DLT. One patient voluntarily withdrew consent at day 22. Another non-compliant patient in the first cohort did not receive two of the four planned doses of temsirolimus. The two missed doses could not be attributed to toxicity. Thus, this patient was considered not evaluable for DLT. One patient in DL1 experienced two DLT events (grade 3 oral mucositis and grade 3 maculo-papular rash), prompting treatment of three additional patients at this dose level. No additional DLT events were observed in the expanded DL1 allowing for further dose escalation to DL2 (n=3). In DL2 and DL3, 0/3 patients initially treated experienced DLT. An additional 3 patients were treated at DL3 to better evaluate safety of this dose level. No DLTs were observed (0/6 patients treated in DL3), leading to a declaration of 20 mg of oral RO4929097 in a 3-days-on/4-days-off schedule along with 37.5 mg of temsirolimus as the RP2D.

Safety and compliance: All 17 treated patients were evaluable for toxicity. The most frequently reported treatment-related AEs are listed in **Table 3**. Fatigue (82 %) and oral mucositis (71 %) were the most frequently reported non-hematological treatment-related AEs. These were grade 1 and 2 in most cases and were easily managed with standard supportive measures. Only one episode of grade 3 mucositis was observed. Maculo-papular rash was observed in 7 patients (41 %), with only one grade 3 episode. Three patients (2 patients with grade 2; 1 patient with grade 3) required treatment interruption due to rash. Rash was reversible in all cases and no dose modification was needed when treatment was resumed. Gastrointestinal toxicities, including anorexia (47 %), nausea (41 %), vomiting (29 %), and diarrhea (18 %) were also frequently observed. Consistent with the single agent safety profile of RO4929097 and temsirolimus, hypophosphatemia was frequently observed (47 %). However, most episodes were grade 2, and were managed with oral and/or intravenous supplementation. Prolongation of the QTc interval has been reported with RO4929097. In this study, three patients (17 %) experienced grade 1 asymptomatic transient QT interval prolongation. This was not associated with electrolyte abnormalities. No clinically significant rhythm abnormalities were noted. Metabolic disturbances and pneumonitis have been frequently described with temsirolimus. In this study, hypertriglyceridemia was the most commonly reported (n=10, 59 %) metabolic AE. Most cases were grade 1 and 2 in severity, and no intervention was required. A few instances of hyperglycemia (n=4, 23 %) and hypercholesterolemia (n=2, 12 %) were noted. Hyperglycemia was of grade 1 or 2 severity in all cases, and was managed with diet and/or oral antidiabetic medication. No dose modifications or study treatment interruption was required for any metabolic abnormalities. No episodes of pneumonitis were observed in this study. Neutropenia (59 %) and anemia (59 %) were the most common hematological toxicities. There were two grade 3 episodes of neutropenia that were not associated with infection. No patient discontinued study treatment due to hematological toxicity.

Antitumor activity: No objective responses were observed. Eleven patients (73 %) had stable disease as their best response and six patients (40 %) completed at least six cycles of treatment (4 months) without experiencing disease progression. The median progression-free survival was 4.2 months (95 % confidence interval [CI], 1.5–8.7 months). Two patients, diagnosed with a metastatic high-grade synovial sarcoma and a metastatic gastrointestinal stromal tumor respectively, who had disease progression to their last regimen, remain on study treatment at cycle 13 and 17 at the time of data cutoff (Nov 5, 2012).

Pharmacokinetics: Plasma exposure of RO4929097 was measured at day 8 and day 22 (**Figure 4**). A dose-proportional increase in RO4929097 exposure was not observed. Temsirolimus clearance was significantly increased from cycle 1 day 1 to cycle 1 day 15 across all doses levels ($p<0.01$), but not between cycle 1 days 1 and 8 (**Figure 5a**). Correspondingly, there was a significant reduction in temsirolimus area under the curve (AUC) from cycle 1 day 1 to cycle 1 day 15 ($p<0.01$),but not between cycle 1 days 1 and 8 (**Figure 5b**). No difference in peak temsirolimus plasma concentrations (Cmax) was observed among different dosing days in cycle 1. These findings are consistent with a cumulative effect of repetitive RO4929097 dosing on CYP3A4 induction leading to increased temsirolimus clearance and reduced AUC.

Figure 4. Plasma exposure of RO4929097 (area under the curve [AUC]) at day 8 and day 22, at the different dose levels

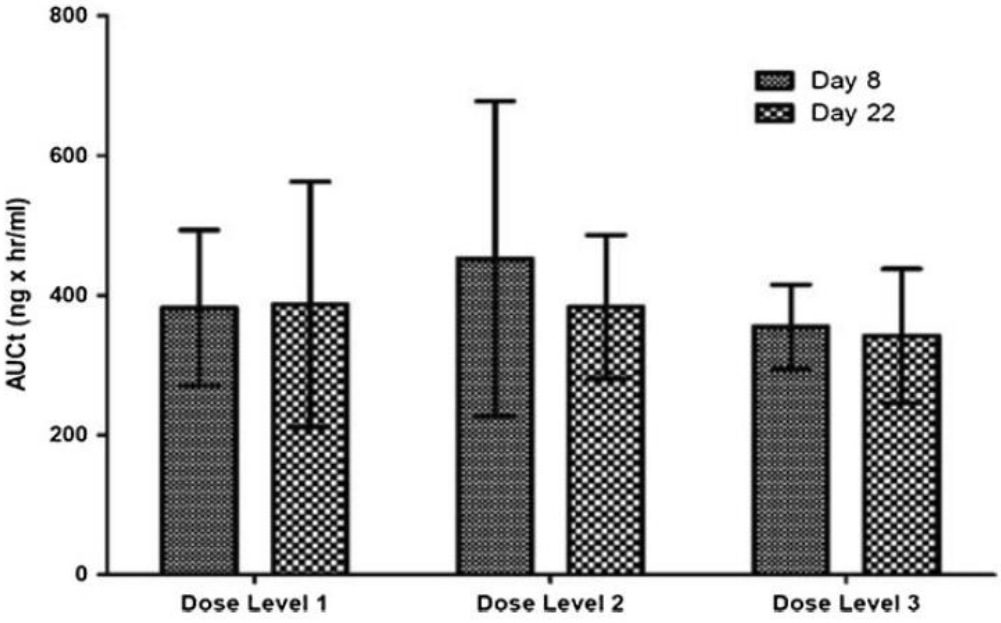
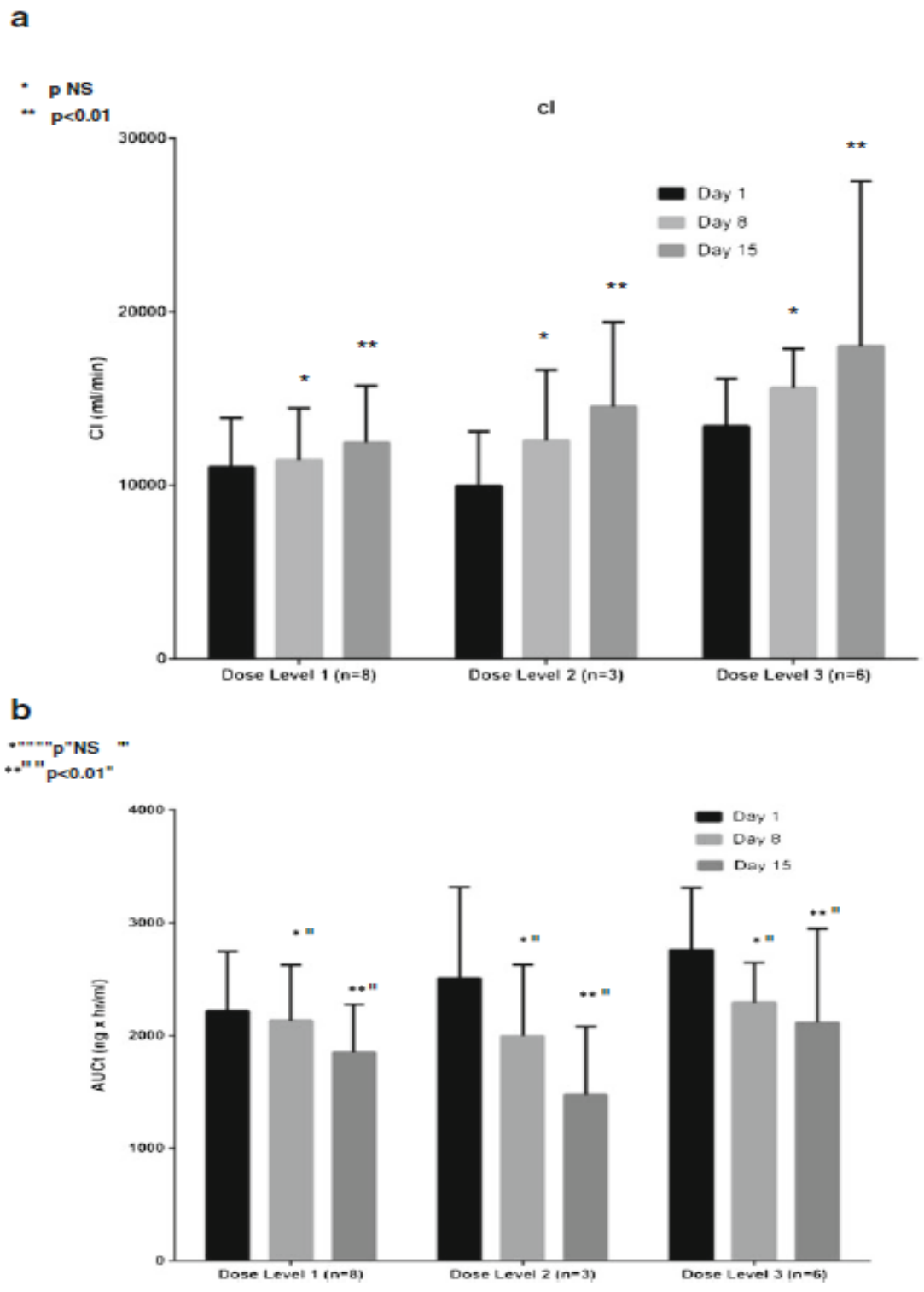


Figure 5. a. Temsirolimus clearance (Cl) observed at cycle day 1, day 8 and day 15 for the different dose levels. b. Temsirolimus area under the curve (AUC) observed at cycle 1, day1, day 8 and day 15 for the different dose levels.



Notch pathway biomarkers: Archival pathology specimens were available from 14 patients. Immunohistochemical (IHC) expression of Notch receptor ligand Jagged-1, Notch-3 receptor, and Notch intracellular domain (NICD) was performed on paraffin-embedded tumor tissue. The median Jagged-1 IHC intensity score was 6 (range 3–7). The median Notch-3 score was 2 (range 0–7), and the median NCID score was 5 (range 0–8). No significant differences in the median protein expression score were seen between patients who remained progression-free after four cycles of treatment and those who progressed prior to cycle 5. No significant association was detected between protein expression of any of the Notch biomarkers evaluated and time to progression. Of 14 tumors with archived pathology specimens and sufficient DNA available for further analysis, mutations were identified in 5 patients. No correlation between mutational status (mutation(s) vs no mutation(s)) and TTP was found.

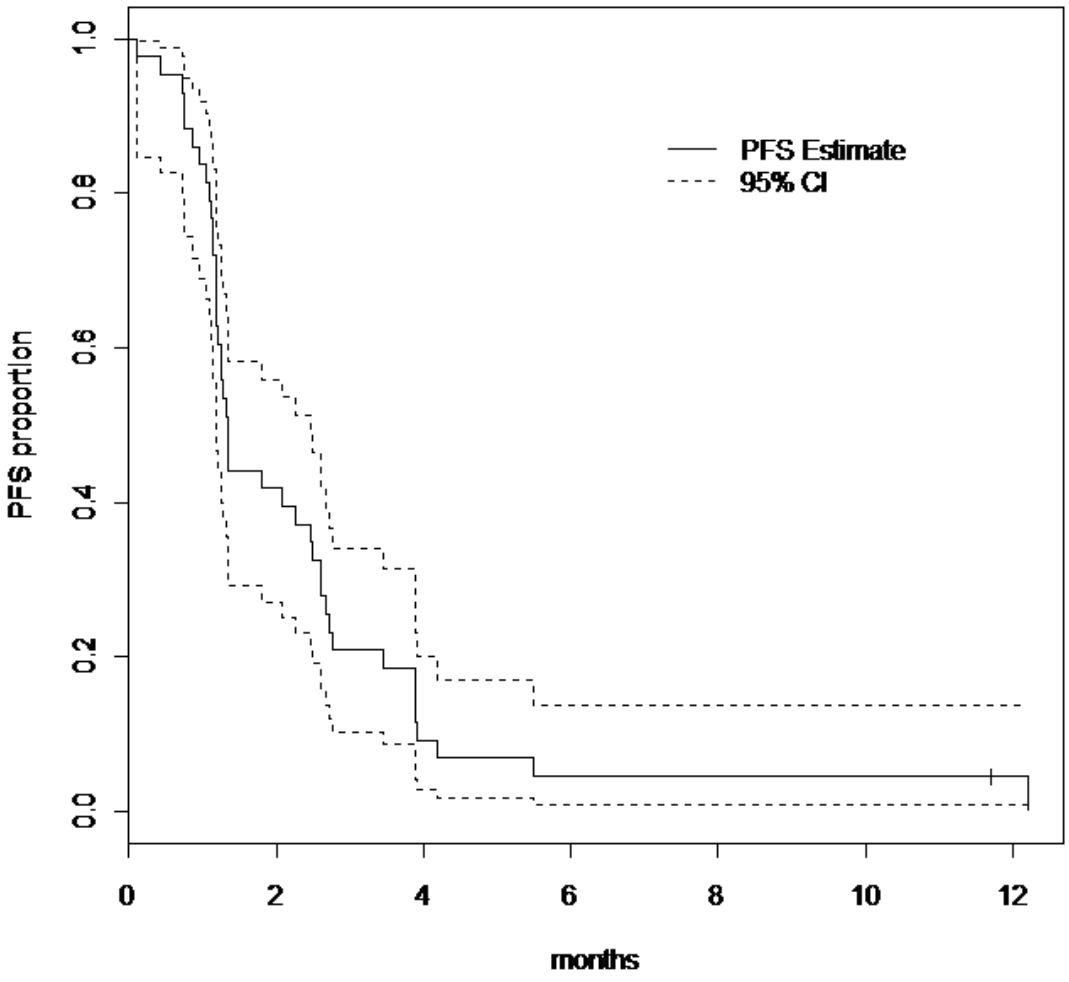
3.2. Results from Study # 2

A phase II study of single-agent RO4929097, a gamma-secretase inhibitor of Notch signaling, in patients with recurrent platinum-resistant epithelial ovarian cancer: a study of the Princess Margaret Cancer Centre, Chicago and California phase II consortia.

Patient characteristics (**Table 4**): Forty-five patients were enrolled from six participating centers between July 2010 and March 2012. After the first 17 patients, the stage I bar was met and the study entered the second stage. Forty-four patients were eligible and evaluable for toxicity, with 40 patients evaluable for response. The majority of patients had serous histology (n=42, 93%). High grade histology by the two-tier system was seen in 82% of patients (n=37). Four patients had progressive disease prior to completing the first cycle of therapy. At the time of data cutoff (June 17 2014), the median follow-up duration was 2.6 months. All patients are off study.

Clinical outcomes: Among 40 evaluable patients, 34 underwent at least one follow-up scan. Nine patients did not complete the second cycle of treatment due to PD. No objective radiologic responses were observed. Fifteen patients (38%) had SD and 24 patients (60%) experienced PD as their best response. Median duration of SD was 3.1 months (2.1-22.7 months). One patient with low-grade serous ovarian cancer had prolonged stabilization of her disease (12.2 months). The median PFS was 1.3 months (95% CI, 1.2-2.5; **Figure 6**) but the median OS has not been reached.

Figure 6. Kaplan-Meier estimate of progression-free survival (patients evaluable for response; n=40).



Safety profile: In total, 164 cycles of therapy were administered to 44 patients. The median number of cycles were two (range 1-33). RO4929097 was generally well tolerated with the most common toxicities (at least possibly related) described being nausea (34%) and fatigue (27%) (**Table 5**). Six patients experienced grade 1-2 transient QT interval prolongation with no associated electrolyte imbalances. Three grade 3 toxicities were described: diarrhea, headache and hypophosphatemia. The episode of grade 3 hypophosphatemia required oral and intravenous phosphate supplementation, but did not necessitate a dose modification. Anemia was the most common hematological toxicity (n=5, 11%), but no grade ≥3 episodes were observed. One patient experienced grade 4 liver transaminitis requiring a treatment interruption during cycle 2. Liver enzymes returned to normal within 2 weeks after treatment cessation. Four patients stopped therapy due to AEs.

Notch pathway biomarkers: An exploratory analysis for potential predictive biomarkers was performed on archival, paraffin-embedded tumour tissue. Twenty-five patients were assessed for Jagged-1 expression. Twenty-three were positive (92%), with a median intensity score of 7 (range 3-8). Twenty-five patients were assessed for NICD expression, of which 17 had high-grade serous histology. Of these 17, six were positive (35%) for NICD (**Figure 7**). A trend towards longer PFS was observed for patients with positive NICD expression in contrast to patients with negative expression of NICD (3.3 vs 1.3 months, p=0.09) (**Figure 8**).

Figure 7. Immunohistochemical expression of NICD in two samples of high-grade serous ovarian carcinoma. A. Absence of NICD protein expression. B. Strong NICD protein expression

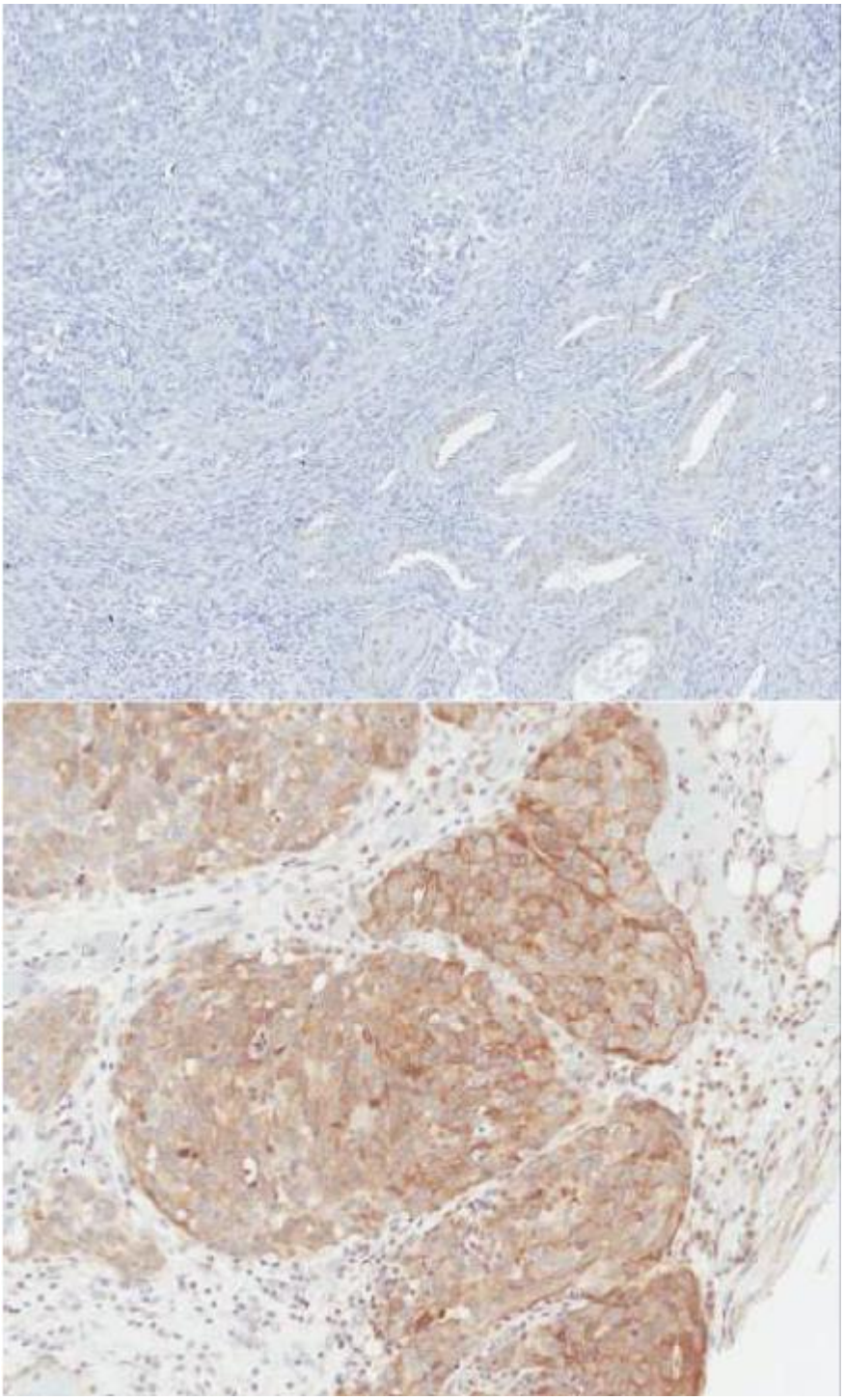
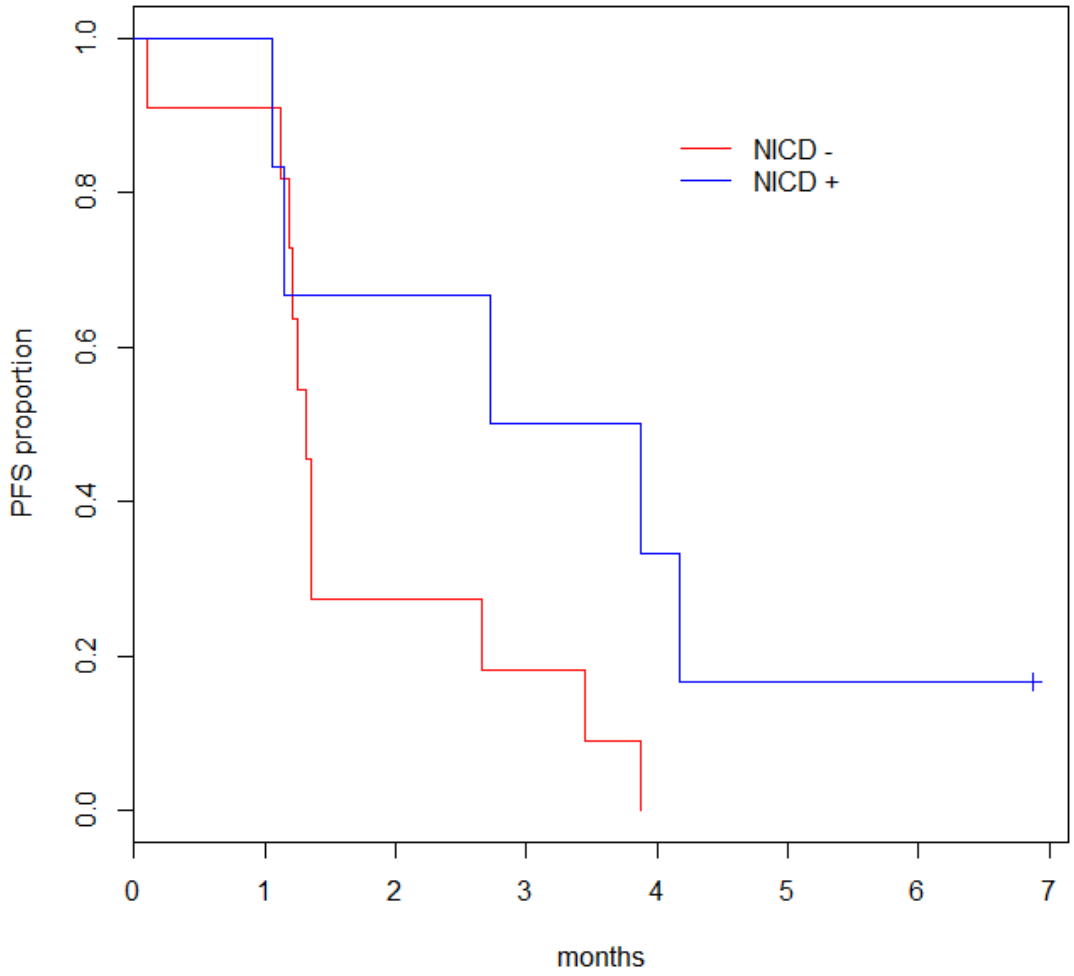


Figure 8. Kaplan-Meier estimate of progression-free survival comparing high intracellular Notch (NICD) protein expression to low NICD (3.3 versus 1.3 months p=0.09). Seventeen patients were evaluable for response. The score was based on the percentage of cells and the intensity of staining. The final scores were classified as negative (0-4) or positive (5-9).



Circulating angiogenic factors: An exploratory analysis was performed investigating potential circulating angiogenic biomarkers on peripheral blood samples prior to therapy and post cycle 2. Forty-four patients had at least one sample available for analysis, 38 of whom had paired samples collected. No association between baseline levels of IL6 (p=0.94), IL8 (p=0.66), SDF (p=0.58), bFGF (p=0.52) and PFS were detected, With respect to VEGFA, C and D, only higher baseline levels of VEGFA appeared to correlate with a better PFS (HR 0.996 p=0.04) but the clinical significance of this was uncertain. No statistically significant correlation was found between change in expression with treatment and clinical outcome.

4. DISCUSION

4.1. Study # 1

A phase Ib combination study of RO4929097, a gamma-secretase inhibitor, and temsirolimus in patients with advanced solid tumors.

Tumor relapse and metastasis remain major obstacles for improving overall cancer survival, which may be due at least in part to the existence of CSCs. CSCs are characterized by tumorigenic properties and the ability to self-renew, form differentiated progeny, and develop resistance to therapy.

Recent searches for therapeutic agents specifically targeted at CSCs in cancer has been classified into three main classes based on their mode of action: (i) agents that specifically target CSCs with defined expression of markers; (ii) agents that specifically target CSC-mediated pathways; and (iii) high throughput screening of drugs that will specifically target CSC-enriched populations. CSCs use many of the same signaling pathways that are found in normal stem cells, such as Wnt, Notch, and Hedgehog (Hh).

The present work has focused on the second strategy, aiming at targeting the Notch pathway as a potential new therapeutic option in advanced solid tumors (in combination with another targeted agent, temsirolimus [clinical study #1 of this thesis]), and in ovarian cancer (clinical study #2 of this thesis).

The clinical study #1 evaluated the feasibility of the combination of RO4929097, an oral gamma-secretase inhibitor, and temsirolimus in patients with advanced solid tumors. The recommended phase 2 dose (RP2D) of such combination was determined to be 20 mg of RO4929097 in 3-days on/4-days off weekly schedule along with the weekly administration of 37.5 mg of temsirolimus. The most common treatment-related toxicities of the combination were fatigue and mucositis. Other toxicities commonly seen with temsirolimus were not exacerbated by the addition of RO4929097

It has been observed that RO4929097 exposure decreases after repeated oral administration due to reversible CYP3A4 auto-induction at doses as low as 24 mg in the 3-days on/4-days off schedule. Thus, it was decided to start RO4929097 dosing at 10 mg, and RO4929097 dose was only escalated to 20 mg. However, even at these relatively low doses, RO4929097 appears to induce CYP3A4 activity, resulting in a significant increase in temsirolimus clearance and a significant decrease in temsirolimus exposure.

No responses were seen with the combination of RO4929097 and temsirolimus. Six evaluable patients (40 %) received at least six cycles of treatment, with SD as best response. Of note, two patients, one with a gastrointestinal stromal tumor and the other with a high-grade synovial sarcoma, remain on treatment after more than 12 cycles.

This study has several limitations. Based on the previously observed auto-induction phenomenon with doses of RO4929097 above 24 mg, RO4929097 was given at low doses (10 mg in DL1; 20 mg in DL2 and DL3). However, human doses of R04929097 ≥ 6 mg achieved plasma concentrations that exceed efficacious exposure levels established in animal models (exposures producing efficacy (AUC24h) were approximately 1,100 ng h/mL after oral daily dosing schedule of 10 mg/kg/day for 21 days in rats, equivalent to 9 mg/day in humans). In fact, the lowest dose level of RO4929097 tested in this study (10 mg) produced serum concentrations above the minimum effective concentration required to inhibit gamma secretase in xenograft studies (data not shown). Secretory diarrhea is a potential pharmacodynamic marker of Notch inhibition due to the critical role of Notch in proper cell differentiation of the rapidly self-renewing crypt cells of the small intestine. Inhibiting gamma-secretase shifts the balance of cell types from nutrient-absorbing cells to mucus-secreting cells leading to secretory diarrhea. Diarrhea was infrequently observed in our study (3 patients, 18 %, all grade 1 episodes), suggesting that the doses and schedule of RO4929097 tested may not have been adequate to inhibit Notch. Other putative pharmacodynamic markers, including

circulating Aß-40 protein and VEGF receptor (VEGFR)-2 protein levels in plasma, changes in interleukin-6 plasma levels, and expression of Notch pathway transcripts in hair follicles, have been evaluated in studies of γ-secretase inhibitors, although it is not clear if these biomarkers reflect intratumoral Notch inhibition. However, this study did not include such pharmacodynamic assessments. Future studies may require pre- and post-treatment paired tumor biopsies in order to assess target engagement and the pharmacodynamic effects of combined pathway inhibition.

Exploratory analysis of selected components of the Notch signaling pathway (Jagged-1, NICD, and Notch-3 receptor) evaluated by immunohistochemistry was performed using paraffin embedded archival tumor specimens. There was no association between protein expression of any of the Notch components and progression free survival beyond 4 cycles, although the number of patients included in this analysis was limited. Most patients had been previously treated with several chemotherapy regimens prior to starting on study treatment. It is unknown whether exposure to prior cytotoxic treatment affects the expression of different Notch pathway components. Future studies should investigate whether expression of Notch pathway components in tumor biopsies acquired before starting treatment is associated with clinical outcome with Notch inhibitor therapy. Our study performed targeted genotyping of tumors with sufficient DNA for analysis. We did not find an association between the mutational status and clinical outcome. This may be due to small numbers and the mixed tumor types of the patients tested.

4.2. Study # 2

A phase II study of single-agent RO4929097, a gamma-secretase inhibitor of Notch signaling, in patients with recurrent platinum-resistant epithelial ovarian cancer: a study of the Princess Margaret Cancer Centre, Chicago and California phase II consortia.

In spite of recent progress in cancer therapeutics and increased knowledge about the cellular and molecular biology of cancer, ovarian cancer still remains a clinical challenge. Chemo-resistance followed by tumor recurrence is a major cause of poor survival rates of ovarian cancer patients. In recent years, ovarian cancer has been described as a stem cell disease. In this scenario, a small percentage of ovarian tumor cells with cancer stem cell-like properties should survive therapeutic treatments by activating the self-renewal and differentiating pathways resulting in tumor progression and clinical recurrence. The mere concept that a small subset of cells in the tumor population drives tumor formation and recurrence after therapies has major implications for therapeutic development.

Resistance to platinum therapy is a major obstacle to treatment for EOC patients and conveys a poor prognosis.[28, 36] Novel therapeutic options are needed. The high rates and patterns of treatment failure have been proposed to be consistent with an accumulation of drug-resistant CSCs.[27, 28] Evidence has shown recurrent EOC is enriched with CSCs and stem cell pathway mediators.[27] Targeting pathways integral to CSC development such as Notch, appears a rational and innovative approach for the treatment of EOC.

CSC marker expression is not static but constantly evolving as a result of differentiation and environmental stimuli. The phenotypical properties of CSCs parallel those seen in chemo-resistant cell populations with high expression of multi-drug resistant transporters, enhanced DNA repair ability and the propensity to proliferate slowly.[28, 37] Repeated courses of chemotherapy have been shown to generate chemo-resistant cell-lines consistent with

CSCs.[38] Importantly, these cells appear to emerge even after a single dose of a chemotherapeutic agent.[39]

The Notch pathway is deregulated in approximately 20% of high-grade serous ovarian carcinomas and appears associated with a worse clinical outcome.[31] As proteolytic cleavage by the gamma-secretase complex represents an important step in the downstream cascade of the Notch pathway, targeting this step either alone or in combination may have important ramifications in recurrent disease. The cleavage releases the intracellular domain of Notch from the membrane, allowing it to move to the nucleus and form a short-lived transcriptional activation complex with the DNA-binding factor RBPJ (also known as CSL) and co-activators of the mastermind-like family. Despite the apparent straightforward nature of this signaling pathway, the outcome of Notch activation varies widely with disease context, from differentiation to maintenance of stemness, apoptosis to cell survival and uncontrolled growth to growth arrest.[40] This incompletely understood pleiotropy emphasizes the complexities inherent to attempts to use Notch inhibition as a therapy.[40]

Gamma-secretase inhibition with drugs including RO4929097 has demonstrated significant anti-tumor activity in preclinical and early clinical studies.[10, 11, 41] Despite this, in the present phase II study in an unselected population of platinum-resistant EOC patients, RO4929097 did not demonstrate meaningful activity to warrant further study. A post-hoc analysis in a limited subgroup of 17 patients revealed a non-significant trend towards better PFS (3.3 vs 1.3 months) in women with positive NICD expression. Positive expression was present in 6 of 17 patients (35%).

In this study, there were no objective responses to RO4929097 and median PFS was 1.3 months (1.2-2.5 months). This does not compare favorably with historical data in platinum-resistant ovarian cancer.[36] Several reasons may explain these negative results. Firstly, whilst the 3-day on, 4-day off dosing schedule of RO4929097 was used, it is unclear whether this is an

optimal dose to cause Notch inhibition.[12] Integration of biopsies pre- and post-treatment would have allowed better assessment of this. The absence of pharmacodynamic and pharmacokinetic evaluation is a weakness of this study. Prior studies have shown plasma concentrations with human doses greater than 6 mg exceed efficacious exposure levels established in xenograft models. Secretory diarrhea has been proposed as a clinical pharmacodynamic marker of Notch inhibition due to the critical role of Notch in differentiation of crypt cells of the small intestine.[10] Inhibiting gamma-secretase shifts the balance of cell types from nutrient-absorbing cells to mucus-secreting cells leading to secretory diarrhea. Diarrhea was infrequently observed in our study (4 patients, 9%), suggesting that the doses and schedule may not have been adequate to inhibit Notch. Furthermore, the development of this drug has been discontinued due to an unfavorable pharmacology profile and concerns over efficacy.[42]

Secondly, similar to differentiated cancer cells, CSCs are likely to be a heterogeneous population. In multi-passaged cancer cell-lines, key CSC populations appear composed of small overlapping CSC groups defined by various arbitrary markers.[26] Given the multitude of pathways and mechanisms involved in drug resistance in CSCs blockade of a single pathway i.e. the Notch pathway may be insufficient. Successful tumor elimination may require a combination of therapies in order to target both the differentiated cancer cells and CSCs.[28, 43] CSCs are likely to represent a minority component of a patient's disease,[44] with the rest of tumor bulk not necessarily sensitive to Notch inhibition alone. Based on this premise, earlier introduction of agents targeting pathways intrinsic to CSC development may be more effective.

Given the limited efficacy seen with monotherapy with GSIs, a combination approach of a Notch inhibitor with either chemotherapy or another targeted agent (or agents) may yield higher response rates. Trials are investigating Notch inhibition in combination with several other inhibitor families including: tyrosine kinases, mammalian target of rapamycin, and

conventional chemotherapeutics. Pharmacodynamic studies from MK-0752 (another GSI) have shown compensatory up-regulation of *Wnt* and other tyrosine kinases at 48 hours, further supporting the hypothesis that combination therapy is necessary. [12, 41] Given that Notch signaling interacts with many other pathways including PI3K/Akt, NF-KB and STAT3, combinational therapy may prove essential for efficacy.[45] In pancreatic cancer cell lines, synergistic anti-tumor effect has been shown with the combination of rapamycin and a GSI.[46] Synergistic activity between platinum therapy and a GSI has also been shown in vivo and in vitro.[28] Additionally, the feasibility of the combination of MK-0752 with docetaxel has been demonstrated in breast cancer.[47] In vivo models have shown the combination of a GSI with paclitaxel was more effective than single agent paclitaxel or single agent GSI at reducing tumor growth.[48] Interestingly, no additional benefit was seen when added to carboplatin and paclitaxel in platinum- sensitive disease. Further exploration of combination therapy may be warranted in platinum-resistant EOC. Other GSI agents are undergoing ongoing investigations as monotherapy (including NCT01292655, NCT01986218).[49] Alternate ways to target the notch pathway are also being investigated, with inhibiting Jag1 of particular interest.[49, 50]

Thirdly, an additional limitation of this study is the lack of drug specificity to the Notch receptors. Given the described pleiotropy, the four Notch receptors may have distinct and even opposite roles depending on cell context and tumor type. Illustrating this, Notch 2 is oncogenic in embryonal brain tumor growth where, in contrast, Notch 1 inhibits it.[8] Notch 3 seems to be of particular interest in EOC. Higher expression of Notch 3 is seen in recurrent disease than in primary tumors, suggesting Notch 3 signaling may be important in chemo-resistance and relapse.[51] Targeting the gamma-secretase component of the Notch pathway may not be specific enough in these circumstances. A further criticism of this study is the inclusion of both low grade and high grade histology in the same cohort, as one would expect

very different Notch signaling and stem-like behavior reflecting the underlying tumor biology of these two distinct diseases.

Finally, whilst Notch signaling seems to be deregulated in approximately 20% of high-grade serous ovarian cancer patients, this study did not pre-screen for the presence of Notch overexpression or gene amplification.[31] It is conceivable that Notch inhibition may only be effective in patients whose tumors are primarily driven by altered Notch signaling. Sensitivity to GSI has been shown in earlier studies to correlate with Notch 3 gene expression.[28] We performed an exploratory analysis of selected components of the Notch signaling pathway (NICD) which to our knowledge, has not been previously reported. The differential response seen in patients with positive expression of NICD versus negative expression warrants highlighting in this study but it is unclear whether the relatively improved PFS demonstrated is due to the predictive or perhaps a prognostic role of NICD. This is something that should be explored further. Recently, contrasting data has demonstrated a worse OS with high notch 1 intracellular domain expression.[52] A PFS of 3.3 months in NICD positive patients appears in keeping with expected outcomes of patients with platinum-resistant disease treated with standard therapy. NICD expression was evaluable in only 25 patients (56%) in our study. The investigation of Notch expression in tumor tissue, CSCs and endothelial cells at baseline and after Notch inhibition should be included in future trials.

A biomarker signature that correlates with response to Notch inhibitors has not yet been identified. In a phase I study by Tolcher *et al* several pharmacodynamic markers were evaluated including Aβ-40 protein and VEGFR2 in plasma and HES1 mRNA expression in hair follicles with only a weak-moderate relationship with pharmacokinetics demonstrated.[11] A further phase I study by Krop *et al* investigating another GSI (MK-0752) developed a nine-gene signature consisting of *ADAM19, CCND1, DVL1, HES4, HES5, HEY1, HEYL, NOTCH1* and *NRARP,* which correlated with PK studies but not clearly with response.[41] To our knowledge, no

studies to date have published data utilizing paired biopsies to evaluate the effect of Notch inhibitors. Notch signaling has been implicated in angiogenesis and has been shown to play an important role in the proliferation and migration of endothelial tip and stalk cells.[53] *In vivo* data has shown that inhibition of DLL4-Notch signaling in endothelial cells led to increased sprouting angiogenesis but reduced tumor growth – so called non-functional angiogenesis.[54, 55] In ovarian cancer, objective response to aflibercept/bevacizumab therapy was observed in patients whose tumors had low expression of DLL4 in contrast to those with high expression.[56] In breast cancer, high expression of DLL4 in the tumor-associated endothelium was thought to evoke resistance and raised the potential need to simultaneously target VEGF and DLL4.[54, 57] In our study, we found there was a suggestion of better outcome and higher baseline expression of VEGFA but the clinical significance of this was unclear (HR 0.996). No correlation between alterations in circulating angiogenic factors post therapy and PFS was found.

In summary, this negative study demonstrated no evidence of objective response to treatment with single-agent RO4929097, a gamma-secretase inhibitor of Notch signaling, in an unselected population of patients with platinum-resistant EOC. Median PFS was less than 1.3 months, suggesting a lack of significant clinical activity of RO4929097 at the study dose and schedule. Further studies with agents targeting the Notch pathway in EOC should investigate combination therapy. Subsequent trials should include biomarker assessment, particularly NICD and/or Notch-3 expression with exploration of potential enrichment designs if its predictive role is confirmed.

5. CONCLUSIONES

En esta sección se detallan las conclusiones de esta Tesis Doctoral de acuerdo con las hipótesis y objetivos que se plantearon para el desarrollo de los dos trabajos que componen la Tesis.

- The administration of RO4929097, a GSI involved in the Notch signaling pathway, in combination with temsirolimus, is feasible, safe, and tolerable in patients with advanced solid tumors.
- The PK profile of RO4929097 makes it a difficult drug for combination studies due to its drug-drug interaction potential.
- The low antitumor activity observed of RO4929097 in combination with temsirolimus does not warrant further investigation of this regimen in a phase II setting.
- Single-agent activity of RO4929097 in unselected patients with platinum-resistant ovarian cancer is insufficient to test this compound in a phase III clinical trial in ovarian cancer
- NICD could potentially represent a predictive biomarker for response to GSIs, and it is worth exploring further.

Enunciados finales de Tesis

- Multiple strategies exist for targeting the Notch pathway in malignancies, but the most attractive therapies will target the specific Notch alteration within tumor types while avoiding Notch signaling in normal tissues.
- Overexpression of Notch receptors and/or ligands might not necessarily imply pathway activation, and pathway activation can lead to tumor-suppressive or oncogenic effects.
- Non-specific inhibition of the Notch pathway with GSI, at least with RO4929097, does not seem to have bright future moving forward, given its lack of specificity and low response rates.
- As for many targeted therapies, selecting the correct patient is critical. Markers such as NICD, expression of ligands, or early dynamic monitoring of response need to be better integrated with early clinical studies.
- More selective and potent inhibitors and selected combinations with chemotherapy or other biologically-targeted drugs should be pursued.
- Future important directions for this signaling pathway may include:

i) the definition of the roles of the Notch pathway at different points in tumorigenesis, metastasis, and self-propagation of cancer stem cells;

ii) the development of biomarkers for sensitivity of cancers and stroma; and

iii) the implementation of rational combination therapies based on robust preclinical data.

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7. TABLAS

Tabla 1. Patient demographics (Study #1)

Table 1 Patient demographics

Characteristic	Patients (n=17), n (%)
Age, years	
Median	62
Range	28–84
Gender	
Male	7 (41)
Female	10 (59)
ECOG PS	
0	4 (23)
1	13 (67)
Type of tumor	
Sarcoma	6 (35)
Neuroendocrine tumor	2 (12)
SCCHN	2 (12)
Other[a]	7 (41)
No. prior chemotherapy regimens	
Median	3
Range	1–8

SCCHN squamous cell carcinoma of the head and neck, *GIST* gastrointestinal stromal tumor

[a] Other tumors included ovarian, lung cancer(non-small cell carcinoma), gastrointestinal stromal tumor, melanoma, hepatocellular carcinoma, endometrial cancer, cholangiocarcinoma

Tabla 2. Patient and Treatment disposition (Study #1)

Table 2 Patient and treatment disposition

Dose level	RO dose (mg)	TEM dose (mg)	No. of pts treated	No. of pts with DLT	DLTs
1†	10	25	8	1	G3 rash, G3 mucositis
2	20	25	3	0	
3^	20	37.5	6	0	

† 2 pts were not evaluable for DLT

^DL3 (RP2D) was expanded to 6 pts

Tabla 3. Treatment emergent adverse events (Study #1)

Table 3 Treatment-related adverse events and laboratory abnormalitiesoccurring in ≥10 % of patients

Dose level (DL)	DL 1		DL 2		DL 3		All	
RO4929097	10 mg		20 mg		20 mg			
Temsirolimus	25 mg		25 mg		37.5 mg			
No. of patients	8		3		6		17	
Grades	All grades	Grades 3/4	All grades	Grades 3/4	All grades	Grades 3/4	All grades (%)	Grades 3/4 (%)
Fatigue	6	1	3	0	5	0	14 (82)	1(6)
Mucositis	5	1	2	0	5	0	12 (71)	1(6)
Anorexia	3	1	1	0	4	0	8 (47)	1(6)
Rash	3	1	2	0	2	0	7 (41)	1(6)
Nausea	3	1	1	0	3	0	7 (41)	1(6)
Dysgeusia	1	0	2	0	4	0	7 (41)	0
Vomiting	2	0	1	0	2	0	5 (29)	0
Diarrhea	2	0	1	0	0	0	3 (18)	0
Flu-like symptoms	0	0	1	0	2	0	3 (18)	0
Headache	0	0	1	0	2	0	3 (18)	0
QTc prolongation	2	0	0	0	1	0	3 (18)	0
Hematology								
Neutropenia	3	1	2	0	5	1	10 (59)	2 (12)
Anemia	5	0	0	0	5	0	10 (59)	0
Thrombocytopenia	2	0	2	0	4	0	8 (47)	0
Chemistry								
Hypertriglyceridemia	5	1	2	0	3	0	10 (59)	1(6)
Hypophosphatemia	4	0	2	1	2	1	8 (47)	2 (12)
Elevated AST	5	0	0	0	0	0	5 (29)	0
Elevated ALT	4	0	0	0	0	0	4 (23)	0
Hyperglycemia	2	0	1	0	1	0	4 (23)	0
Hypercholesterolemia	2	0	0	0	0	0	2 (12)	0
Proteinuria	1	0	0	0	1	0	2 (12)	0

DL dose level, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

Tabla 4. Patient demographics (Study #2)

Clinical and Histological Characteristics		N = 45 (%)
Median Age (Range)		58 (26-81)
ECOG PS	**0** **1** **2**	13 (29) 30 (67) 2 (4)
Disease Site	**Ovarian** **Peritoneal** **Fallopian Tube**	41 (91) 3 (7) 1 (2)
Histology*	**Serous** **Endometrioid**	42 (93) 2 (4)
Grade[§*]	**Low-Grade** **High-Grade**	7 (16) 37 (82)
No. of prior regimens[¶]	**1** **2** **3** **4**	15 (33) 15 (33) 10 (22) 5 (11)
Median number of cycles per patient (Range)		2 (1-18)

Table 5. Treatment-related toxicity at least possibly related to therapy (Study #2)

RO4929097—related toxicity	Any Grade (%)	Grades 3/4 (%)
Nausea	15 (34)	0
Fatigue	12 (27)	0
Hypophosphatemia	7 (16)	1 (2)
Anorexia	6 (14)	0
Anemia	5 (11)	0
Headache	5 (11)	1 (2)
Vomiting	5 (11)	0
ALT increase	5 (11)	1 (2)
AST increase	4 (9)	1 (2)
Diarrhea	4 (9)	1 (2)

8. APENDICES

Apéndice 1. Publicación Original #1

<u>Apéndice 2.</u> Publicación Original #2