



GENERACIÓN DE MODELOS ORTOTÓPICOS AVANZADOS EN RATONES ATÍMICOS DE CÁNCER DE OVARIO EPITELIAL PARA EL ESTUDIO DE RESISTENCIA AL CISPLATINO Y DESARROLLO DE FÁRMACOS

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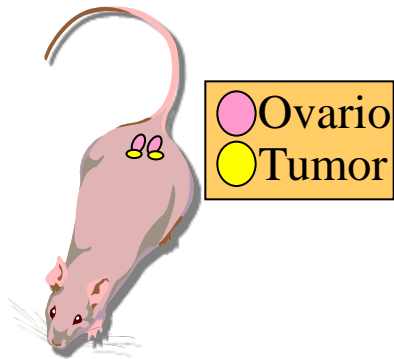
INTRODUCCIÓN

- El cáncer epitelial de ovario es la quinta causa de muerte por cáncer en mujeres y la primera por neoplasias ginecológicas.
- Es un grupo heterogéneo de enfermedades, que abarca carcinomas serosos de alto y bajo grado, endometrioides, de células claras y mucinosos.
- La tasa de supervivencia global a 5 años en estadios avanzados es tan solo del 29%.
- El mal pronóstico viene determinado por el diagnóstico frecuente en estadios avanzados y por la resistencia (intrínseca o adquirida) a la quimioterapia basada en platino.
- Escaso conocimiento de los mecanismos biológicos subyacentes a la resistencia.
- Modelos preclínicos diferentes de la enfermedad humana.

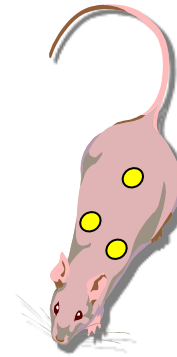
OBJETIVOS

- Generar modelos animales de cáncer epitelial de ovario más parecidos a la enfermedad humana
- Desarrollar modelos tumorales de resistencia adquirida al cisplatino

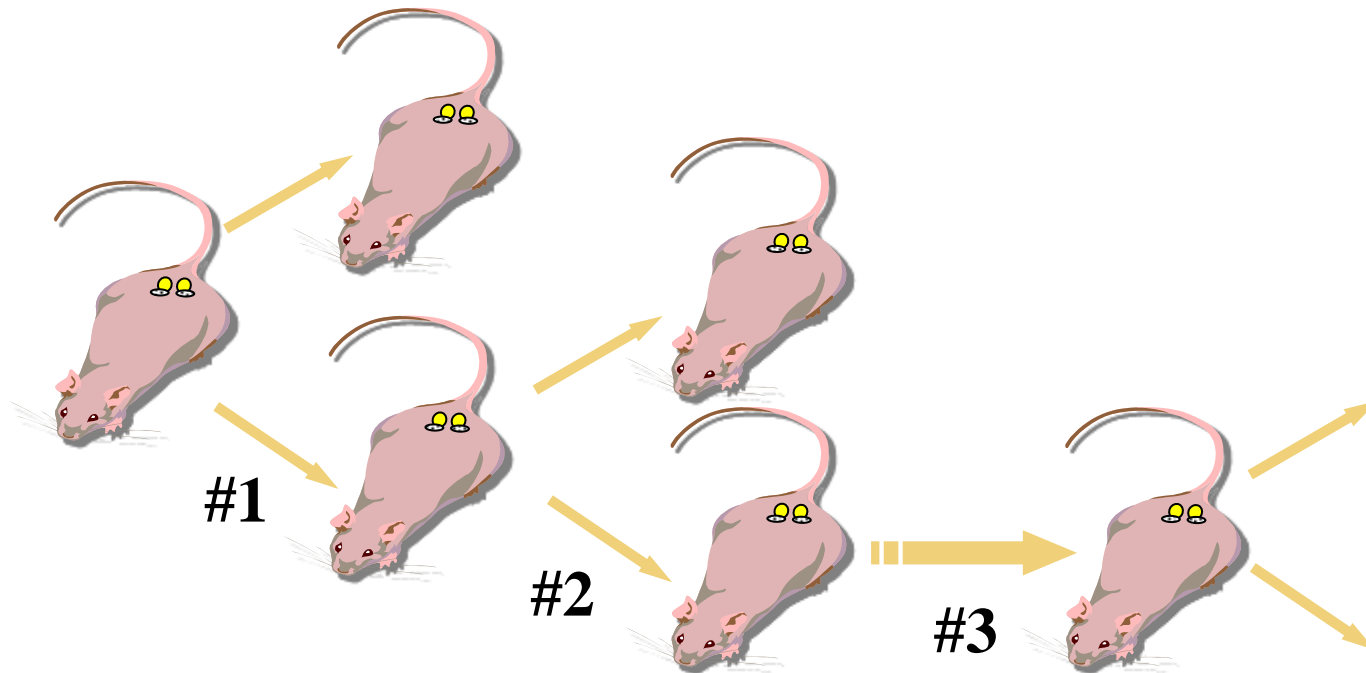
MÉTODO



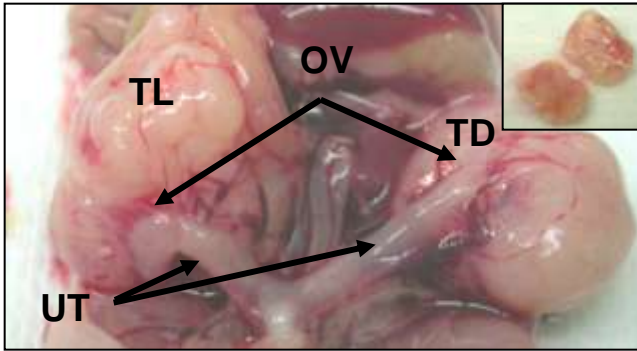
Implantación Ortotópica



Implantación Subcutánea



RESULTADOS



98 tumores implantados

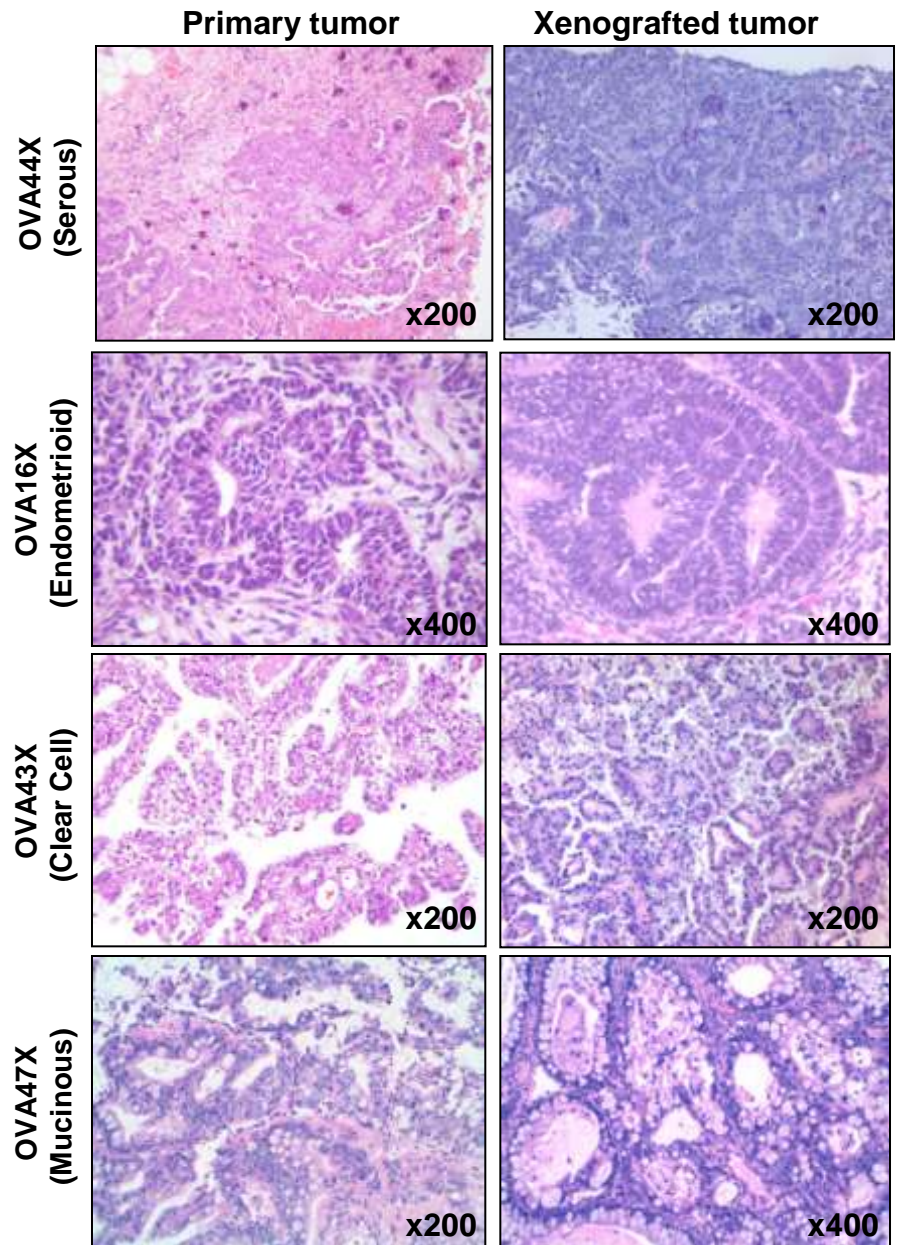
41 Tumores perpetuados:

-35 no tratados (12 serosos, 6 endometrioides, 5 células claras, 6 mucinosos, 6 carcinosarcomas)

-6 tumores post-tratamiento
(3 serosos, 3 endometrioides)

(15 Tumores recientemente implantados o en vías de perpetuación)

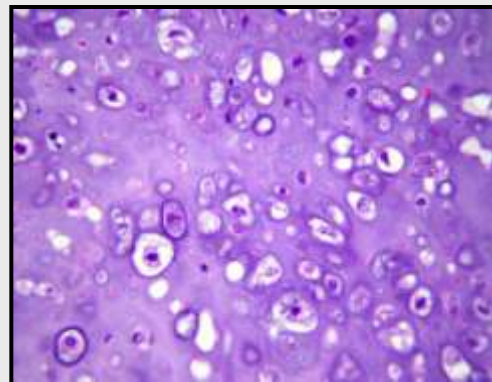
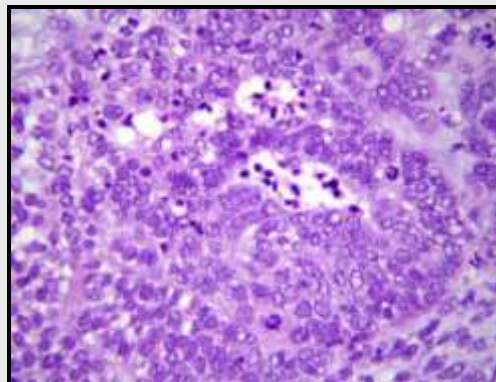
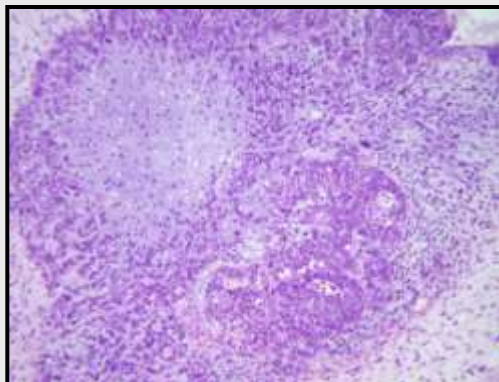
(5 metástasis ováricas)



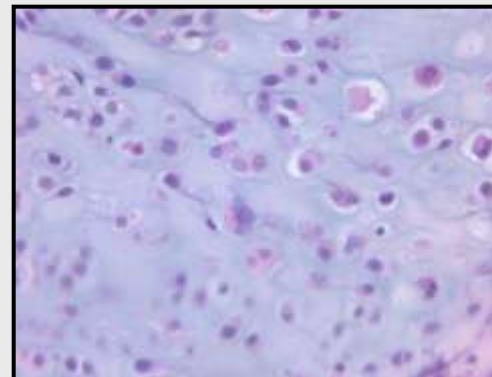
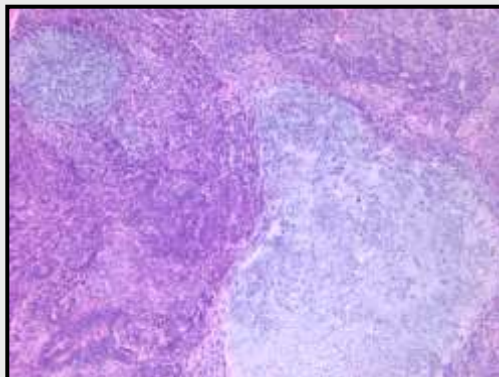
OVA20 (Carcinosarcoma)



Primary tumor



Xenograft



Diseminación peritoneal

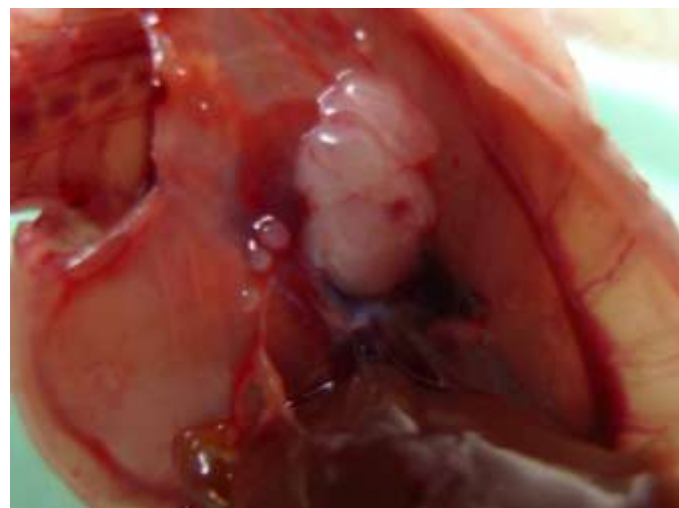


Table 1. Summary of primary epithelial ovarian cancer (EOC) tumors perpetuated as tumors xenografts in nude mice.

Tumor ^a	Histology of the primary tumor	Patient treated with cisplatin	Orthotopically implanted	Subcutaneously implanted ^b	Peritoneal injection of the tumor ^d	Histology of the engrafted tumor	Generation of advanced cisplatin resistant tumor
Xenografts generated after implantation of primary tumor							
OVA1X	Serous	No	Yes	No	nd	Serous	OVA01XR
OVA2X	Endometrioid	No	Yes	No	nd	Endometrioid	OVA02XR
	Serous					+Serous	
OVA5X	Serous	No	Yes	Yes	nd	Serous	OVA08XR
OVA12X	Serous	No	Yes	No	nd	Serous	OVA11XR
OVA13X	Endometrioid	No	Yes	No	nd	Endometrioid	OVA13XR
OVA14X	Mucinous	No	Yes	Yes	nd	Mucinous	OVA14XR
OVA15X	Endometrioid	No	Yes	No	nd	Endometrioid	OVA15XR
OVA17X	Serous	No	Yes	No	nd	Serous	OVA17XR
OVA19X	serous	No	Yes	No	nd	serous	OVA19XR
OVA22X	Serous	No	Yes	No	nd	Serous	OVA22XR
OVA23X	Mucinous	No	Yes	No	nd	Mucinous	-
OVA24X	Serous	No	Yes	No	nd	Serous	OVA24XR
OVA26X	Clear cell	No	Yes	No	nd	Clear cell	OVA26XR
OVA29X	Mucinous	No	Yes	No	nd	Mucinous	OVA29XR
OVA33X	Endometrioid	No	Yes	No	nd	Endometrioid	-
OVA34X	Endometrioid	No	Yes	Yes	nd	Endometrioid	OVA33XR
OVA35X	Serous	No	Yes	No	nd	Serous	OVA35XR
OVA41X	Mucinous	No	Yes	Yes	nd	Mucinous	OVA41XR
OVA42X	Clear cell	No	Yes	No	nd	Clear cell	-
OVA43X	Clear cell	No	Yes	No	No	Clear cell	OVA43XR
OVA45X	Endometrioid	No	Yes	No	No	Endometrioid	-
OVA46X	Clear cell	No	Yes	No	No	Clear cell	OVA46XR
OVA47X	Mucinous	No	Yes	No	No	Mucinous	OVA47XR
OVA57X	Serous	No	Yes	No	No	Serous	OVA57XR
OVA59X	Serous	No	Yes	No	No	Serous	in progress
OVA61X	Serous	No	Yes	No	No	Serous	in progress
OVA66X	Mucinous	No	Yes	Yes	Yes	Mucinous	in progress
OVA68X*	Clear cell	No	Yes	No	No	Clear cell	OVA68XR
OVA75	Serous	No	Yes	No	No	Serous	in progress
Xenografts generated by implantation of viable relapsed tumor masses after patient cisplatin-based treatment							
OVA44X	Endometrioid	Yes	Yes	No	No	Endometrioid	in progress
OVA58X	Serous	Yes	Yes	No	No	Serous	in progress
OVA67X**	Endometrioid	Yes	Yes	No	No	Endometrioid	OVA67XR
OVA69X	Serous	Yes	Yes	No	No	Serous	in progress
OVA74X	Serous	Yes	Yes	No	No	Serous	in progress
OVA78X	Endometrioid	Yes	Yes	No	No	Endometrioid	in progress

^a Nowadays another 15 additional tumors are initially implanted (n=8) or in process of perpetuation (n= 7). In this table only are included those tumors perpetuated (>5 mice to mice passages). So far, we have implanted 98 ovarian human tumors (5 discard by other origins and 6 perpetuated cases were carcinosarcomes of ovarian).

^b A fragment of the primary tumor was simultaneously implanted in the ovary and in the subcutaneous tissues of nude mice. Additionally, for the last 40 cases the primary tumor was also peritoneal injected in mice.

^c Time elapse between passages was calculated based on the first five passages, and in a median of fifteen mice implanted for each tumor.

^d Clinica Mayo approach for growth of EOCs

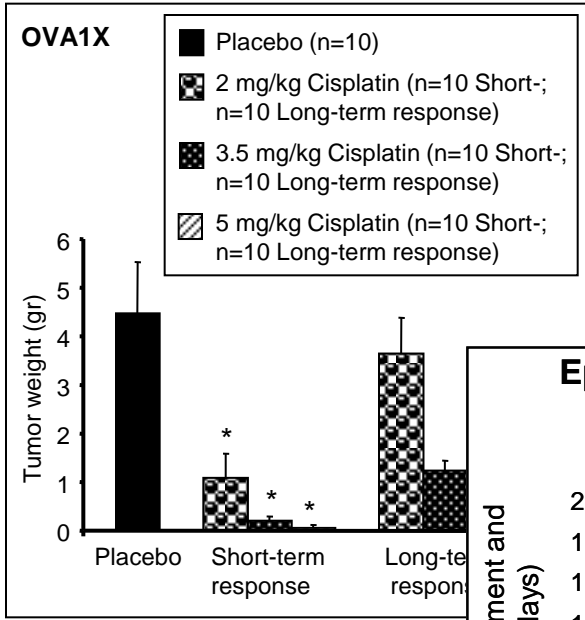
* Lynch syndrome case.

** Tumor relapsed 2 years after chemotherapy treatment (clinically considered a sensitive cases)

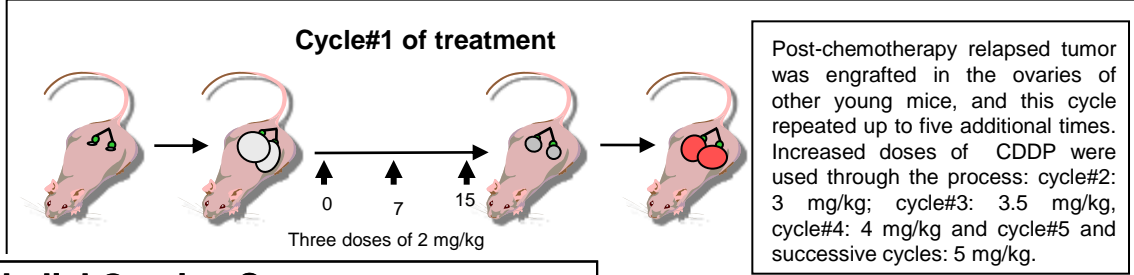
nd, not determined or in progress.

Figure 2

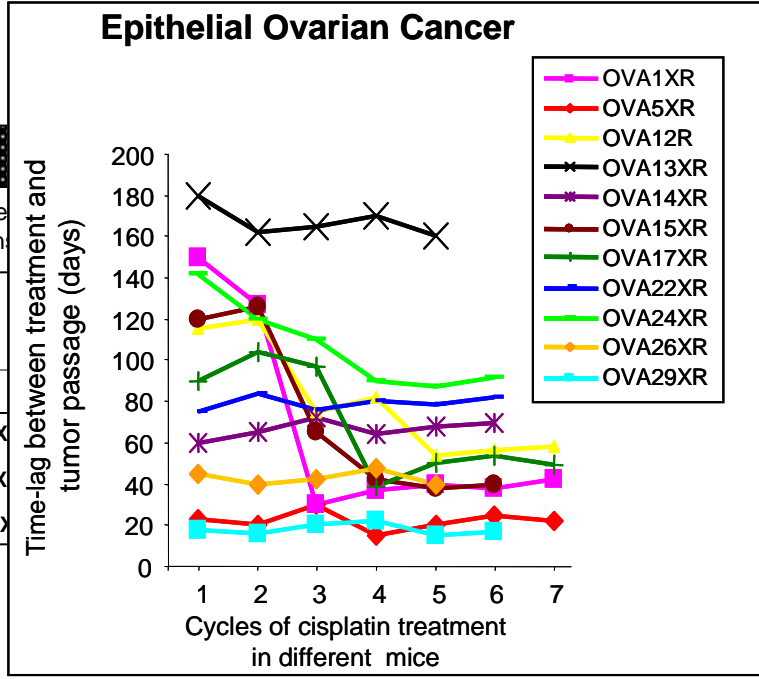
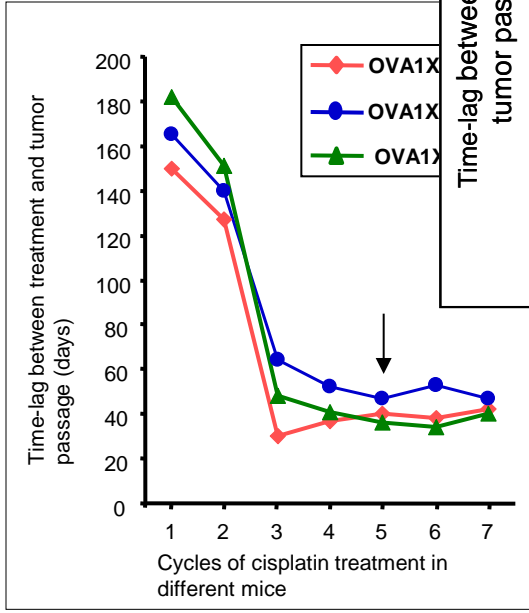
A



B



C



Tumor relapse

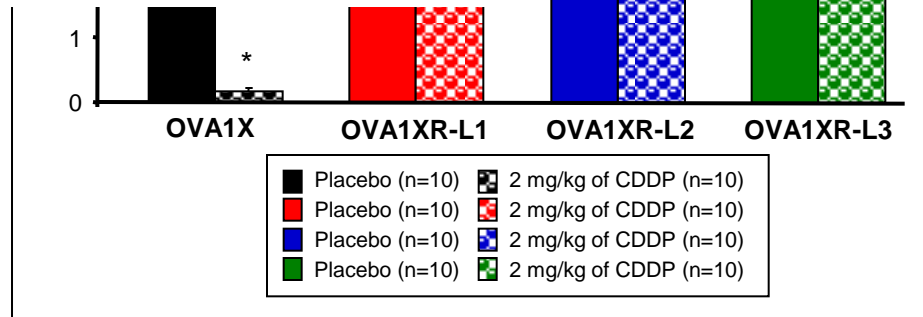
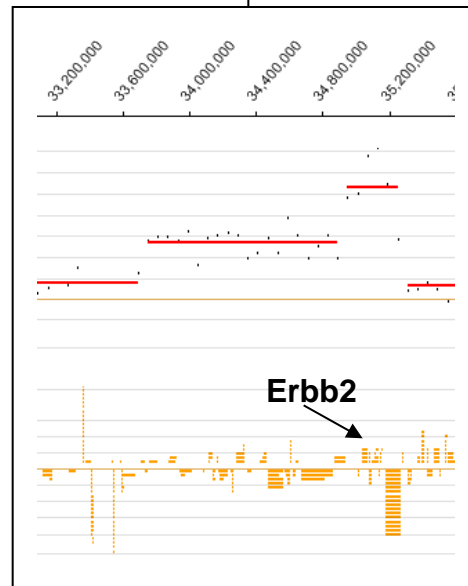
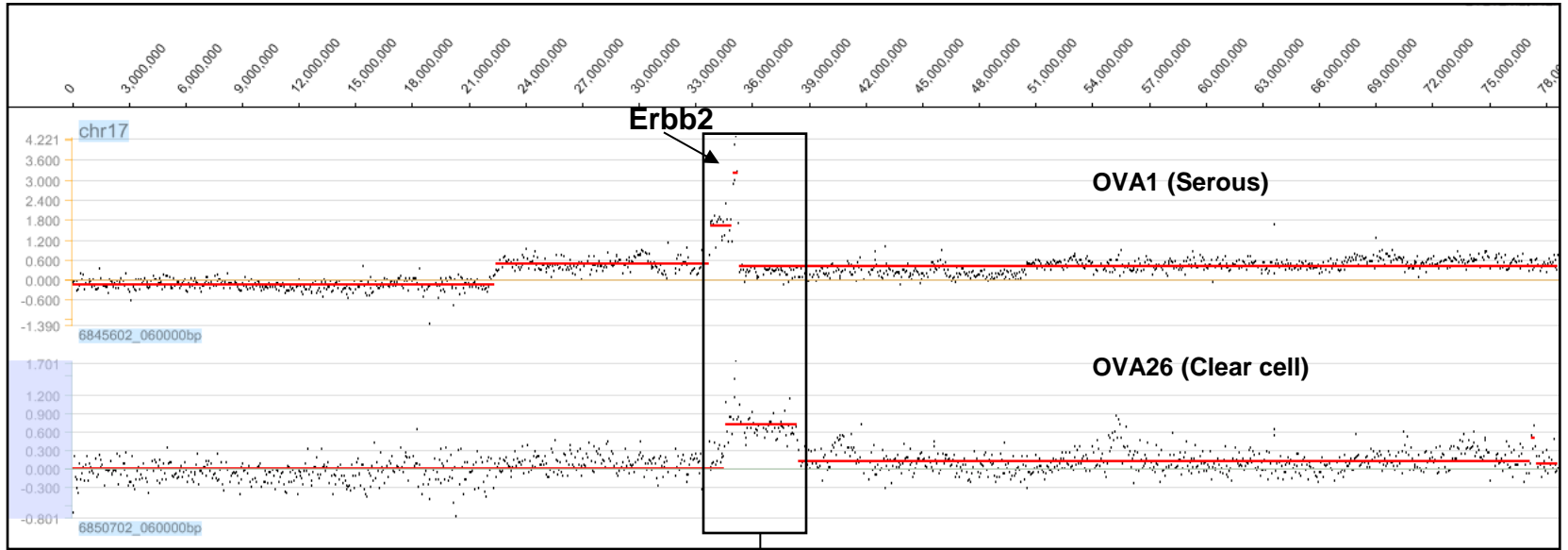
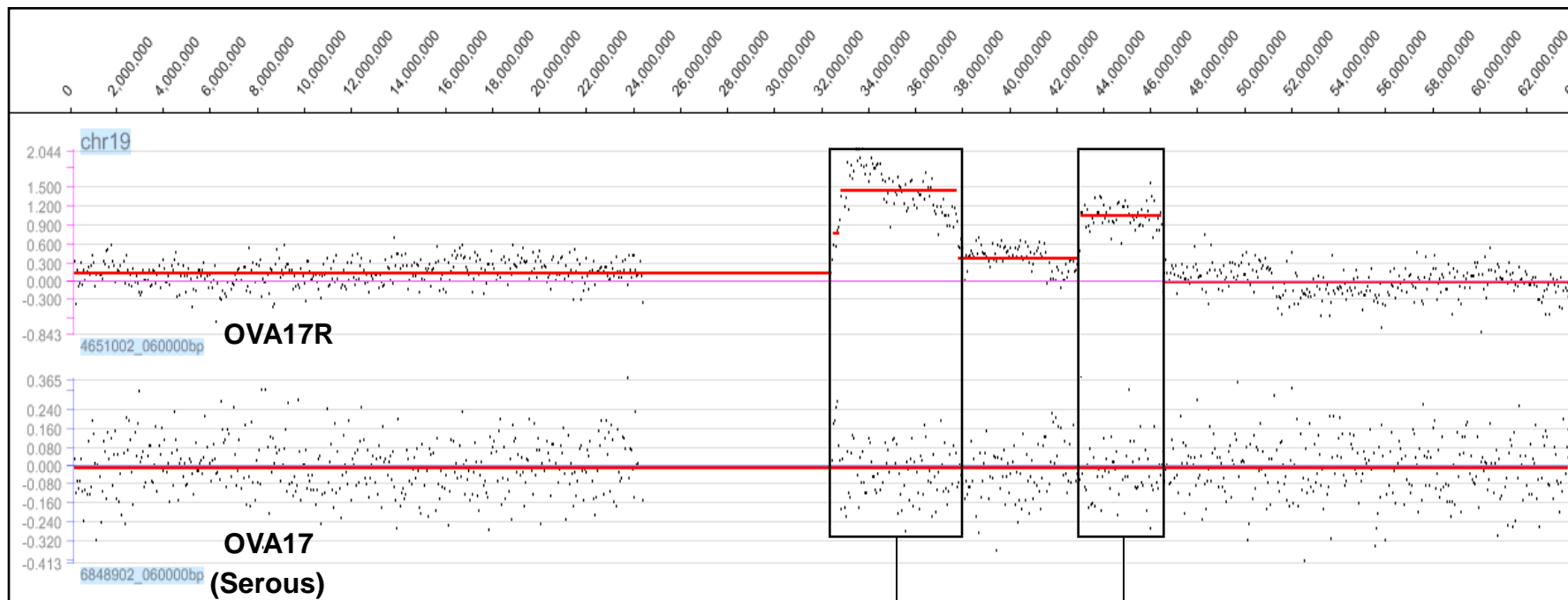


Table 3 Summary of basic genetic characterization (in progress) of primary epithelial ovarian cancer (EOC) tumor growing as xenografts in nude mice.

Tumor	Histology	K-ras	b-raf	PI3KCA	PTEN	P53	CTNNB1	BRCA1/2 (in progress)	MSI (in progress)	ARID1
OVA1X	Endometrioid/ Serous				+	+				
OVA8X	Serous					+		+		
OVA11X	Serous			+		+		+		
OVA13X	Endometrioid						+			
OVA14X	Mucinous	+				+				
OVA15X	Endometrioid			+						
OVA17X	Serous				+	+				
OVA22X	Serous				+	+				
OVA23X	Mucinous	+				+				
OVA24X	Serous					+				
OVA26X	Clear cell	+								+
OVA29X	Mucinous	+				+				
OVA33X	Endometrioid				+		+			
OVA34X	Endometrioid									
OVA35X	Serous					+				
OVA41X	Mucinous	+			+					
OVA42X	Clear cell				+	+				+
OVA43X	Clear cell			+						
OVA46X	Clear cell				+					
OVA47X	Serous	+				+				





19q12-19q13.11

**152 genes including
a region containing
23 zinc fingers**

19q13.12-19q13.2

**158 genes including a
region containing 15 zinc
fingers, and GSK3A,
XRCC1, ERCC2 and ERCC1**

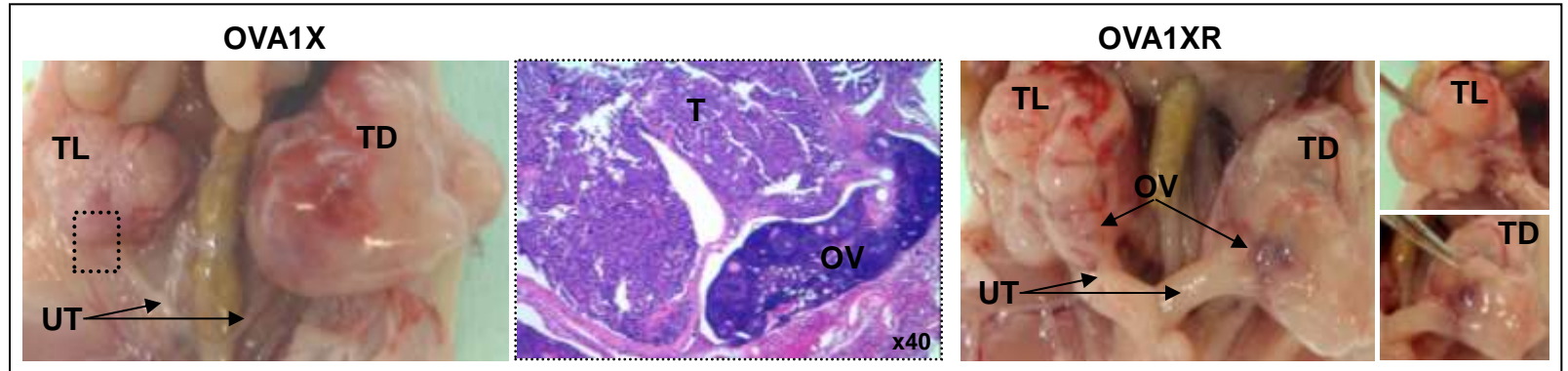
▪ “Exome sequence” de 21 casos de parejas de tumores sensibles / con resistencia adquirida al cisplatino.

- ✓ 10 parejas de carcinomas serosos (3 de ellos de enfermas con mutación de BRCA1/2). [Colaboración Alan Ashworth/Chris Lord, Institute of Cancer Research, London]
- ✓ 6 parejas de carcinomas endometrioides.
- ✓ 5 parejas de carcinomas de células claras (2 con mutación en ARID1)

Gene	Sample	Location
[Redacted]	2339 (OVA17X)	16:49672140
	9084 (OVA24X)	16:49672554
[Redacted]	21916 (5189)	22:25011077
	2339 (OVA17X)	22:25007109
	2339 (OVA17X)	22:25007110
[Redacted]	21916 (OVA11X)	1:62253492
	2339 (OVA17X)	1:62257110
	2339 (OVA17X)	1:62257116
[Redacted]	21916 (OVA11X)	7:91709345
	2339 (OVA17X)	7:91709216

Figure 1

A



B

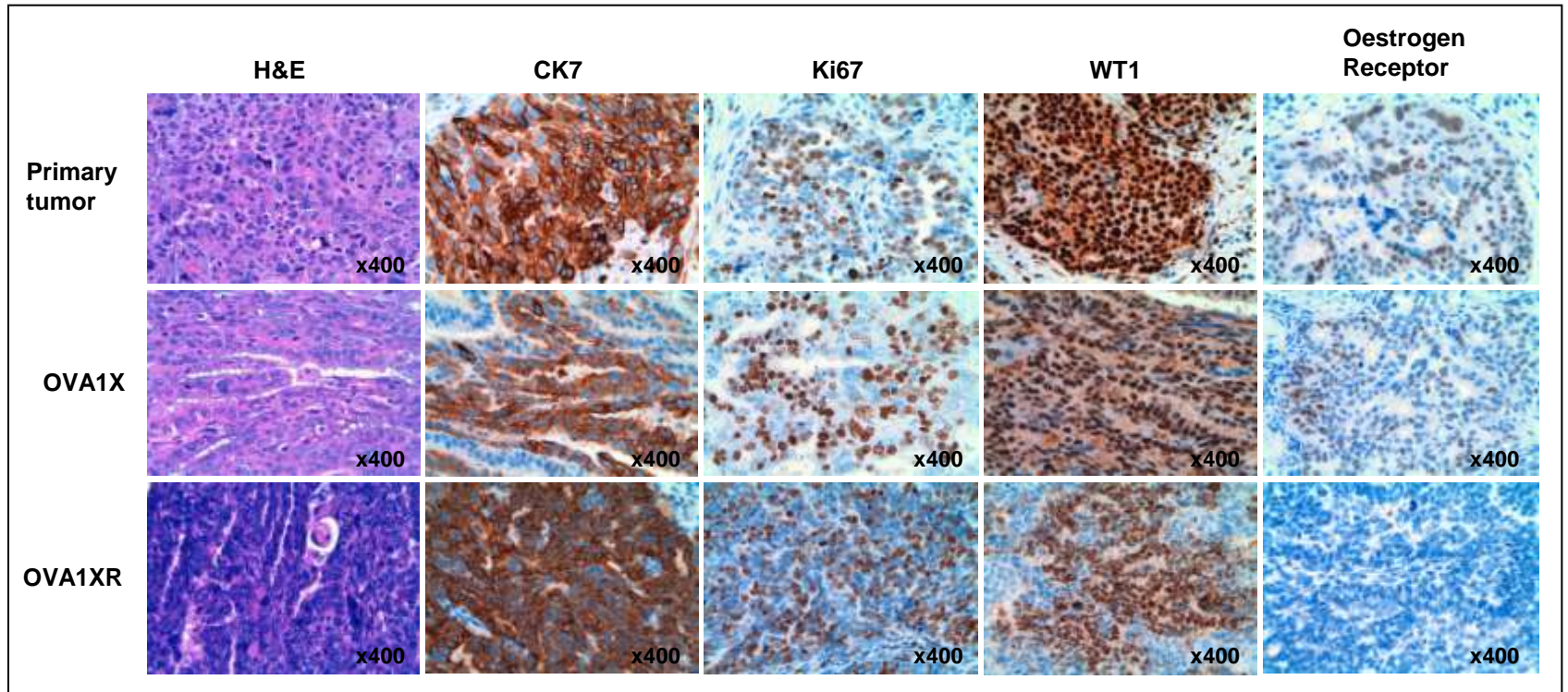
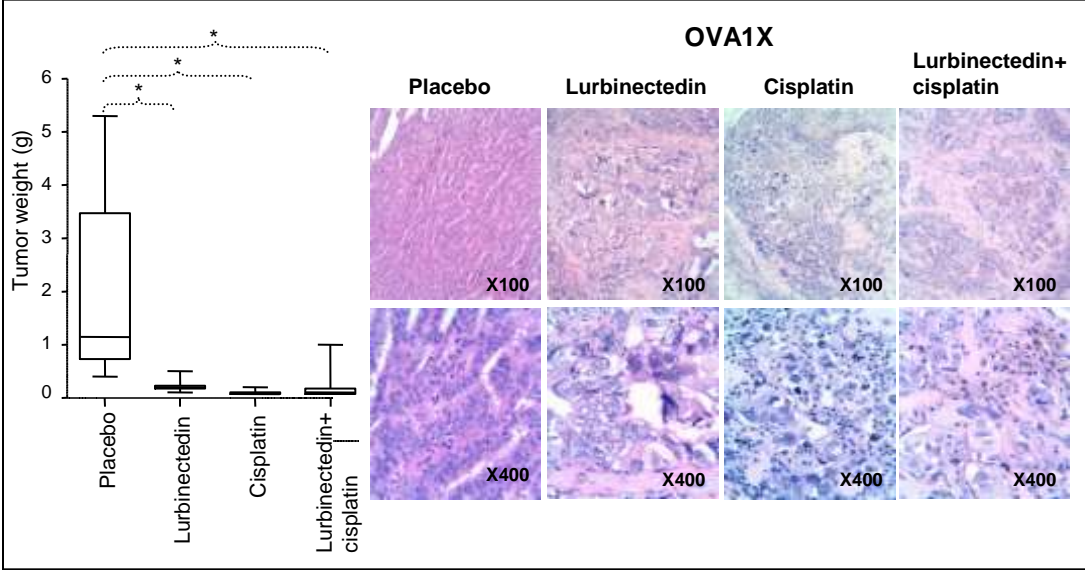
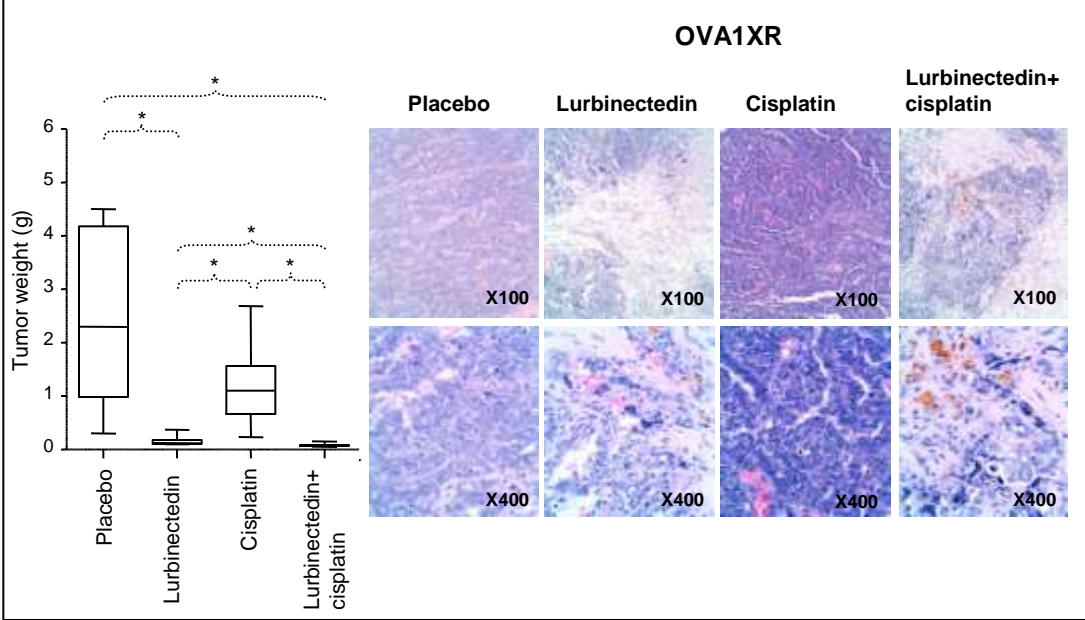


Figure 2

A



B



C

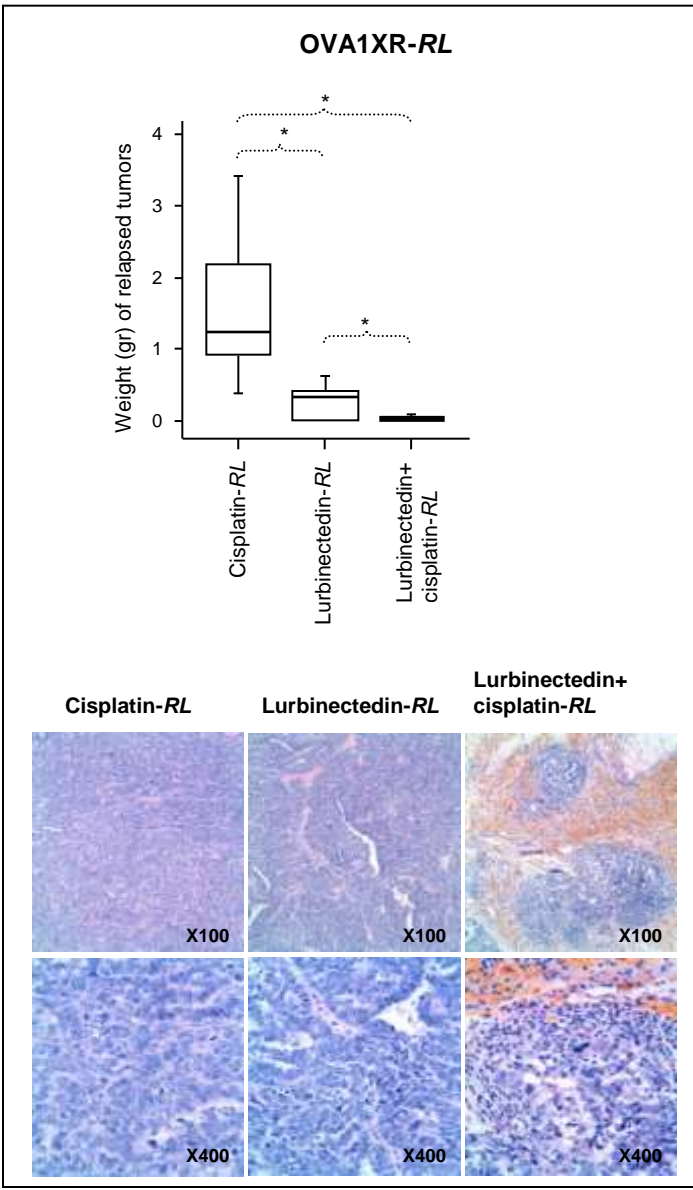


Table 1 Extensive histopathological tumor regression criteria analyses in post-chemotherapy engrafted OVA1XR tumor.

	Mice	Fibrosis	Necrosis	Infiltrant Inflammatory ^b	Foamy macrophages	Calcification	Hemosiderin	Foreign- body giant cells	Giant tumor cells	Pattern of tumor infiltration	Chemotherapy response based on histopathological features ^e
<i>Short-term response^a</i>											
Placebo	1	0	1+	0	0	0	0	0	1+	1+	<i>NHR</i>
	2	0	1+	1+	0	0	0	0	1+	1+	<i>NHR</i>
	3	1+	1+	0	0	CD ^c	0	0	1+	1+	<i>NHR</i>
	4	1+	0	1+	0	0	0	0	1+	1+	<i>NHR</i>
	5	1+	1+	0	0	0	0	0	1+	1+	<i>NHR</i>
Cisplatin	6	2+	0	0	0	0	0	0	0	1+	<i>NHR</i>
	7	2+	0	0	0	0	1+	0	1+	1+	<i>NHR</i>
	8	1+	0	0	0	0	1+	0	1+	1+	<i>NHR</i>
	9	1+	0	0	0	0	0	0	1+	1+	<i>NHR</i>
	10	1+	1+	0	0	0	0	0	1+	1+	<i>NHR</i>
PM01183	11	2+	1+	1+	0	0	2+	0	2+	2+	<i>NHR</i>
	12	2+	0	1+	0	CD	2+	0	2+	2+	<i>NHR</i>
	13	2+	0	2+	0	0	2+	0	2+	2+	<i>NHR</i>
	14	1+	0	1+	0	0	1+	0	1+	1+	<i>NHR</i>
	15	2+		1+	0	0	2+	0	2+	1+	<i>NHR</i>
PM01183+Cisplatin	16	3+	0	2+	0	0	2+	0	3+	2+	<i>MHR</i>
	17	3+	0	1+	0	0	2+	0	3+	3+	<i>GHR</i>
	18	3+	0	2+	0	CD	2+	0	3+	3+	<i>GHR</i>
	19	1+	0	2+	0	0	1+	0	3+	3+	<i>MHR</i>
	20	3+	0	1+	1+	1+	CD	2+	0	3+	3+
<i>Long-term response^a</i>											
<i>Cisplatin-RL</i>	21	1+	1+	0	0	0	0	0	1+	1+	<i>NHR</i>
	22	1+	2+	1+	0	0	0	0	1+	1+	<i>NHR</i>
	23	1+	0	0	0	0	0	0	1+	1+	<i>NHR</i>
<i>PM01183-RL</i>	24	1+	0	1+	0	0	1+	0	1+	1+	<i>NHR</i>
	25	1+	0	1+	0	0	0	0	1+	1+	<i>NHR</i>
	26	3+	0	1+	0	0	2+	0	3+	2+	<i>MHR</i>
	27	NV ^d	NV	NV	NV	NV	NV	NV	NV	NV	<i>GHR</i>
<i>PM01183+Cisplatin-RL</i>	28	3+	0	2+	0	0	2+	0	3+	3+	<i>GHR</i>
	29	3+	0	1+	0	0	0	0	3+	3+	<i>GHR</i>
	30	NV	NV	NV	NV	NV	NV	NV	NV	NV	<i>GHR</i>
	31	NV	NV	NV	NV	NV	NV	NV	NV	NV	<i>GHR</i>

^a For short-term response studies, animals were sacrificed on day 21 of treatment, while for long term-response studies, mice were sacrificed 42 days after the end of treatment when the tumor had relapsed.

^b Pattern and extent of tumor infiltration was classified as follows: 1+, macroscopic large confluent tumor mass(es); 2+, multiple small tumor foci; 3+, scattered solitary tumor cells or complete absence of residual tumor. The remaining regression criteria were graded as follows: 0/1+, no or only minimally presence of the regression criterion within the specimen; 2+, focal occurrence of the respective regression criterion; 3+, widespread occurrence of the respective regression criterion.

^c CD, Dystrophic calcification.

CONCLUSIONES

- Hemos generado un modelo de carcinoma de ovario basado en la implantación ortotópica de tumores en ratones que reproduce la enfermedad humana
- Nuestro modelo de carcinoma de ovario puede contribuir a la identificación de mecanismos y/o marcadores de resistencia al cisplatino.
- Estos modelos preclínicos pueden ser útiles para desarrollar nuevas estrategias terapéuticas en enfermas con neoplasias resistentes al cisplatino.