Asian Society of Gynecologic Oncology

3rd International Workshop on Gynecologic Oncology

Patient-derived Tumor Xenograft (PDX) Model for Gynecologic Cancer

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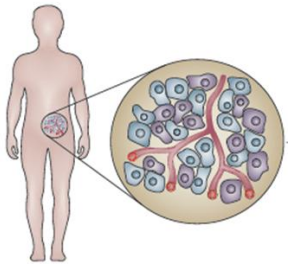
Agenda

- Introduction: Patient-derived Tumor Xenografts (PDX) Models
- Methods and Results for the Development of PDX
  - Ovarian Cancer / Endometrial Cancer / Cervical Cancer
- Limitations
- Summary

PDX = AVATAR Mouse



Need of PDX model

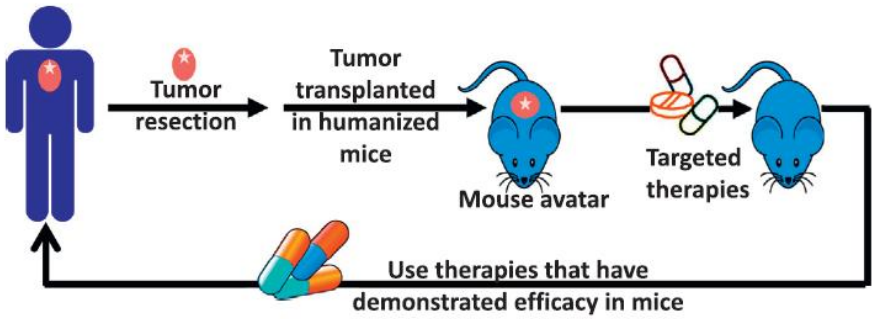
- As animal cancer model, **xenografts of established cancer cell lines**

  → not accurately mimic the behavior in cancer patients

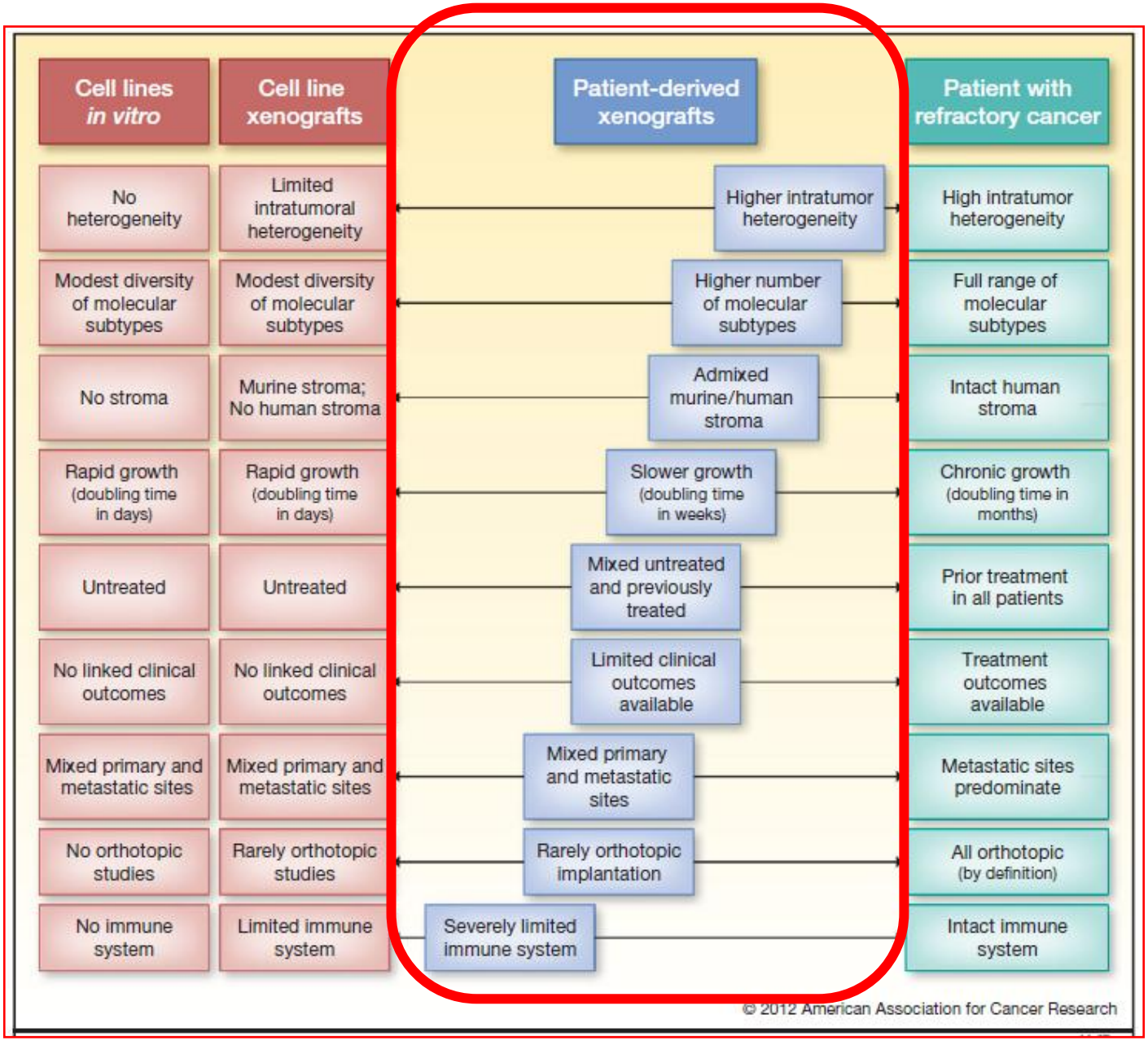
  → not properly predict the clinical efficacy of anticancer agents.

- **PDX models** are built by *transplanting original patient's tumor tissue into immunodeficient mice.*

  → can **better preserve the morphology & gene expression of patient's tumor**

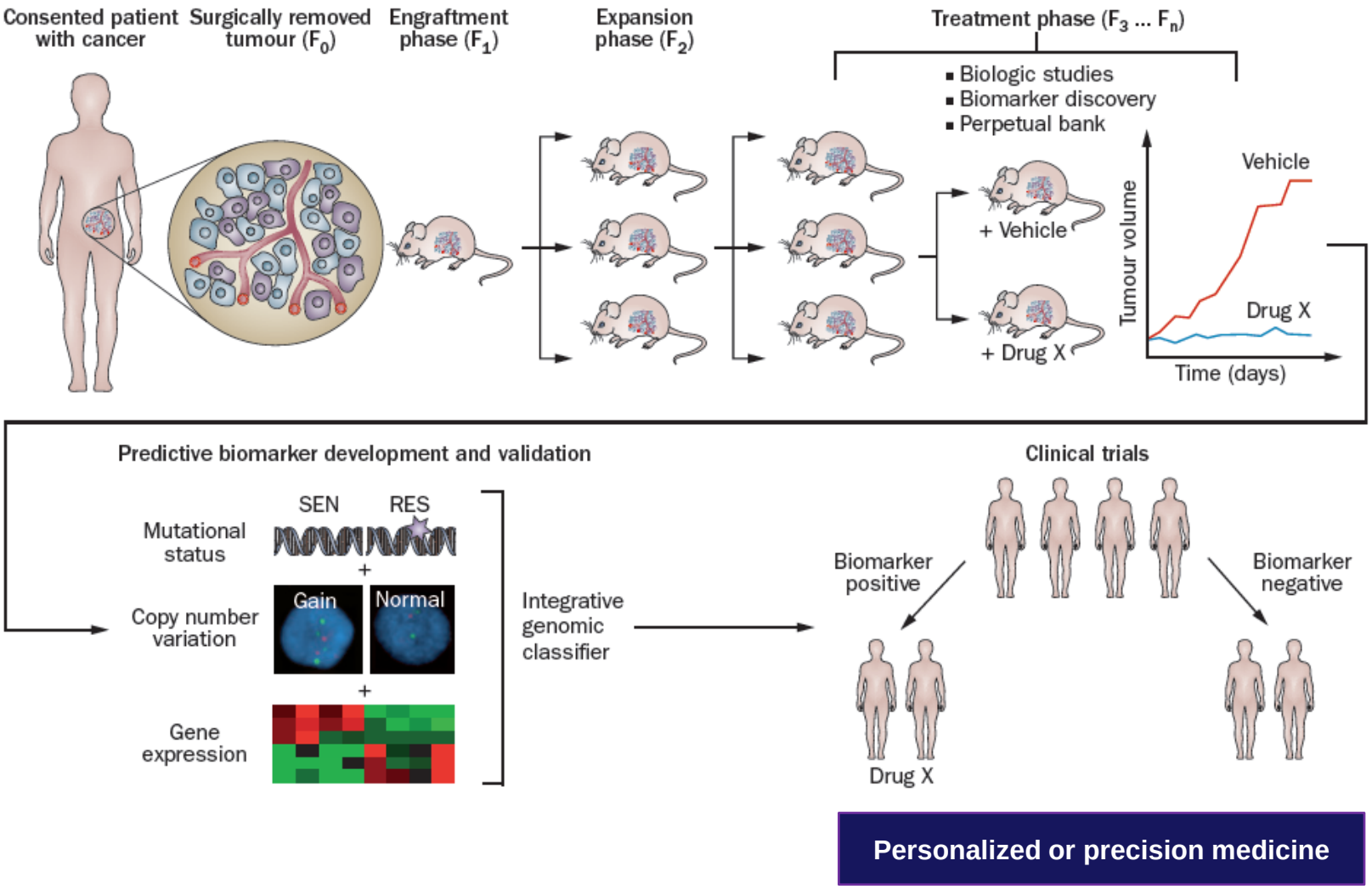


"Avatars / PDX" : mice or other animals with human tissue implanted onto them



The promise of patient-derived xenografts, CCR 2012

Establishment and testing of PDX models.

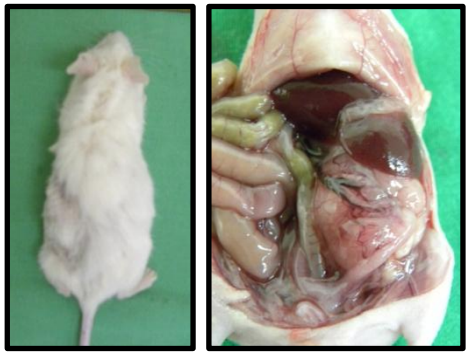


Tentler J.J. et al. (2012) Nat. Rev. Clin. Oncol.

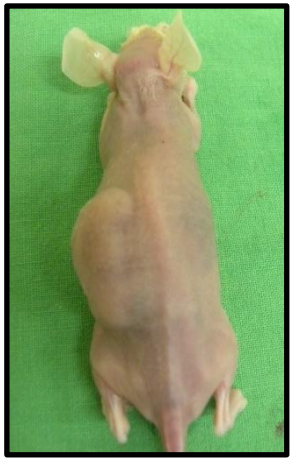
Pilot study

- **Implantation site ?**
  - *Intraperitoneal*
  - *Gonadal fat pad*
  - *Ovary intrabursa*
  - √ *Subrenal capsule*
- **Mouse ?**
  - √ *NOG*
  - √ *Nude*
- **Patient samples ?**
  - *Ascites*
  - √ *Primary tissue*

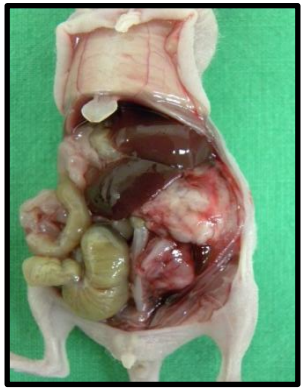
Gonadal fat pad

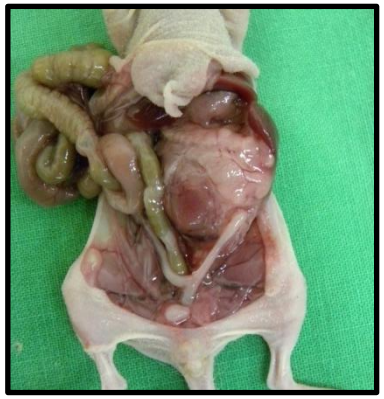


Subrenal capsule



Ovary intrabursa





SMC | IRCR
Samsung Medical Center,
Institute for Refractory Cancer Research

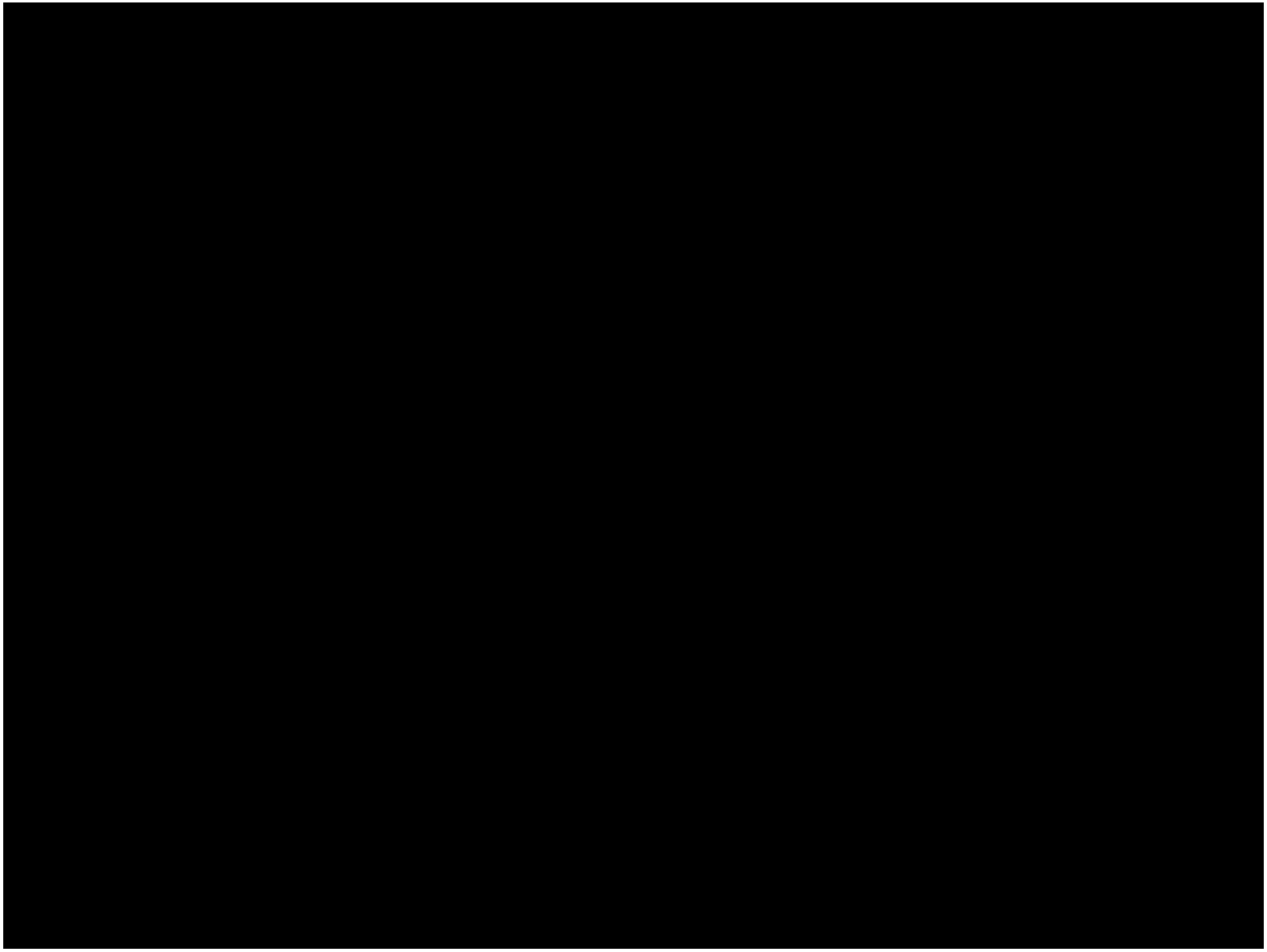
Development of PDX models for gynecologic cancer

using subrenal implantation

in immunodeficient mice (athymic nude or NOG mice)

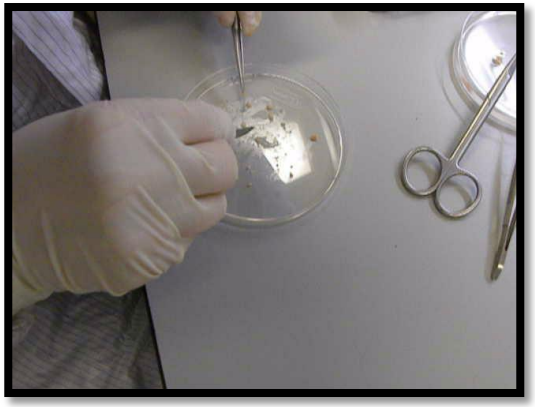
Hospital-centered Personalized Animal Model Development
(Moving Closer to Bedsides)

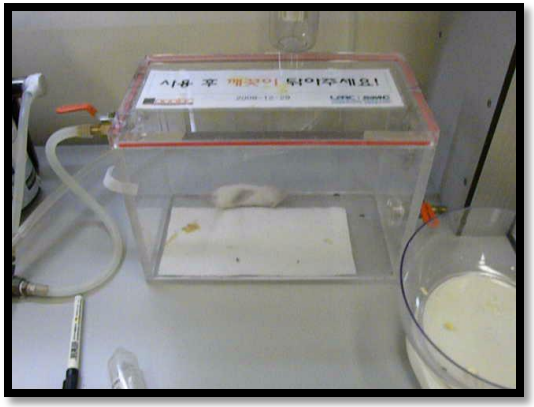
난치암 연구 사업단
난치암 치료 새로운 길을 개척하다.

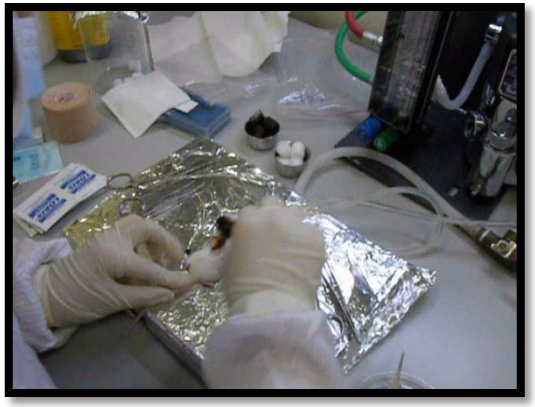


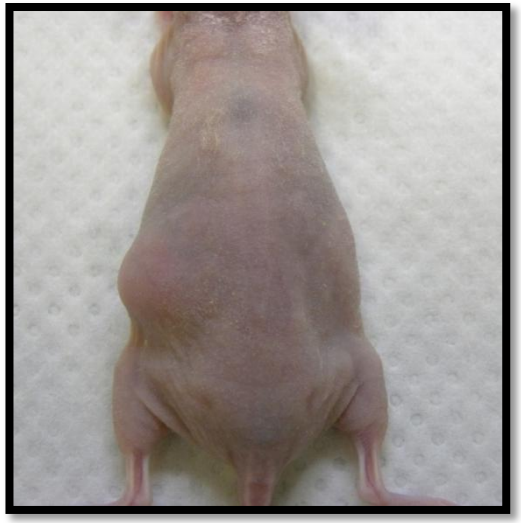
NOG (NOD/Shi-*scid*/IL-2Rγ^{null}) mouse: can be the best model as a highly efficient recipient of human cells to engraft, proliferate and differentiate.

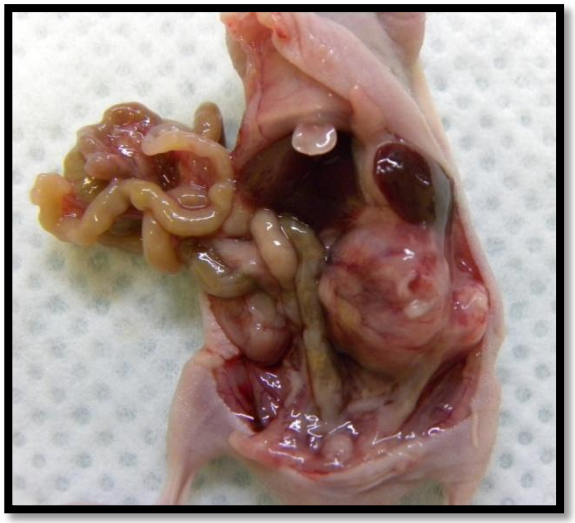
Subrenal Implantation of Patient-derived Tumor

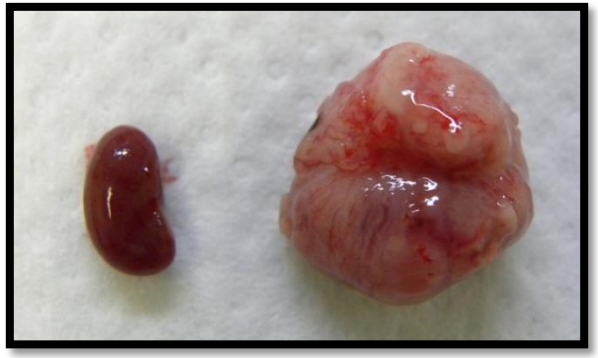








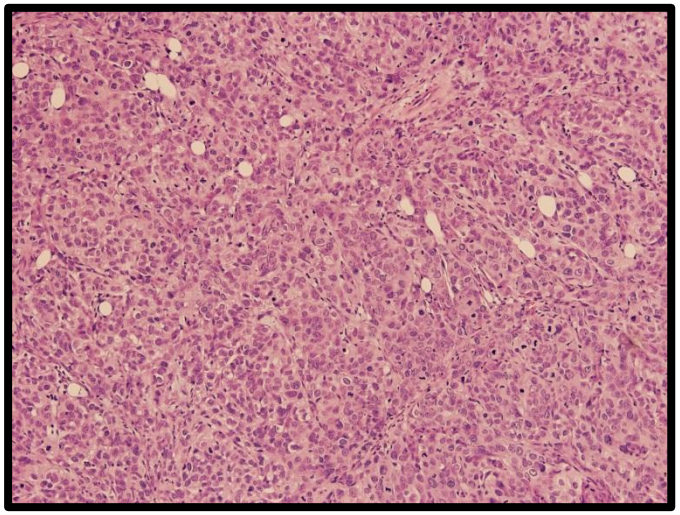


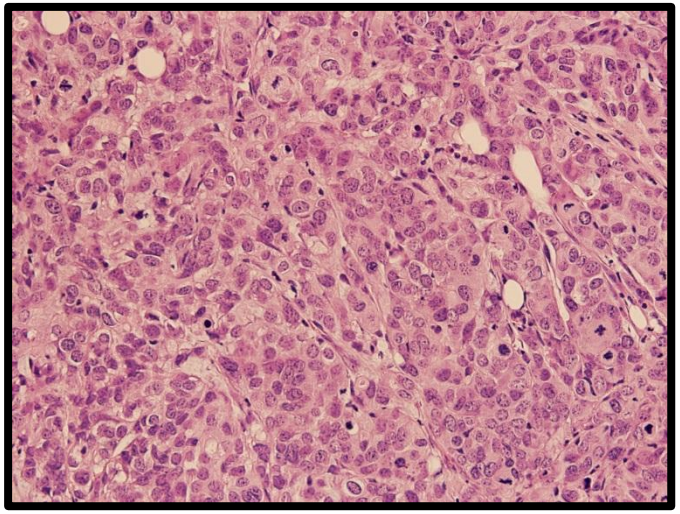


Tissues from Cell Line vs. PDX → *quite different* (1)

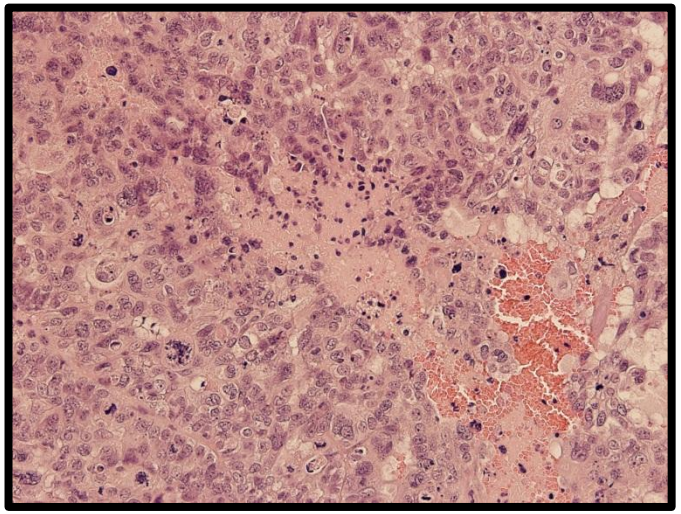
Serous AC

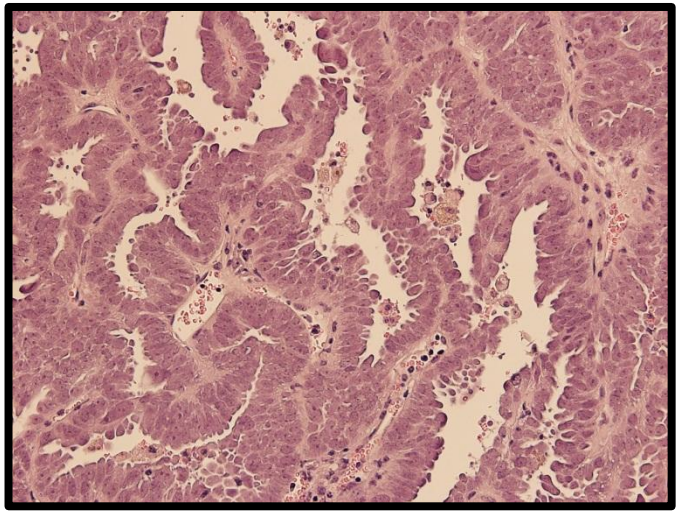
Cell line (HeyA8)





PDX

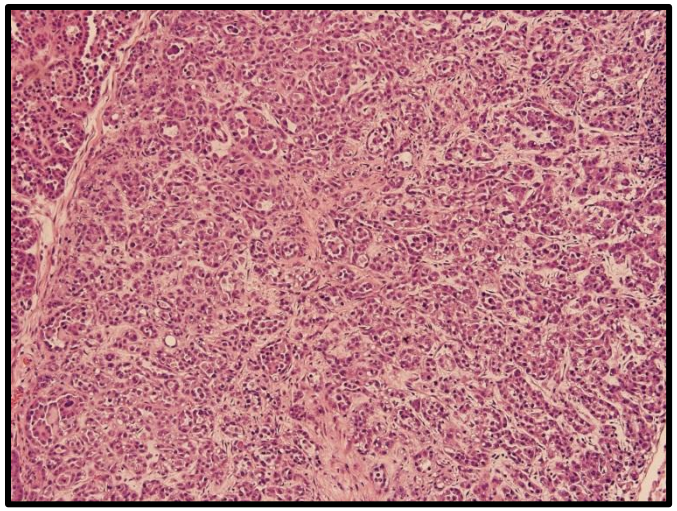


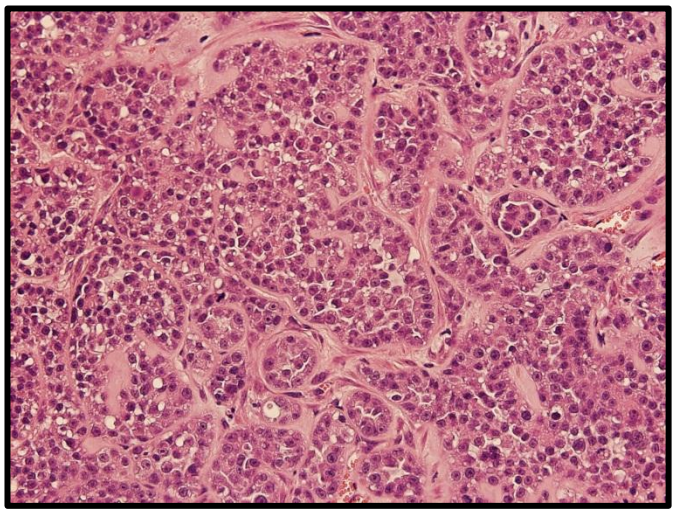


Tissues from Cell Line vs. PDX → *quite different* (2)

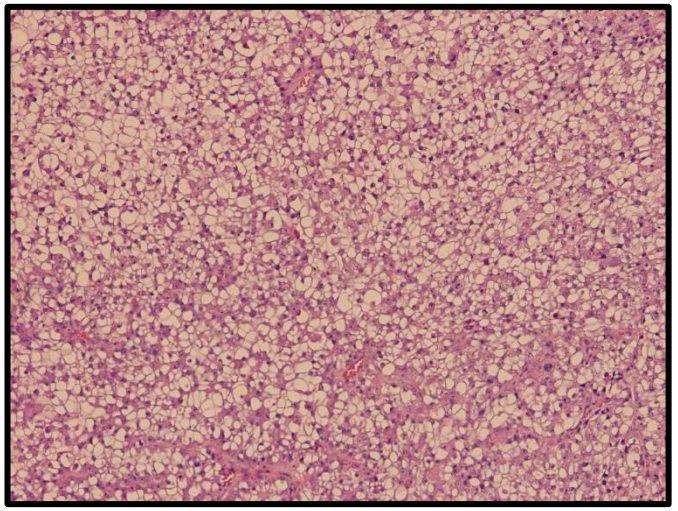
Clear cell carcinoma

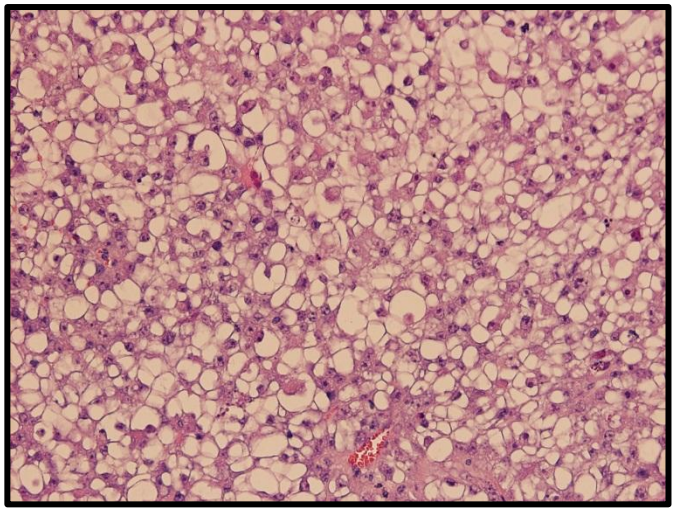
RMG1





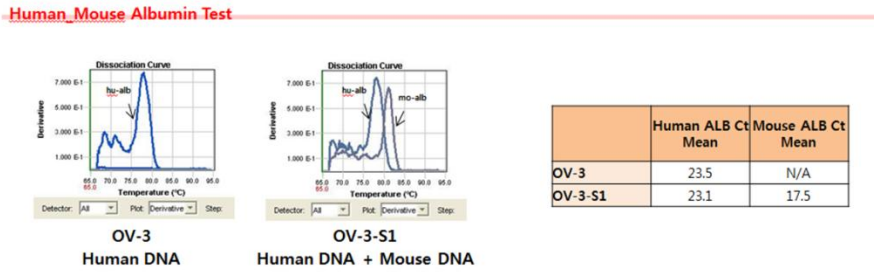
PDX





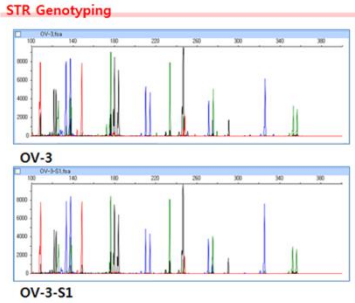
QC process for developed PDX mice

1. Human Mouse Albumin test



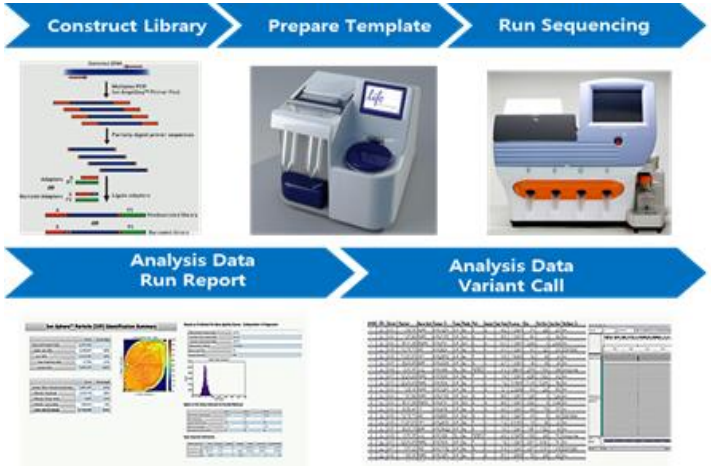
	Human ALB Ct Mean	Mouse ALB Ct Mean
OV-3	23.5	N/A
OV-3-S1	23.1	17.5

2. STR test

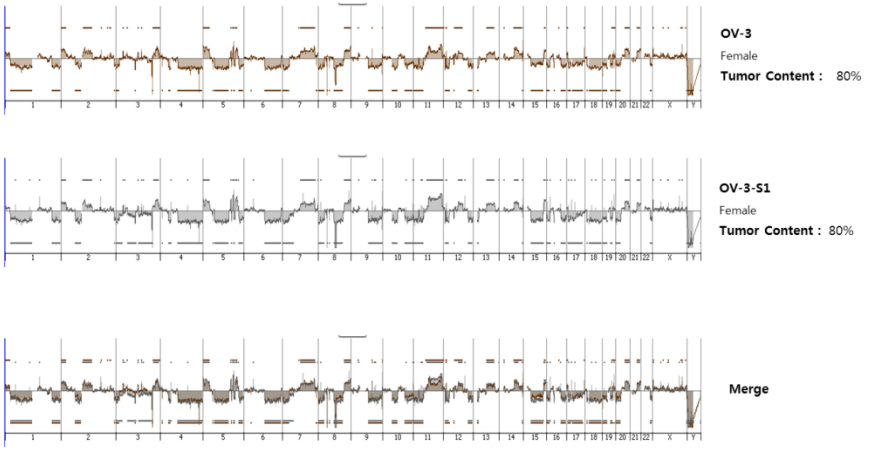


Locus	Chromosome Location	OV-3	OV-3-S1
D8S1179	8	10, 11	10, 11
D21S11	21q11.2-q21	30, 31	30, 31
D7S820	7q11.21-22	9, 10	9, 10
CSF1PO	5q33.3-34	11	11
D3S1358	3p	15, 18	15, 18
TH01	11p15.5	7	7
D13S317	13q22-31	11	11
D16S539	16q24-qter	10	10
D2S1338	2q35-37.1	26, 27	26, 27
D19S433	19q12-13.1	14, 14.2	14, 14.2
vWA	12p12-pter	17, 18	17, 18
TPOX	2p23-2per	11	11
D18S51	18q21.3	13	13
X	Xp22.1-22.3 Yp11.2	X	X
D5S818	5q21-31	10	10
FGA	4q28	23	23

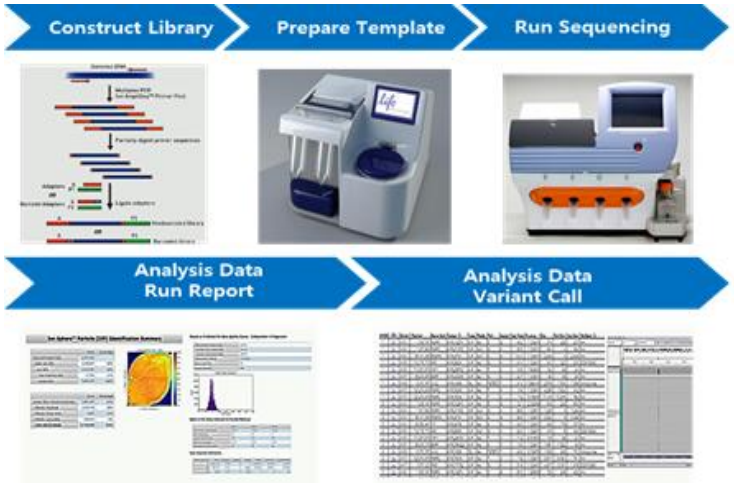
3. IonTorrent (AmpliSeq Cancer Panel)



4. Array CGH



IonTorrent (AmpliSeq Cancer Panel)



- 790 hotspots
- 190 amplicons
- 46 genes

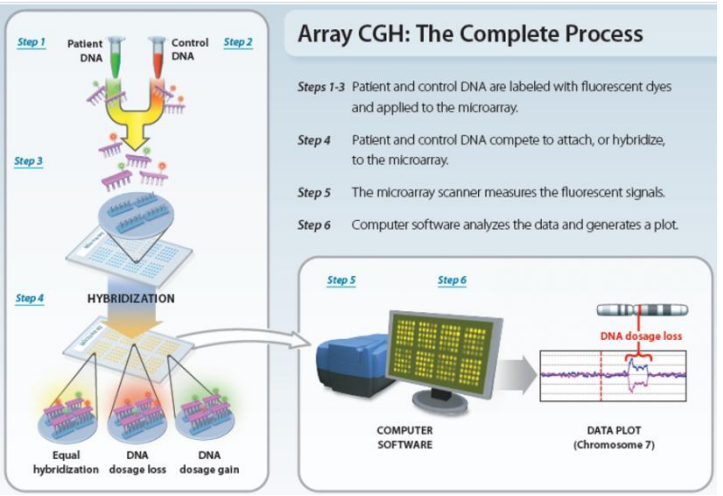
ABL1	CSF1R	FGFR3	KDR	NRAS	SMARCB1
AKT1	CTNNB1	FLT3	KIT	PDGFRA	SMO
ALK	EGFR	GNAS	KRAS	PIK3CA	SRC
APC	ERBB2	HNF1A	MET	PTEN	STK11
ATM	ERBB4	HRAS	MLH1	PTPN11	TP53
BRAF	FBXW7	IDH1	MPL	RB1	VHL
CDH1	FGFR1	JAK2	NOTCH1	RET	
CDKN2A	FGFR2	JAK3	NPM1	SMAD4	

Primary ovarian cancer & M1 tissues

Sample	FGFR3	MLH1	PIK3CA	RB1	TP53
Ov10-002T					
Ov10-005T					
Ov11-023T			Q546R	L670P	C238Y
Ov11-031T					R248Q
OV-7					C176R
OV-22	A797_P799del				
OV-0					
OV-3		V384D			V173L

Array CGH

→ primary tumor vs. M1



No.	human	mouse	Pathology	Classification		amplification	deletion
				Tumor	Ascites		
23	O	O	**Mixed mucinous & serous AC, G3**	O		19q12 (CCNE1)	
16A		O	**Serous AC, G3**		O		9p21 (CDKN2A) 18q12 (NOL4)
31	O	O	**Leiomyosarcoma, G3**	O		16q12.1	13q13 (RB1) 14q23 (RAD51L1)
005	O	O	**Serous papillary AC, G2**	O			10q23 (PTEN)
002	O	O	**Serous AC, G3**	O			18q12 (NOL4)
0	O	O	**Serous papillary AC**	O	O	3q21-22 20q13	
3	O	O	**Serous papillary AC**	O			8q21.1
7	O	O	**Serous AC**	O			

PDX models (Ovarian cancer)

NO.	Op. date	Type	Sample ID	Transplantation (2014.08.14)					
				Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6
1	2011.08.05	Serous papillary AC, G3	OV-0	○	○	○	○	○	○
2	2011.11.17	Serous papillary AC, G2	OV-3	○	○	○	○	○	
3	2012.02.23	Carcinosarcoma, G3	OV-22	○	○	○	○	○	○
4	2012.02.15	Serous papillary AC, G3	OV-20	○	○	○	○		
5	2011.12.16	Serous AC, G3	OV-7	○	○	○	○		
6	2012.04.24	Carcinosarcoma, G3	OV-27	○	○	○	○	○	
7	2012.01.03	Serous papillary AC, G2	OV-11	○	○	○	○		
8	2012.09.03	Serous papillary AC, G3	OV-40	○	○	○	○		
9	2012.01.19	Serous papillary AC, G2	OV-15	○	○	○	○		
10	2013.02.05	Serous papillary AC, G3	OV_52	○	○				
11	2012.10.30	Serous AC, G3	OV_46	○	○	○	○		
12	2012.11.22	Undifferentiated CA	OV-49	○	○	○	○	○	
13	2012.12.04	Serous papillary AC, G2	OV-50	○	○	○	○		
14	2012.07.05	Recurrent serous AC	OV-35	○	○				
15	2012.09.05	Serous papillary AC, G3	OV-41	○	○	○	○		
16	2012.07.26	Serous papillary AC, G2	OV-36	○	○	○	○		
17	2012.07.04	Serous papillary AC, G2	OV-33	○	○	○			
18	2012.10.12	recurrent AC (LN)	OV-43	○	○	○	○		
19	2013.01.31	Serous papillary AC, G3	OV-55	○	○	○			
20	2012.12.04	Serous papillary AC, G3	OV-51	○	○	○			
21	2012.10.22	Surface serous AC, G3 (LN)	OV-45-1	○	○				
22	2013.08.13	Clear cell ca	OV-64	○	○	○	○	○	○
23	2012.08.20	Primary peritoneal serous AC	OV-39	○	○				
24	2012.10.22	Surface serous AC, G3	OV-45	○	○				
25	2012.03.25	Serous carcinoma, G3	OV-60	○	○				
26	2014.03.26	Clear cell ca	OV-68	○	○				
27	2013.02.04	Clear cell ca	OV-56	○	○				

PDX model (Endometrial & Cervix cancer)

NO.	Operating Date	Type	Sample ID	Transplantation (2014.08.14)								
				Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6	Mouse 7	Mouse 8	Mouse 9
1	2011.10.31	Carcinosarcoma	EM-0	○	○	○	○	○				
2	2011.12.26	Endometrioid AC, G3	EM-2	○	○	○	○	○	○	○	○	○
3	2012.08.23	Endometrioid AC, G1	EM-4	○	○	○						
4	2013.03.21	Endometrioid AC, G1	EM-8	○	○	○	○					
5	2013.10.07	Metastatic serous AC	EM-11	○	○							

1	2011.12.19	Invasive SCC	CX-4	○	○	○	○	○	○	○	○	
2	2011.12.21	Invasive SCC	CX-6	○	○	○	○	○	○			
3	2012.04.26	Invasive SCC	CX-8	○	○	○	○	○	○	○		
4	2012.03.09	Invasive SCC	CX-7	○	○	○						
5	2012.11.16	Invasive SCC	CX-15	○	○							
6	2012.11.15	Endocervical AC	CX-14	○	○	○	○					
7	2012.05.11	Invasive SCC	CX-10	○	○	○	○	○	○			
8	2012.06.12	Invasive SCC	CX-11	○	○							
9	2012.11.29	Invasive SCC	CX-17	○	○	○	○	○				
10	2012.10.04	Metastatic SCC (LN)	CX-13	○	○							
11	2013.07.10	Invasive SCC	CX-21	○	○	○	○	○				
12	2013.05.02	Invasive SCC	CX-19	○	○	○	○					

In vivo tumorigenicity rate

	OV CA	CX CA	EM CA
1. Total cases	57	26	14
2. Engraftment rates (%)	47.4% (27)	46.2% (12)	42.9% (6)
3. Mean time to M1	5.7 (1.9 – 14.4) month	5.5 month	4 month
4. Mean no. mouse developed	3/5		

Pathology of tumors

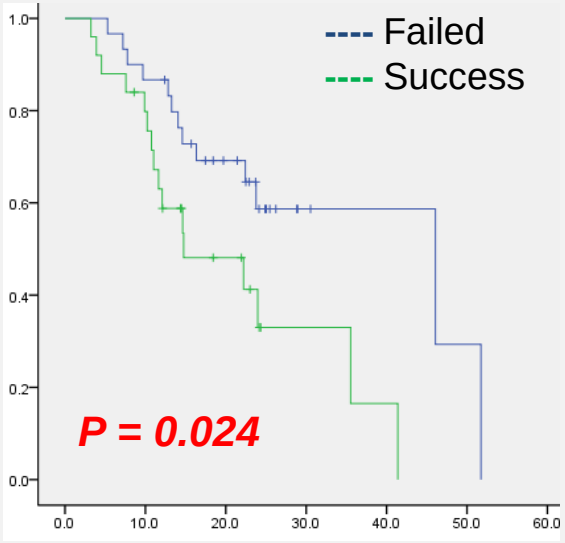
*2011.11~2014.8

	Histopathology of primary tumor / Grade	Generation of PDX
1	Serous papillary adenocarcinoma / 3	19
2	Invasive squamous cell carcinoma / -	11
3	Endometrioid adenocarcinoma / 3	4
4	Carcinosarcoma / 3	1
5	Malignant mixed Mullerian tumor / 3	3
6	Undifferentiated carcinoma	1
7	Endocervical adenocarcinoma	1
8	Metastatic adenocarcinoma / 3	1
9	Clear cell carcinoma	3
10	Mixed serous and clear cell adenocarcinoma, endometrium	2
	Total	**45**

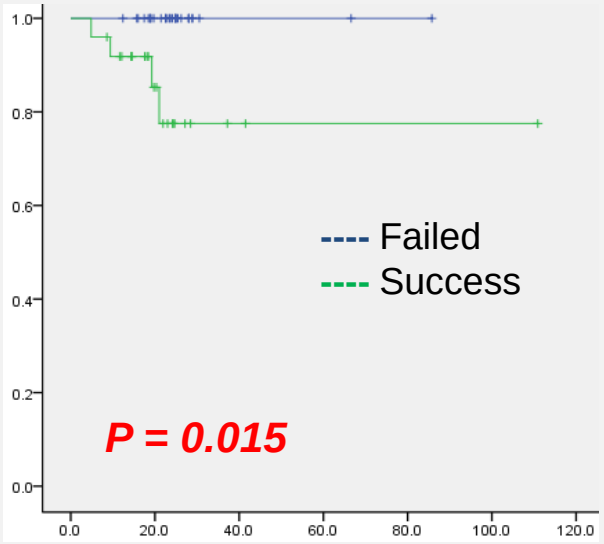
In vivo Tumorigenicity & Clinical Aggressiveness of Parental Tumors

- **N=63, EOC**
- Successful engraftment: 25
- Ongoing: 8
- Failed engraftment: 30, engraftment rate: 25/55 (45.6%)
- Median M1 day: 171 (58-431) days, 5.7 (1.9-14.4) months

Progression-free survival



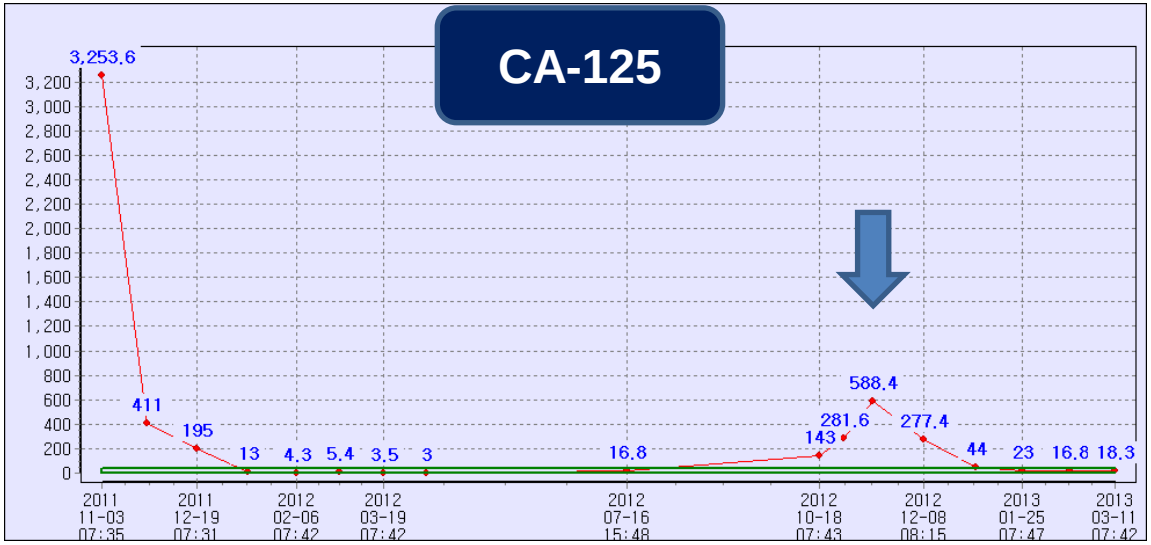
Overall survival

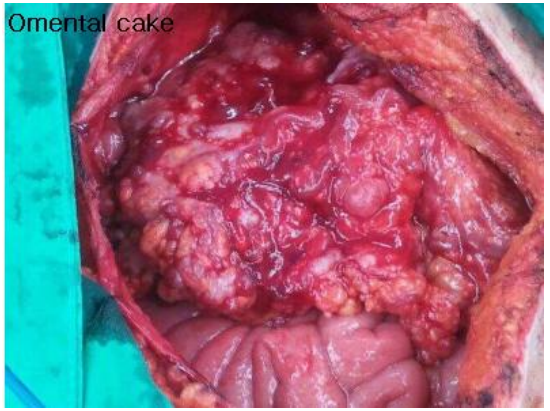


PDX OVCA-03

- **Serous ca, G 2, Stage IIIC**
- **Platinum-resistant group**

Mouse Model # OV-03





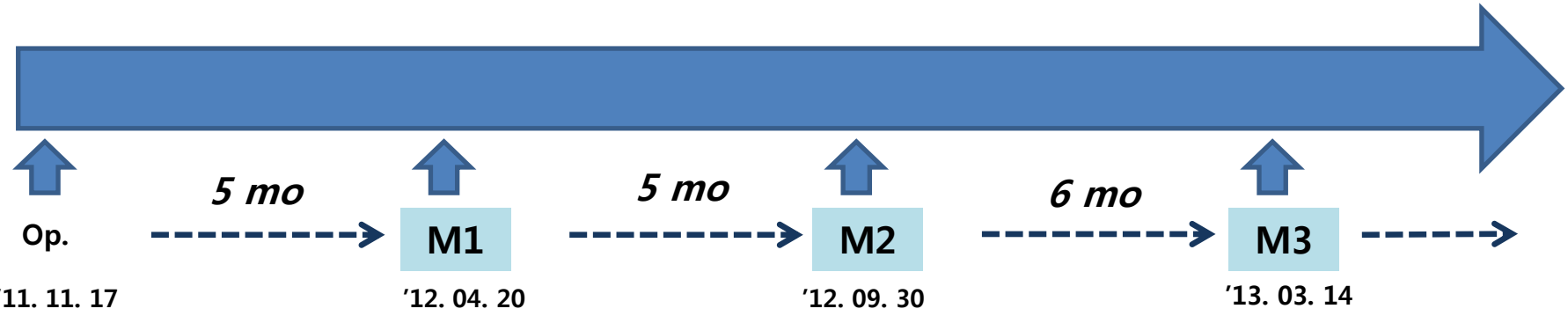
PDS

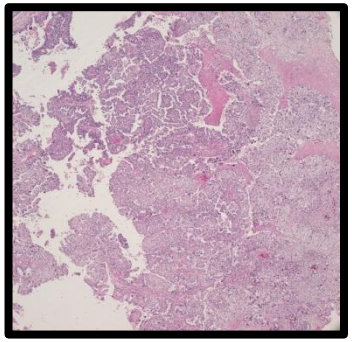
Recur < 6 mo

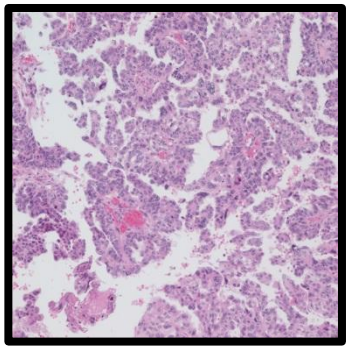
1st line CTx.
(Paclitaxel-carboplatin # 1 - 6)

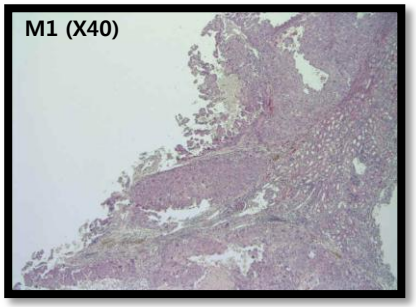
CR

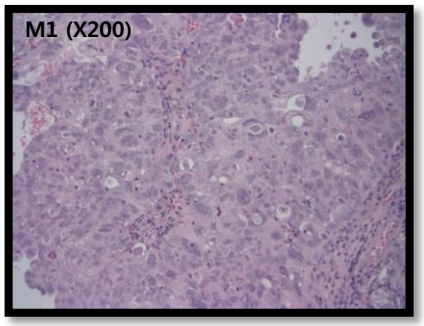
2nd line CTx.
(Docetaxel-carboplaitn #1 – 6)

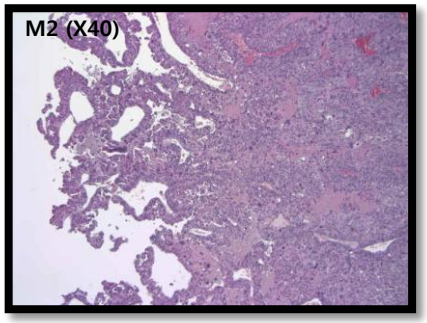

Op.
5 mo
M1
5 mo
M2
6 mo
M3
'11. 11. 17
'12. 04. 20
'12. 09. 30
'13. 03. 14

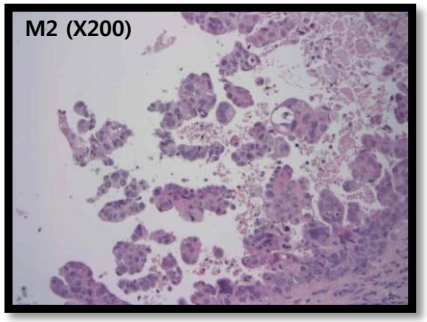


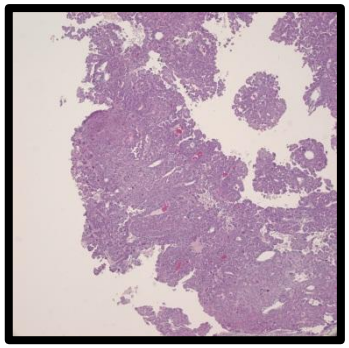


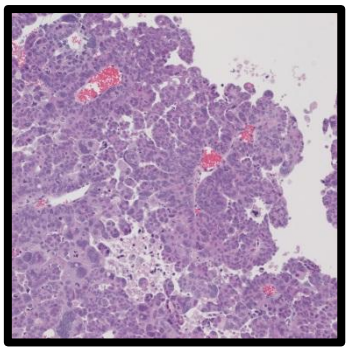

M1 (X40)

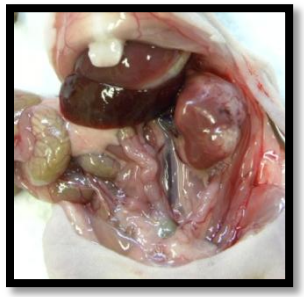

M1 (X200)


M2 (X40)


M2 (X200)



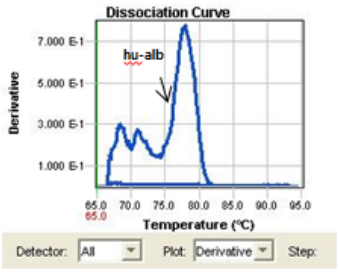




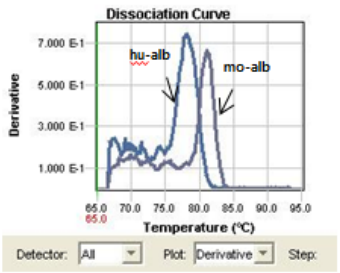
OV-3

Human Mouse Albumin Test : **OK**
STR Genotyping : **OK** (동일환자 pair로 확인)

Human_Mouse Albumin Test



OV-3
Human DNA

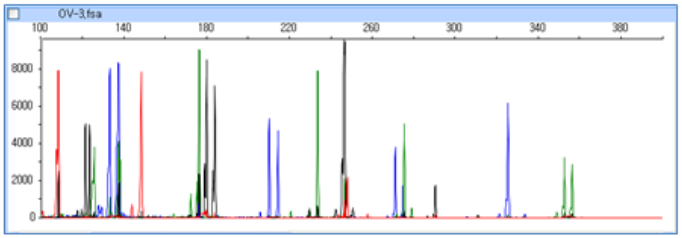


OV-3-S1
Human DNA + Mouse DNA

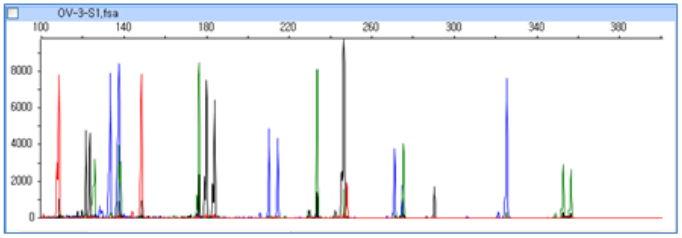
	Human ALB Ct Mean	Mouse ALB Ct Mean
OV-3	23.5	N/A
OV-3-S1	23.1	17.5

Pass

STR Genotyping



OV-3

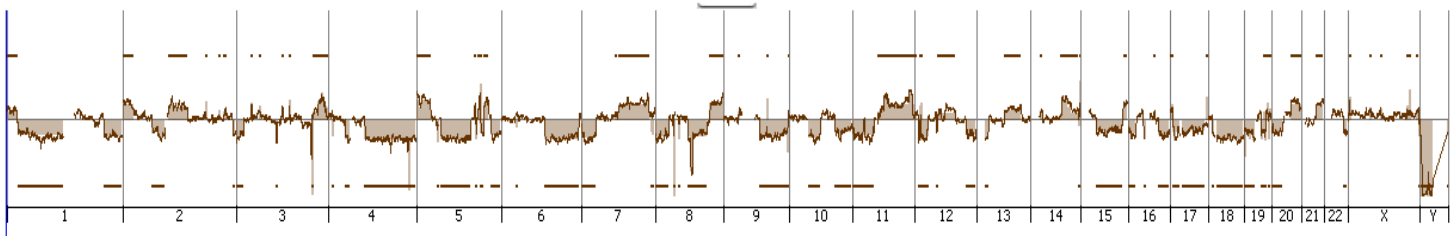


OV-3-S1

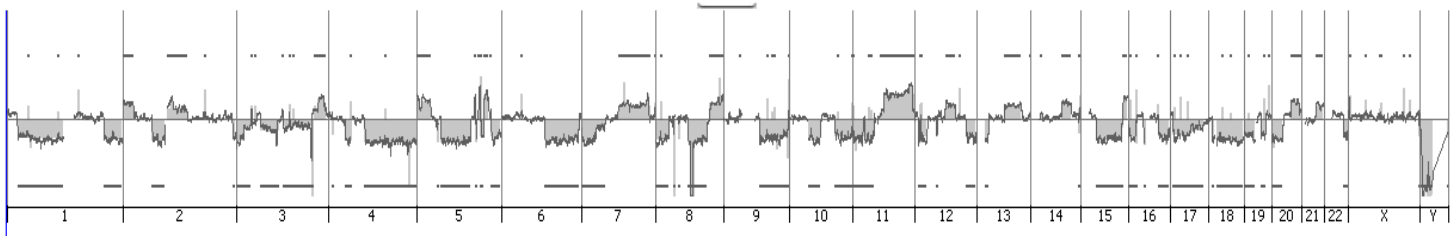
Locus	Chromosome Location	OV-3	OV-3-S1
D8S1179	8	10, 11	10, 11
D21S11	21q11.2-q21	30, 31	30, 31
D7S820	7q11.21-22	9, 10	9, 10
CSF1PO	5q33.3-34	11	11
D3S1358	3p	15, 18	15, 18
TH01	11p15.5	7	7
D13S317	13q22-31	11	11
D16S539	16q24-qter	10	10
D2S1338	2q35-37.1	26, 27	26, 27
D19S433	19q12-13.1	14, 14.2	14, 14.2
vWA	12p12-pter	17, 18	17, 18
TPOX	2p23-2per	11	11
D18S51	18q21.3	13	13
X	X:p22.1-22.3 Y: p11.2	X	X
D5S818	5q21-31	10	10
FGA	4q28	23	23

Pass

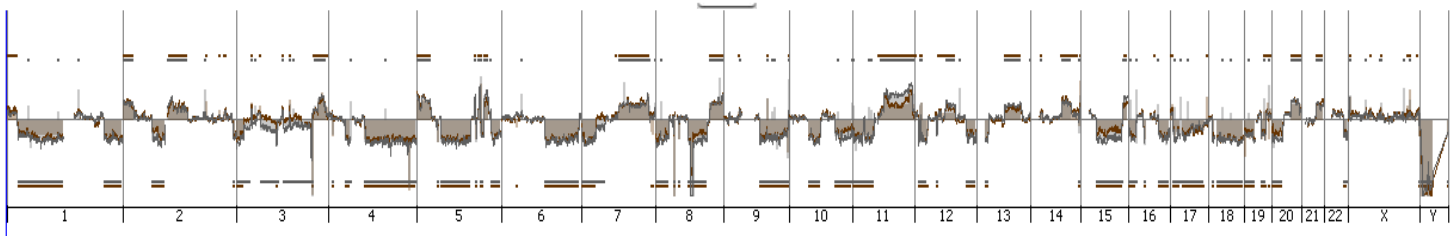
OV-3 **Patho:** Serous papillary adenocarcinoma



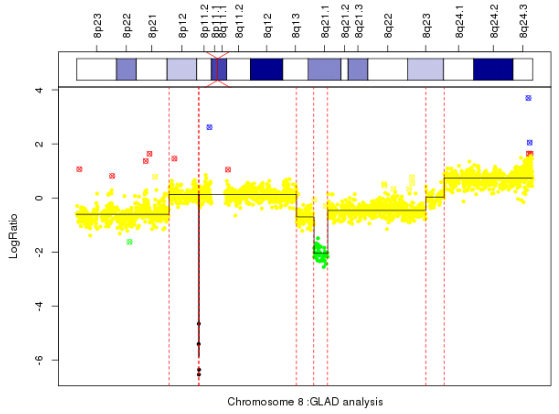
OV-3
Female
Tumor Content : 80%



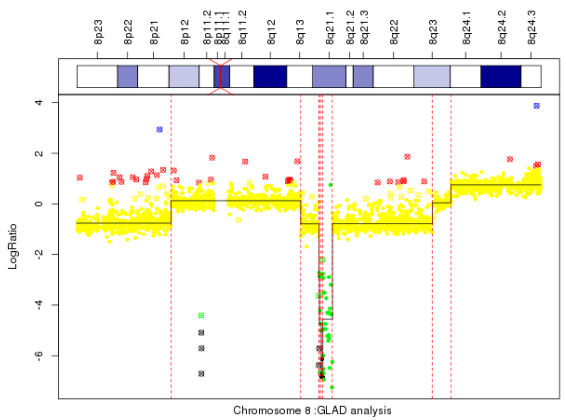
OV-3-S1
Female
Tumor Content : 80%



Merge



OV3



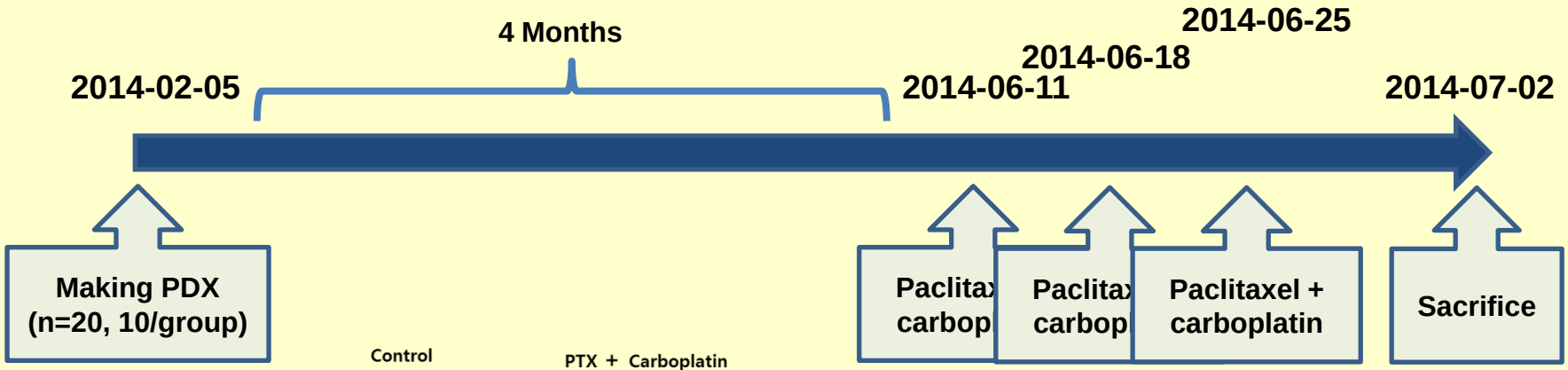
OV3-M1

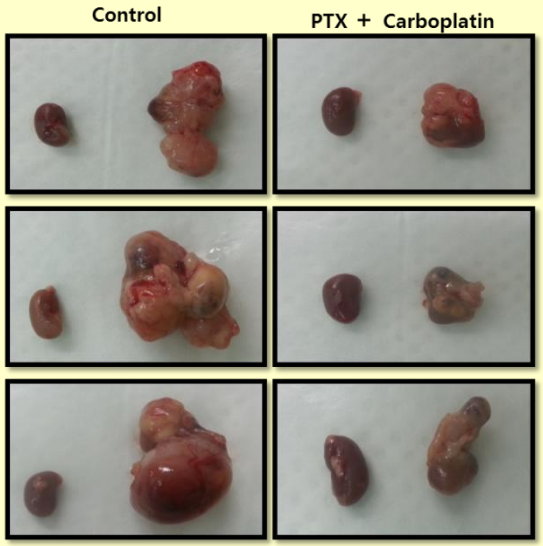
Pass

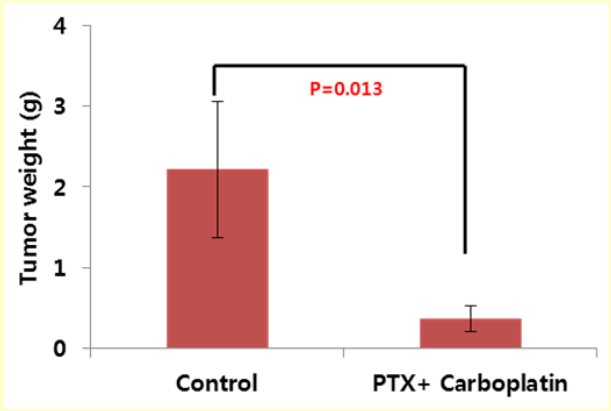
8q21.1 deletion

Paclitaxel-carboplatin combination therapy in PDX OVCA Model

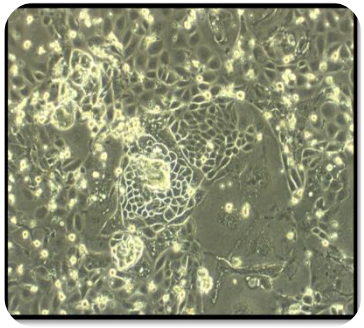
- Sample: **OV-41-M3 (Serous AC, G3), platinum-sensitive case**
- Drug: **Taxol® (6mg/kg) + Neoplatin® (8mg/kg),** once a week for 3 weeks



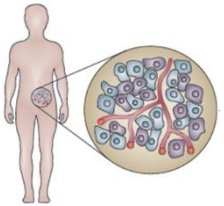




Platform of Experimental Therapeutics (SMC)



PDX (AVATAR)



In vitro

- Survival
- Proliferation
- Apoptosis
- Invasion
- Migration
- etc

In vivo with cell lines

- Orthotopic, Heterotopic
- Drug-sensitive: HeyA8, SKOV3ip1, A2780
- Drug-resistant: HeyA8-MDR, SKOV3-TR, A2780-CP20
- Clear cell ca: RMG1, ES2

PDX testing

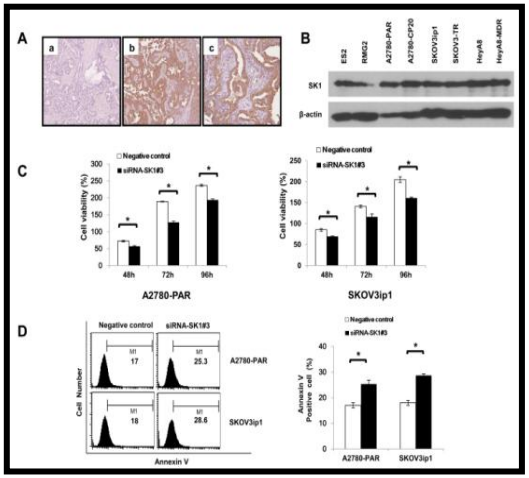
- Subrenal implant
- Histology
- Cancer type

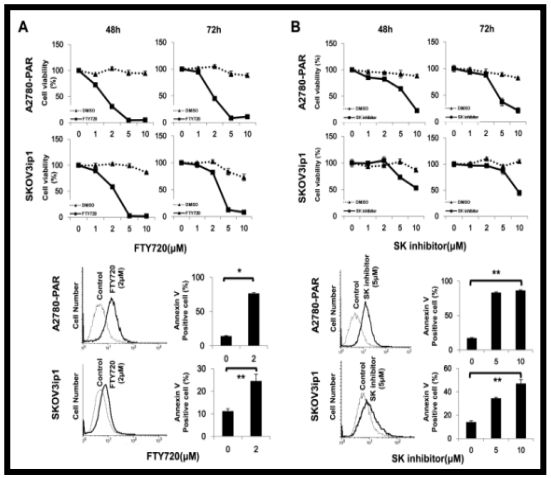
In revision, Int. J. Cancer

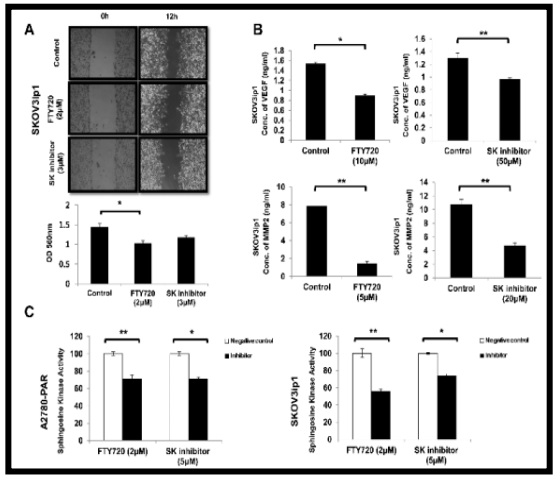
Sphingosine Kinase 1 Inhibitor (FTY720) as Novel Target Agent in EOC

Protein expression, Survival, Proliferation, Apoptosis, Invasion, Migration, etc

In vitro

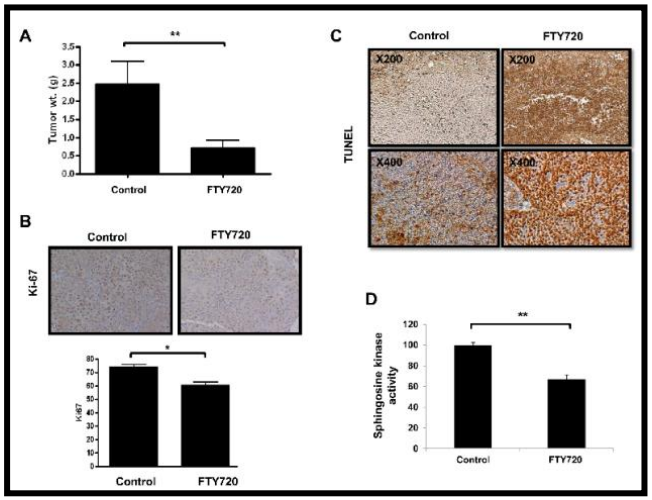




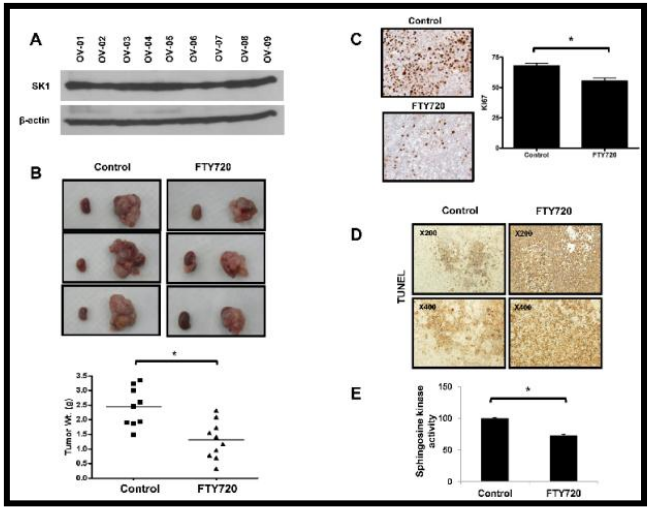


In vivo

Cell line model - orthotopic



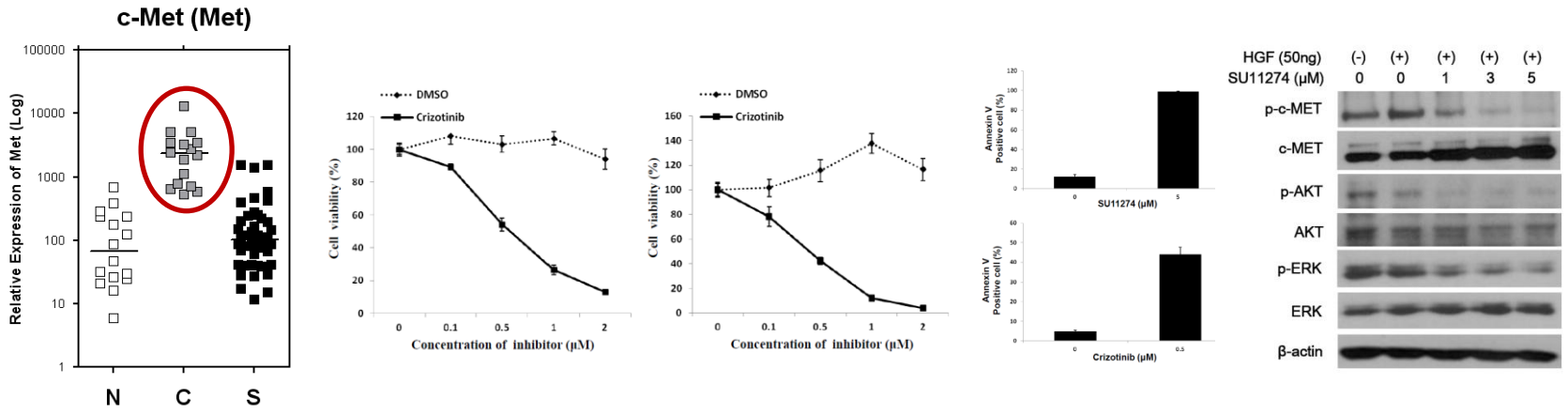
PDX model - subrenal



C-met inhibitor (SU11274) as Target Agents in OCCC

Protein expression, Survival, Proliferation, Apoptosis, Invasion, Migration, etc

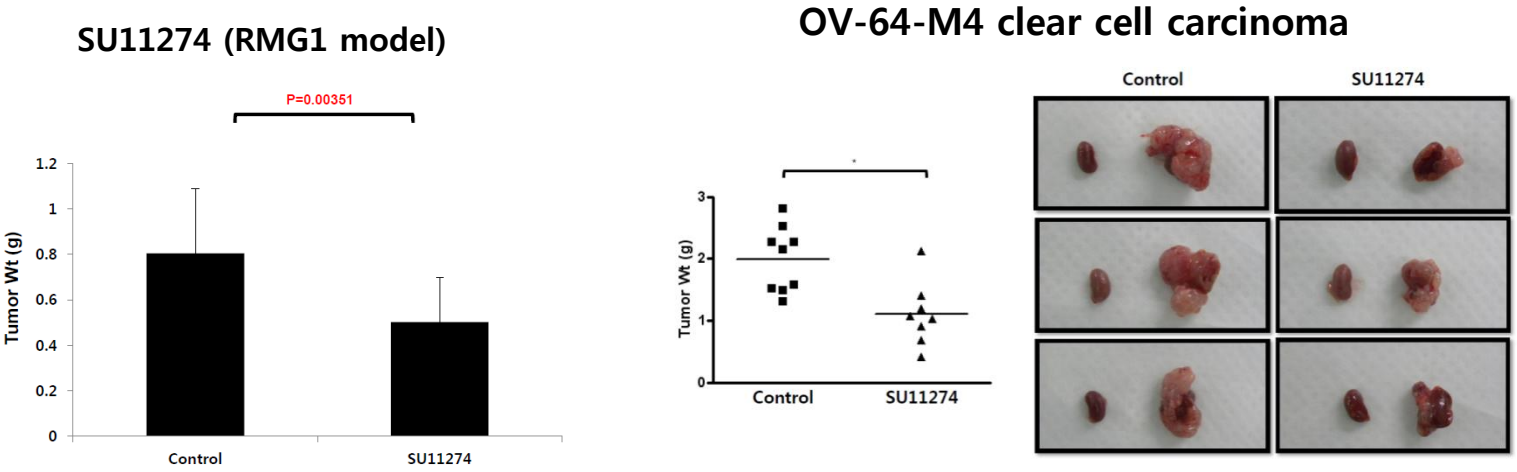
In vitro



Cell line model - orthotopic

PDX model - subrenal

In vivo

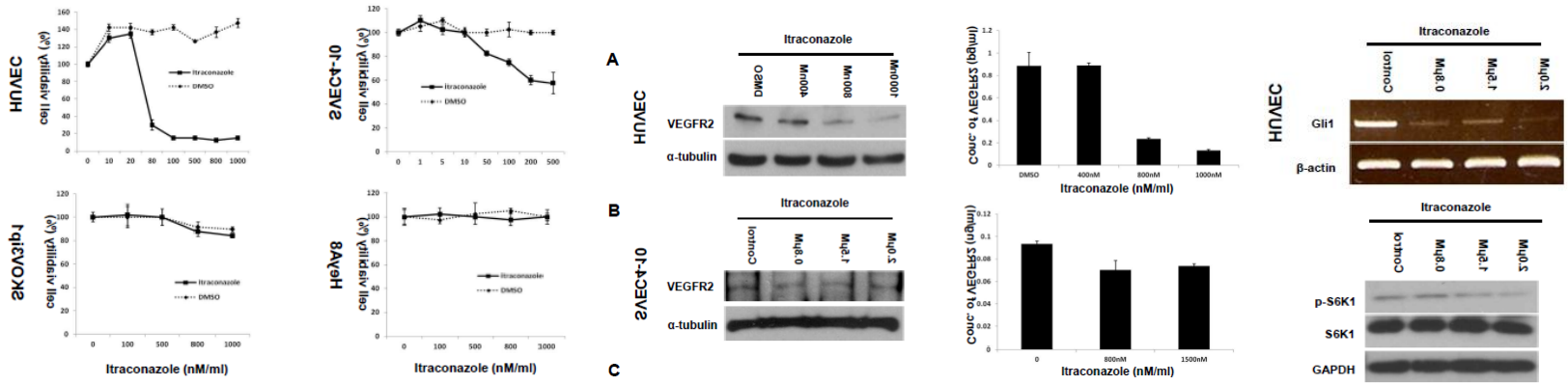


Unpublished data

Itraconazole as anti-angiogenic agent in EOC

Protein expression, Survival, Proliferation, Apoptosis, Invasion, Migration, etc

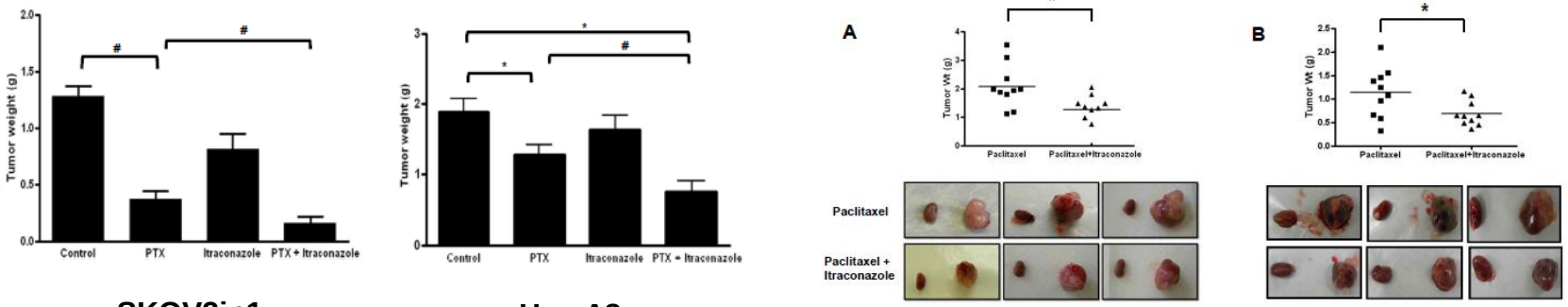
In vitro



Cell line model - orthotopic

PDX model - subrenal

In vivo



SKOV3ip1

Hey-A8

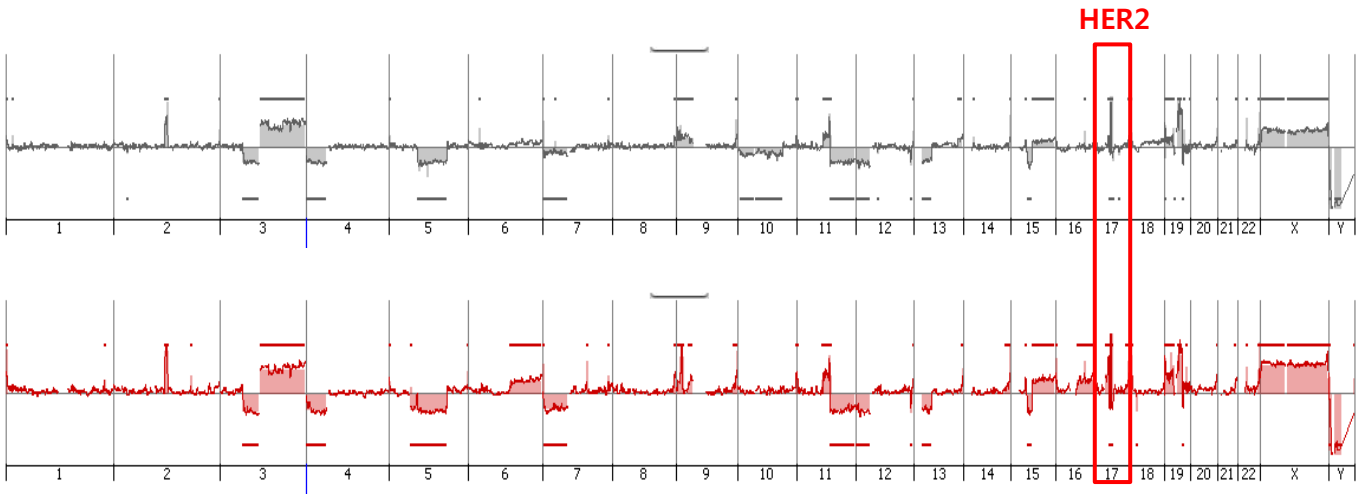
OV-22-M5, MMMT

OV-20-M3, Serous AC, G3

Unpublished data

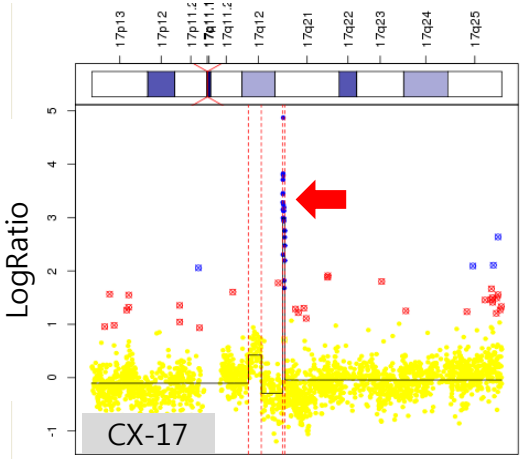
PDX (Avatar) Models of Cervical Cancer

HER2 amplification in CX-17 human & PDX tumor (aCGH)

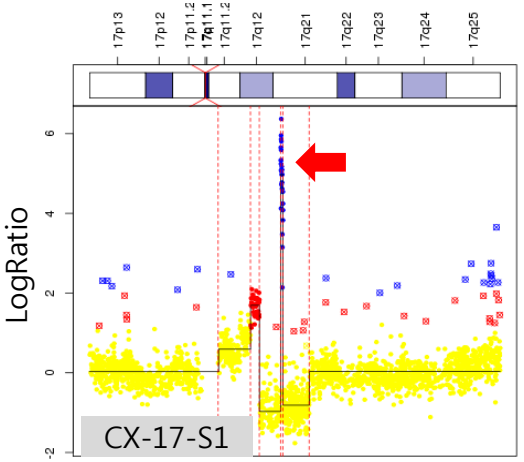


CX-17
Tumor Content : 90%

CX-17-S1
Tumor Content : 80%



Chromosome 17

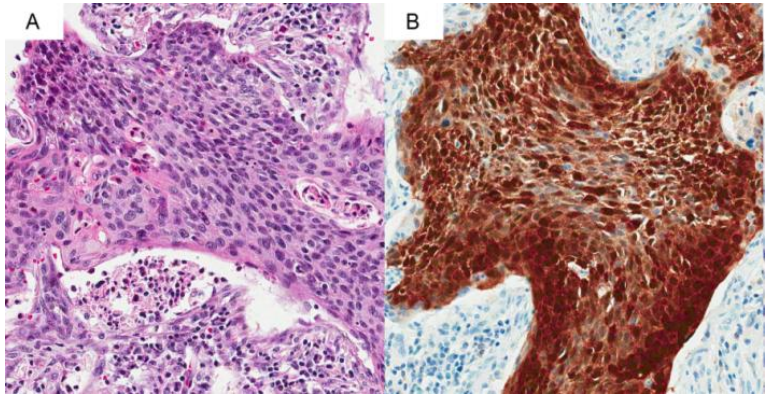


Chromosome 17

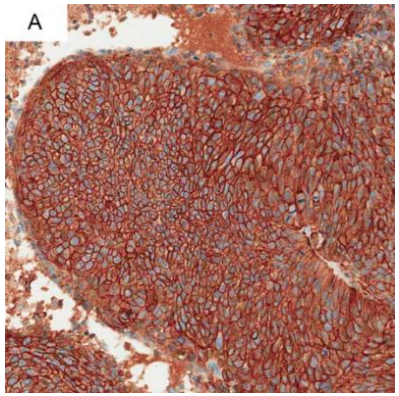
	HER2	ALB	HER2/ALB
Sample	Ct mean	Ct mean	Copy Number
Normal Blood	24.57	24.77	1.00
SKBR3	21.36	25.10	11.73
BT20	25.37	25.76	1.15
CX-17	19.38	22.97	10.56
CX-17-S1	17.62	23.37	47.01

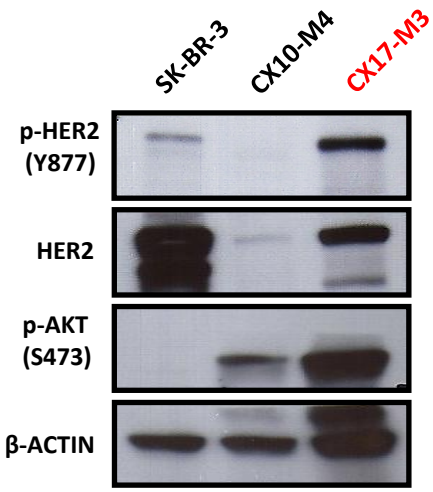
HER2 amplification in CX-17 human & PDX tumor

Patient's histology



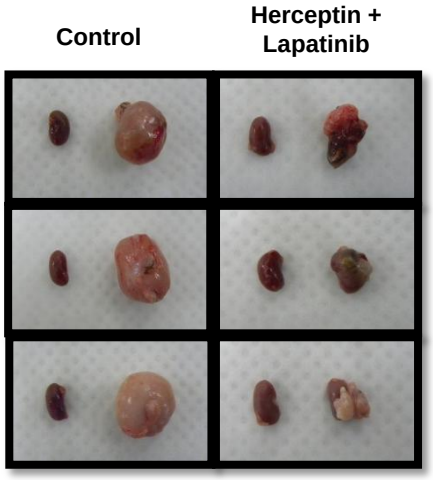
PDX's histology

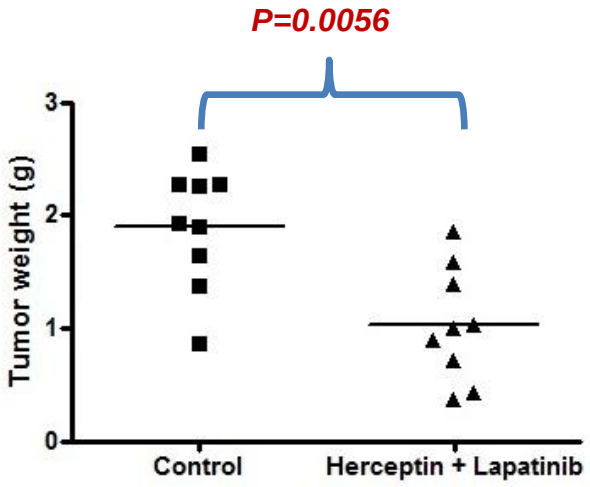




***PDX testing*:**

The effects of Herceptin & Lapatinib on tumor growth in PDX (CX-17-M4)





Limitation of PDX model

- The requirement to use **immunosuppressed mice**.
- **Heterotopic** → Orthotopic ?
- Tumor graft **latency** (4-6 Mo)
- **Engraftment rates** → 45-50% depending on the tumor type
- Cost and labor intensive ?

Summary

- PDX models may be **superior** to cell line xenograft because of maintaining similarities for pathologic and genetic features to the parental tumors.

- **Genomic characterization** with exome sequencing or NGS

- Focus to **refractory & recurrent cancer.**

- PDX models offer **a powerful tool** for studying **tumor biology** & **good platform** to provide **personalized medicine**

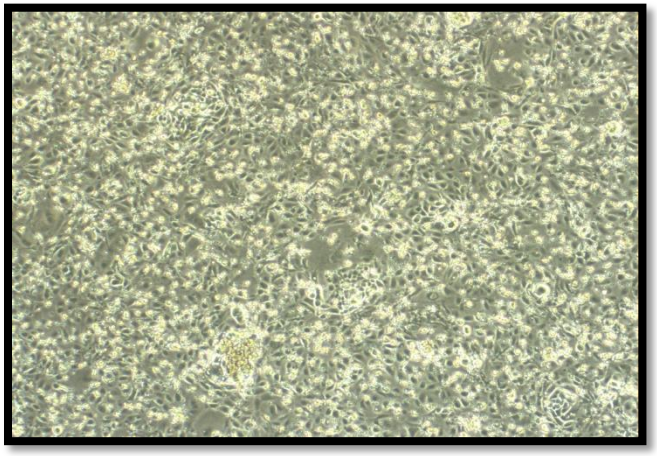
Thank you for your attention

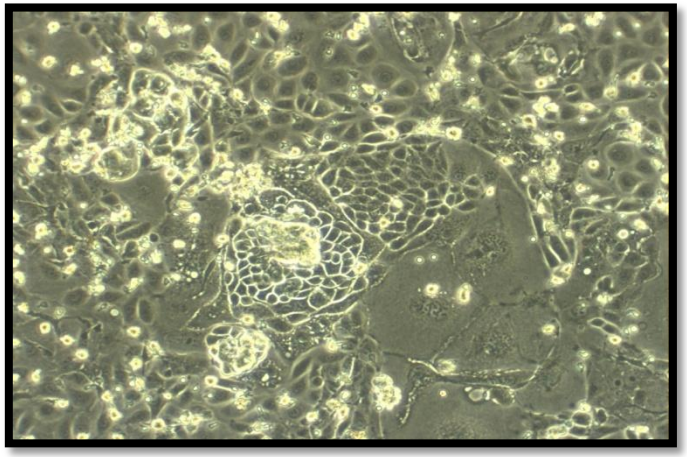
Translational Research Team of Gynecologic Cancer, SMC

Pilot Study for the development of PDX (OV CA)

Samples	Method	Tumorigenesis
1. Ascites cultured cells (6 cases)	Intraperitoneal injection	1 case
2. Primary ovarian cancer tissues (8 cases)		0

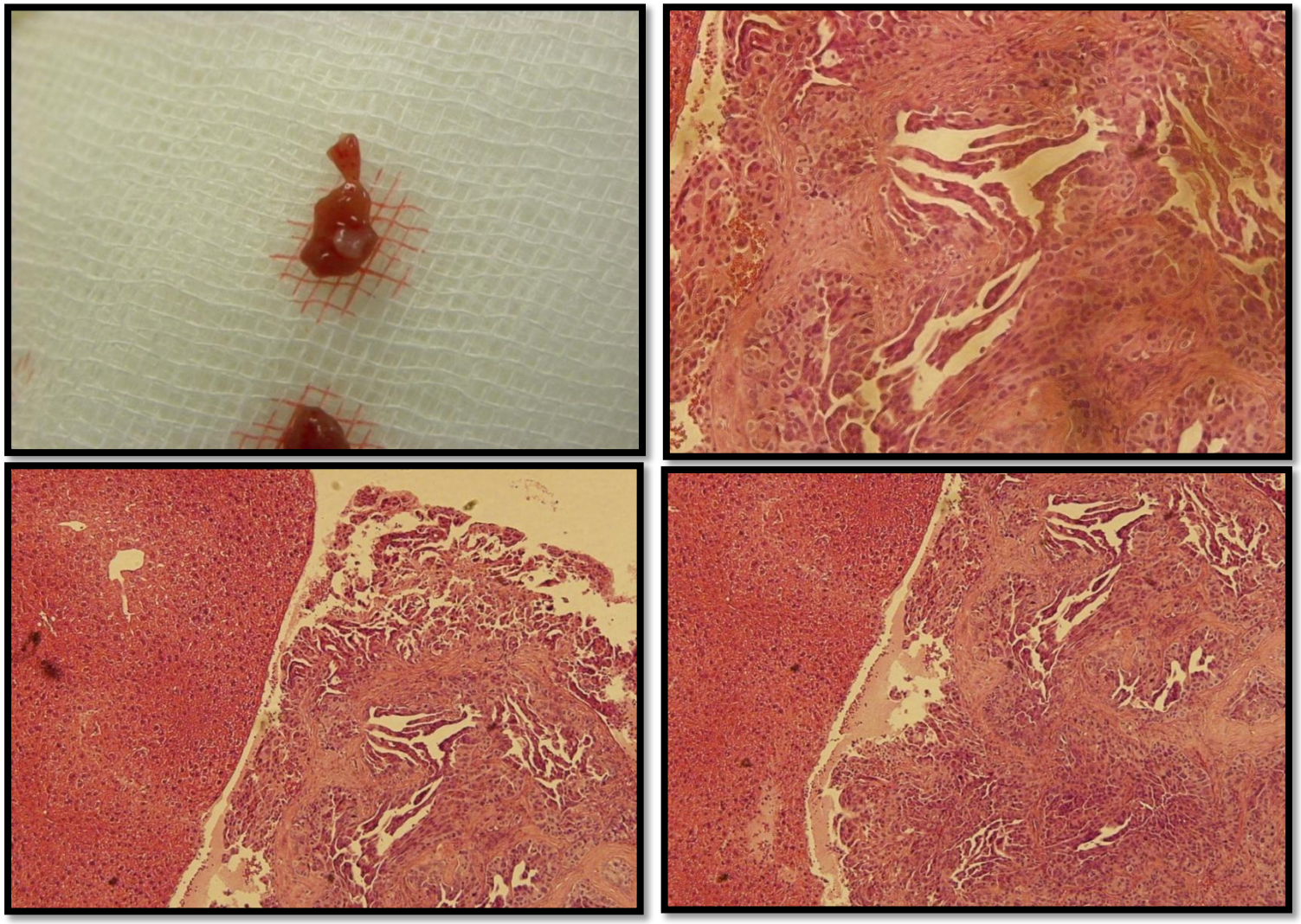
Ascites cell (cultured, passage 0)





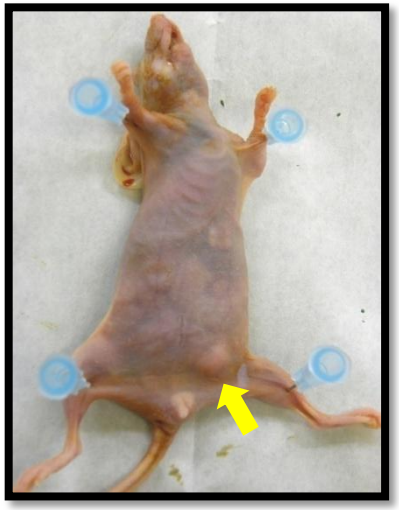
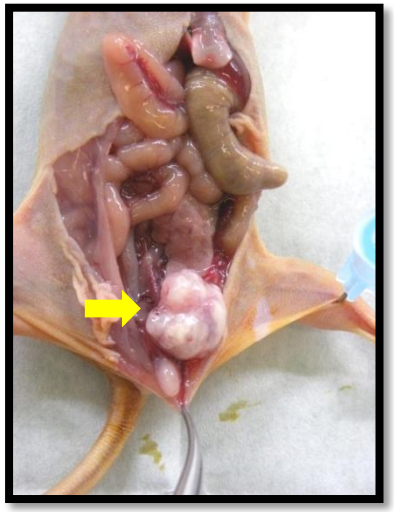
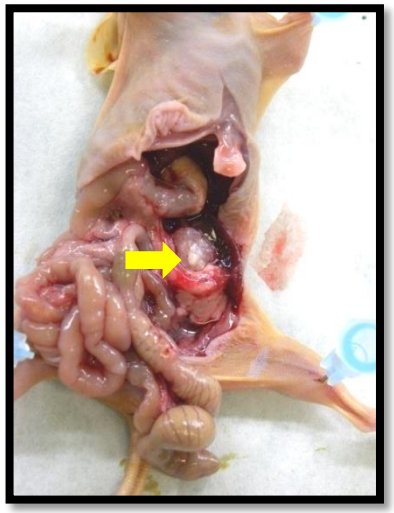
Tumor development from patients-derived OV CA ascites cells (IP injection)

1st Sacrifice (2010.11.4, 143days) : mouse #1



Tumor development from patients-derived OV CA ascites cells (IP injection)

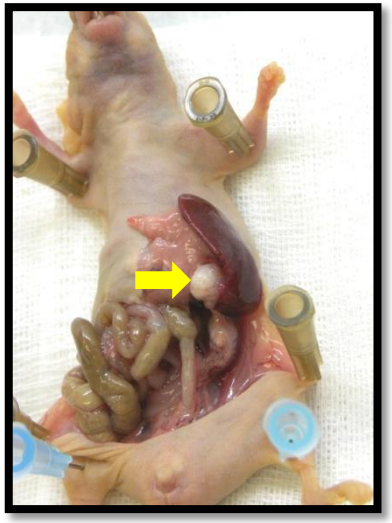
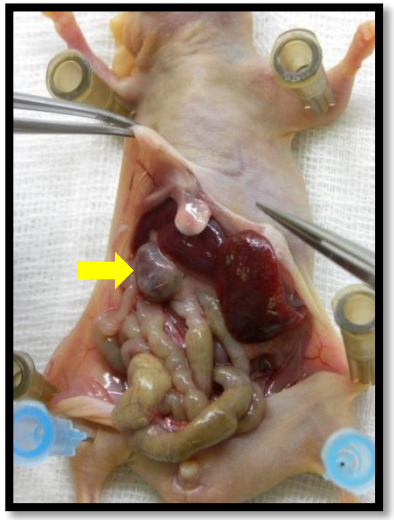
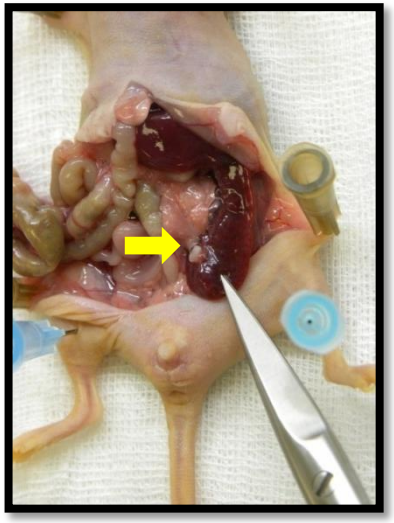
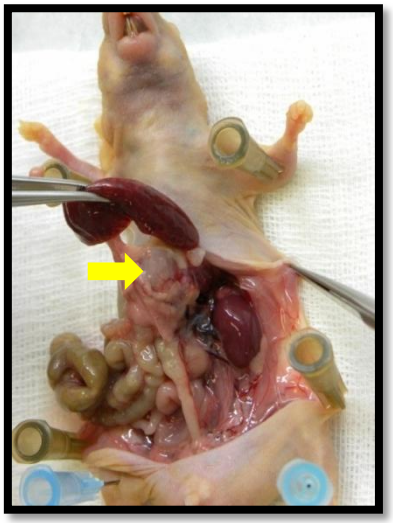
2nd Sacrifice (2011.1.25, 224 days) **: 2 mice**

Mouse#2
liver, spleen,
pelvis, mesentery

Mouse#3
liver, spleen,
pancreas, omentum

Take 7-8 month

PDX Therapy: C-MET Inhibitor in Ovarian CCC

- PDX Nol : OV-64-M4
- Cell type : Clear cell carcinoma