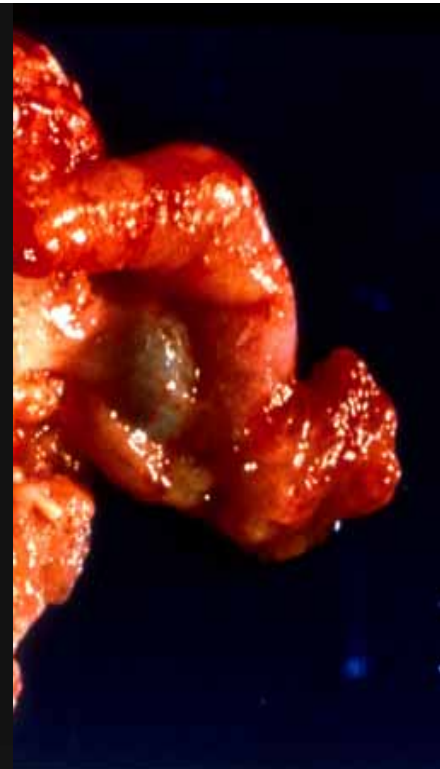
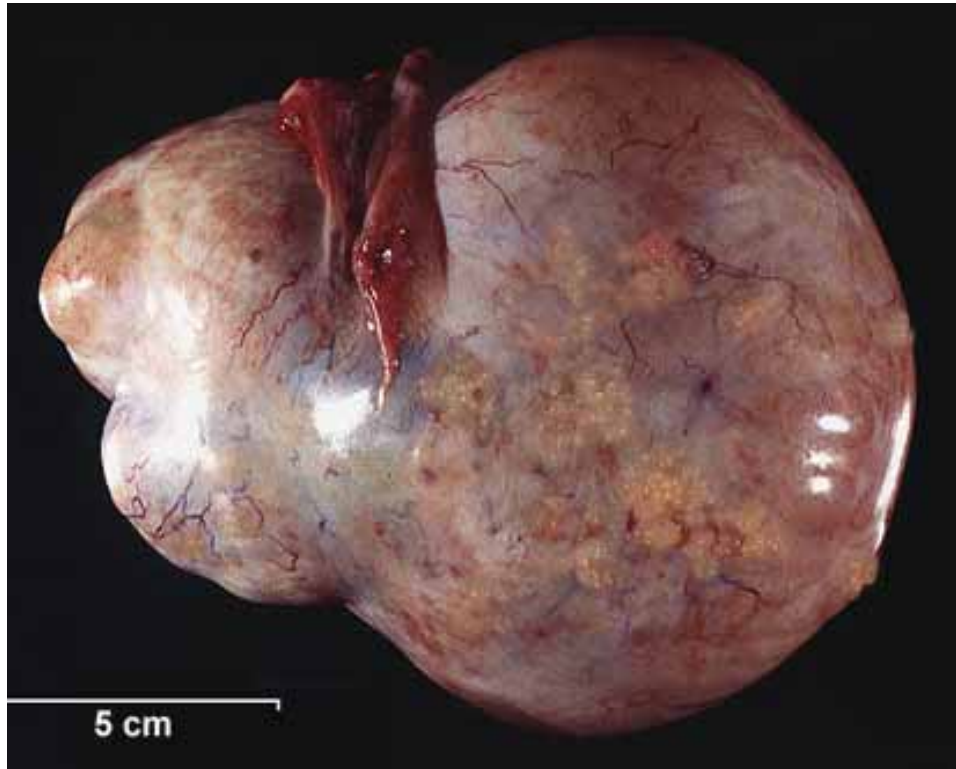


Using Mouse Models to Study Ovarian Tumor Biology and Therapy

Denise C. Connolly, PhD



Epithelial Ovarian Cancer (EOC): The clinical problem

- ~22,000 new cases diagnosed annually
 - 8th most common cancer among women in the US
- >15,000 deaths annually
 - 5th most common cause of cancer death
 - Most common cause of death from gynecologic malignancy
- Survival rate at early diagnosis: > 90%
- 75% diagnosed at late stage: ~30-40% survival past 5 years
- 80% respond to chemotherapy
- Vast majority of patients relapse and develop drug resistant disease

Histogenesis of ovarian neoplasms

- Epithelium – (85-90%)

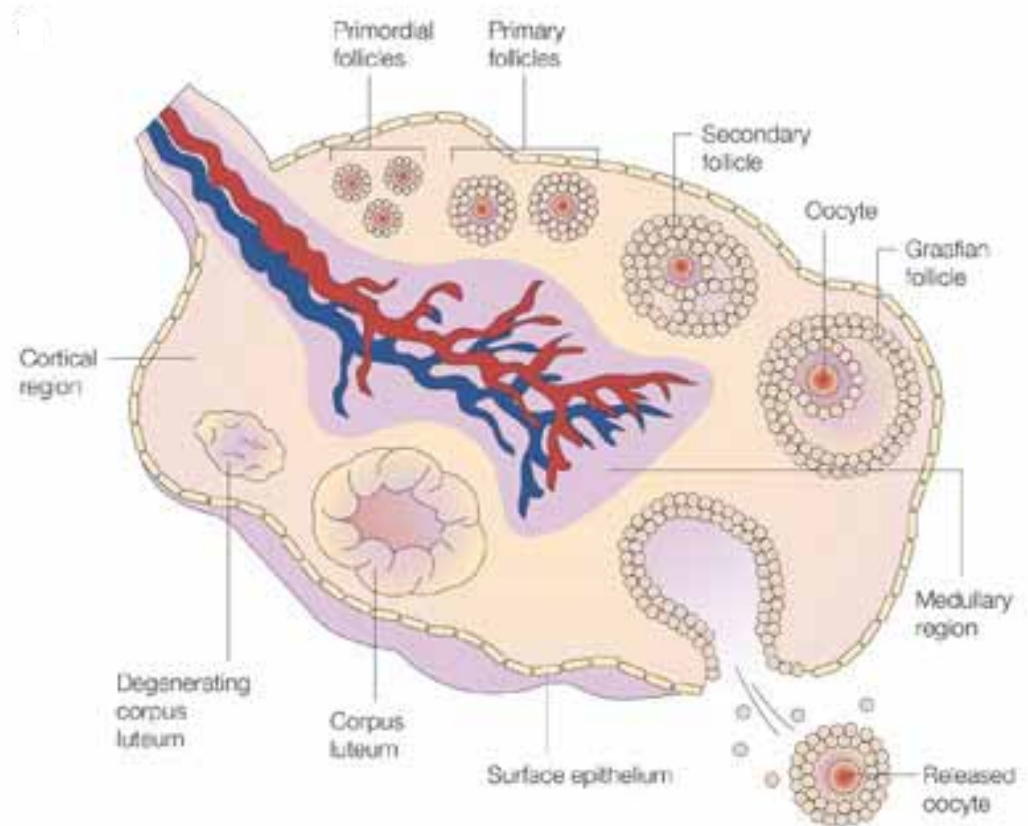
- ovarian carcinomas:
serous, endometrioid,
mucinous, clear cell

- Follicles, cortical stroma, hilum

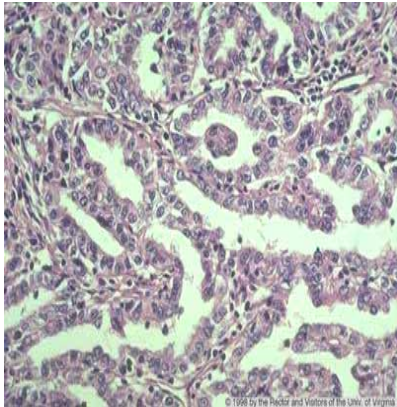
- sex cord stromal tumors:
granulosa, Sertoli, Leydig,
theca

- Oocytes

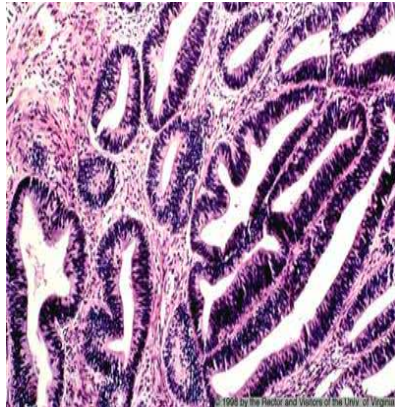
- germ cell tumors:
dysgerminoma, teratoma,
embryonal CA



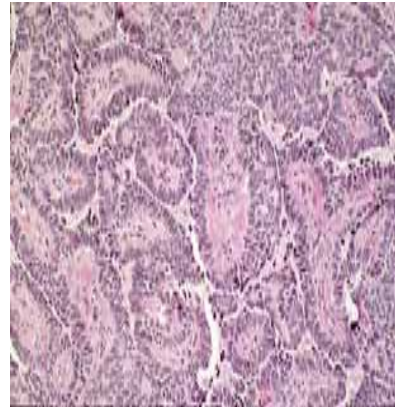
Histologic subtypes: Invasive carcinomas



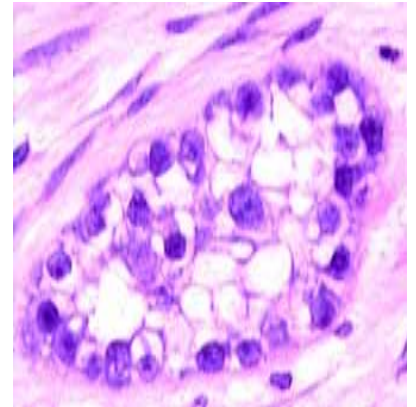
serous
(51%)



endometrioid
(17%)

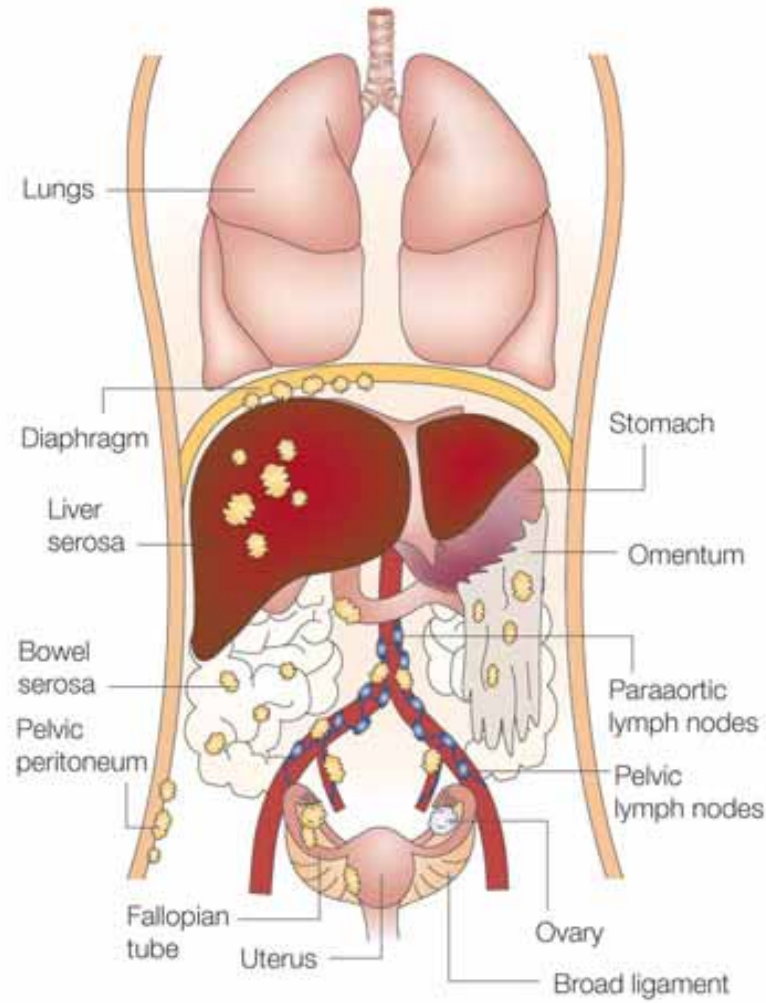


mucinous
(11%)

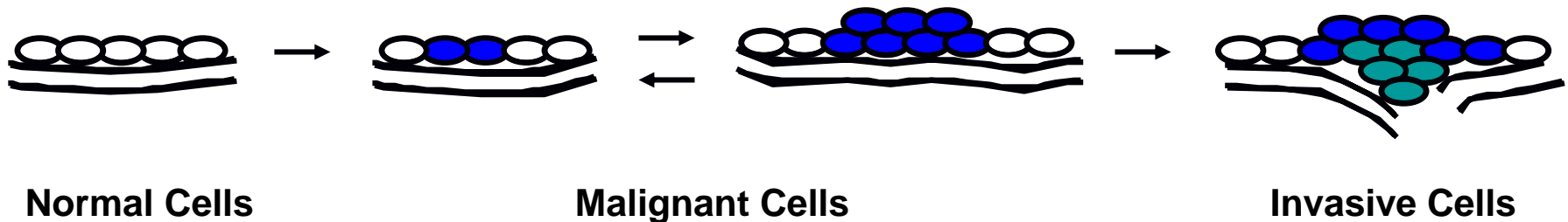


clear cell
(11%)

Spread of ovarian cancer



Multistage progression of ovarian carcinoma?

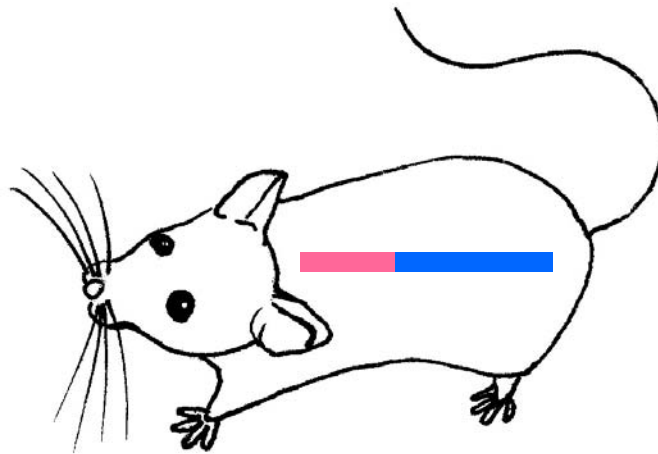


Controversies and unknowns:

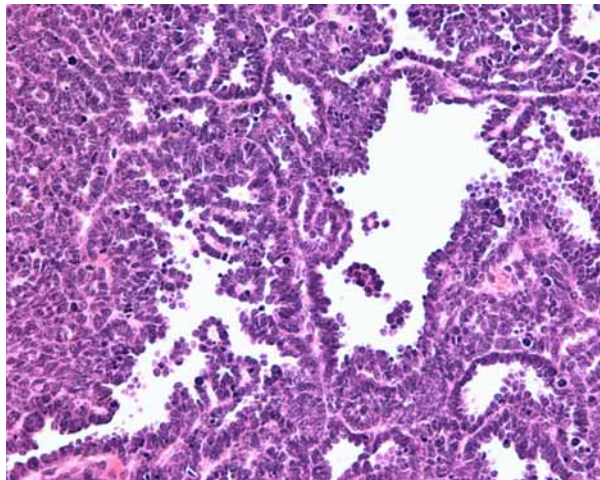
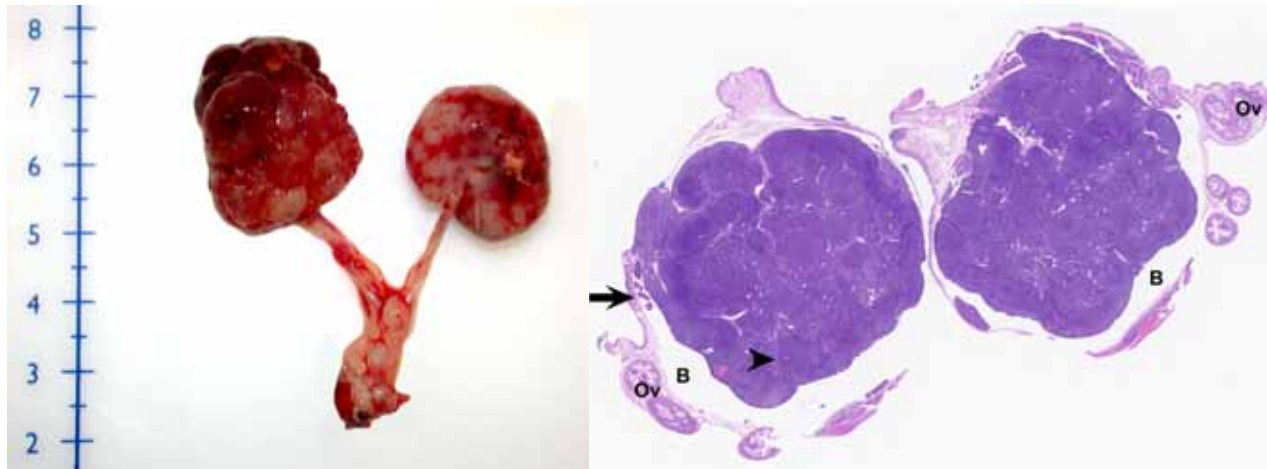
- Epithelial precursor
- Early tumor lesions
- Tumor initiating pathways
- Tumor progression and maintenance pathways
- Mechanisms of tumor invasion and metastasis

Tg*MISIIR*-TAg transgenic mice

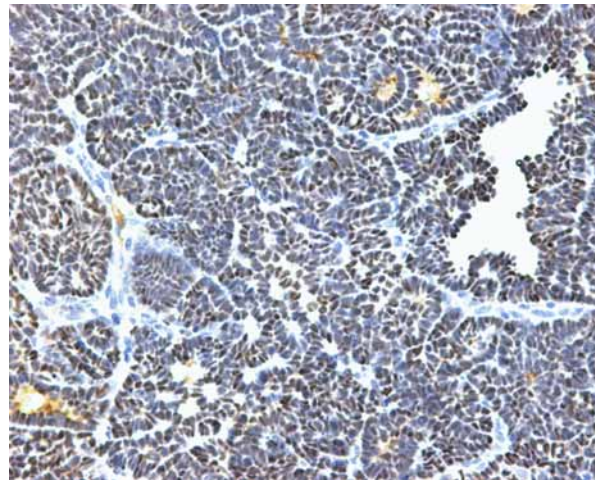
- 5' upstream regulatory region of the murine Müllerian inhibiting substance type II receptor (*MISIIR*) gene PCR cloned and fused to the early region of SV40 including the large and small T antigen genes



Bilateral ovarian tumors in TgMISIR-TAg transgenic mice



H&E



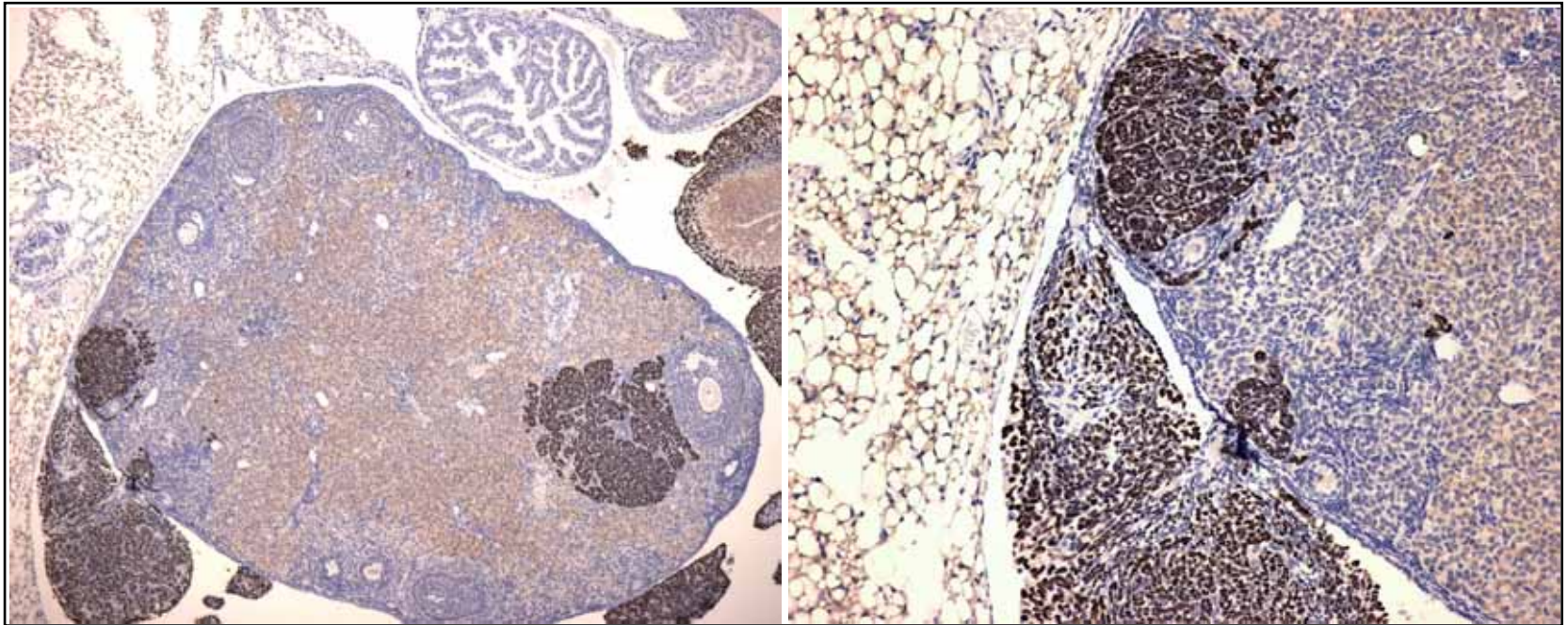
TAg



Similarities between TgMISIIR-TAg ovarian tumors and human EOC

- Disease presentation
 - Spontaneous
 - Asymptomatic, advanced stage, peritoneal spread, ascites
- Histology:
 - Serous carcinoma
- Molecular features
 - Cytokeratin 8 and 19 expression
 - Expression of ER and responsive to E2 and SERMs
 - Muc16 (murine CA125)
 - pStat3
 - pSrc
 - pFak
 - pAkt2
 - Vegf
 - Cox-1
 - Claudin 3
 - Common genes/pathways altered in mouse and human ovarian cancers (cDNA arrays)
- Advantages
 - Stochastic autochthonous model
 - Immunocompetent
 - **MOVCAR cell lines derived from ascites- can be grown as allografts**

Tumor implants in ovary (MOVCAR cells in SCID mouse)



TgMISIR-TAg model of EOC as pre-clinical model

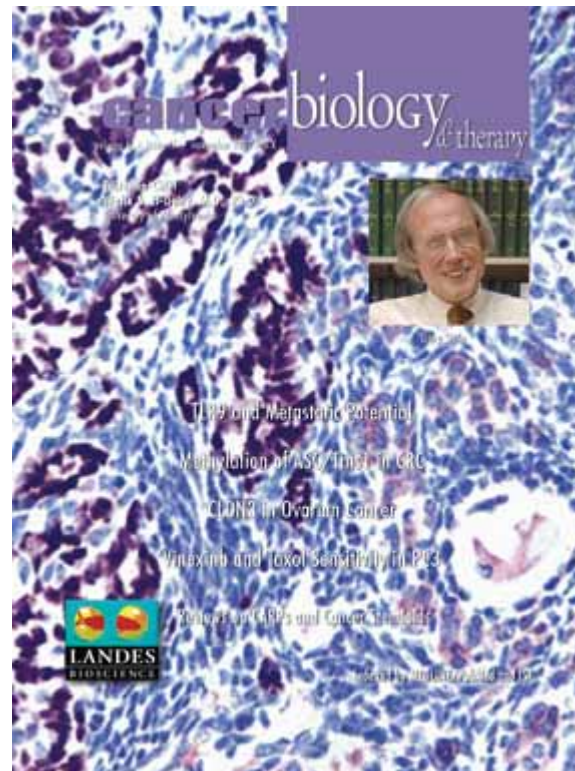
- Determine study group size based on statistical concerns
- Response assessment
 - Tumor latency/multiplicity/regression
 - Tumor growth rate
 - Metastasis
 - Survival
 - Histological changes
 - Changes in gene expression, protein biomarkers
- In vivo imaging to facilitate evaluation
 - Bioluminescence, fluorescence
 - microPET, CT
 - Ultrasound
 - MRI

In vivo Magnetic Resonance Imaging

Research Paper

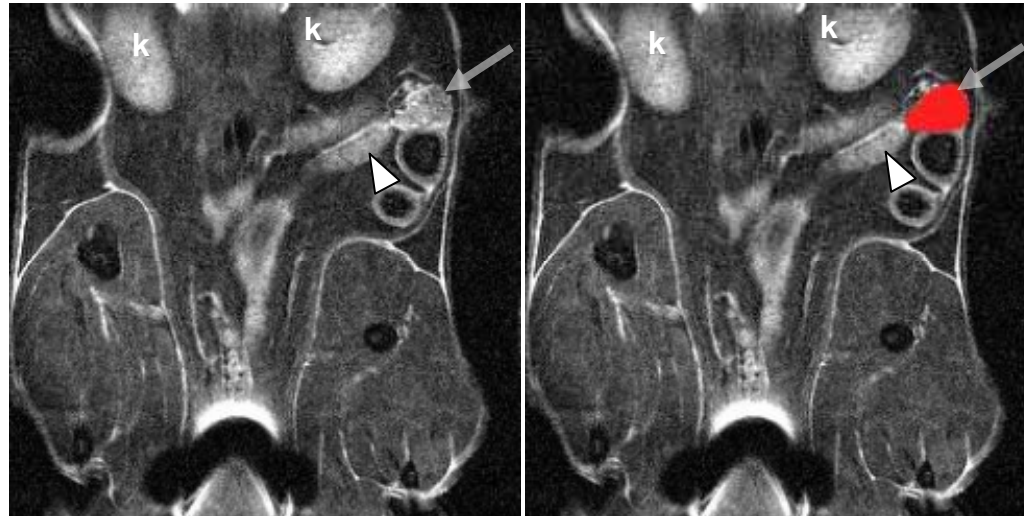
Magnetic Resonance Imaging for Detection and Determination of Tumor Volume in a Genetically Engineered Mouse Model of Ovarian Cancer

Harvey Hensley^{1,†}
Bridget A. Quinn^{1,†}
Ronald L. Wolf²
Samuel L. Litwin¹
Seiji Mabuchi¹
Stephen J. Williams¹
Christine Williams¹
Thomas C. Hamilton¹
Denise C. Connolly¹

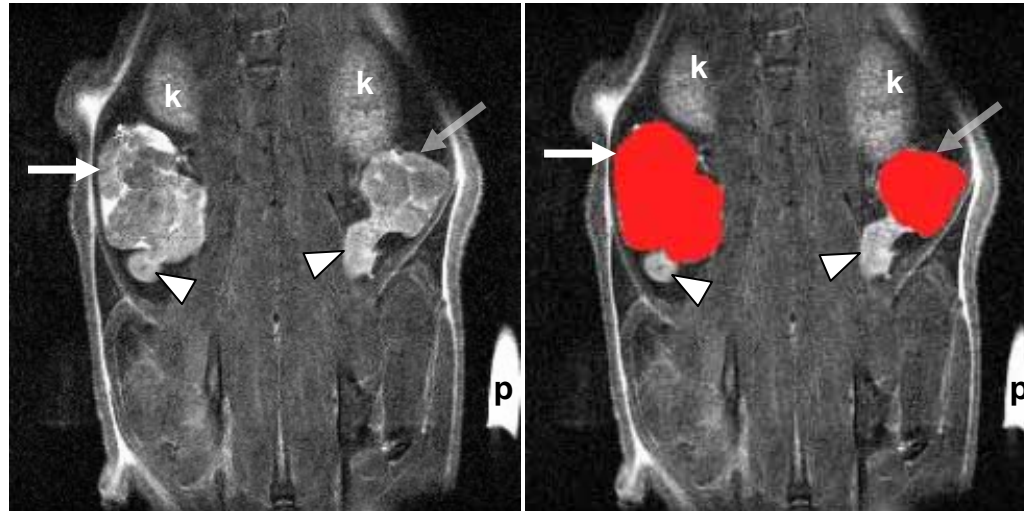


Detection of ovaries by MRI

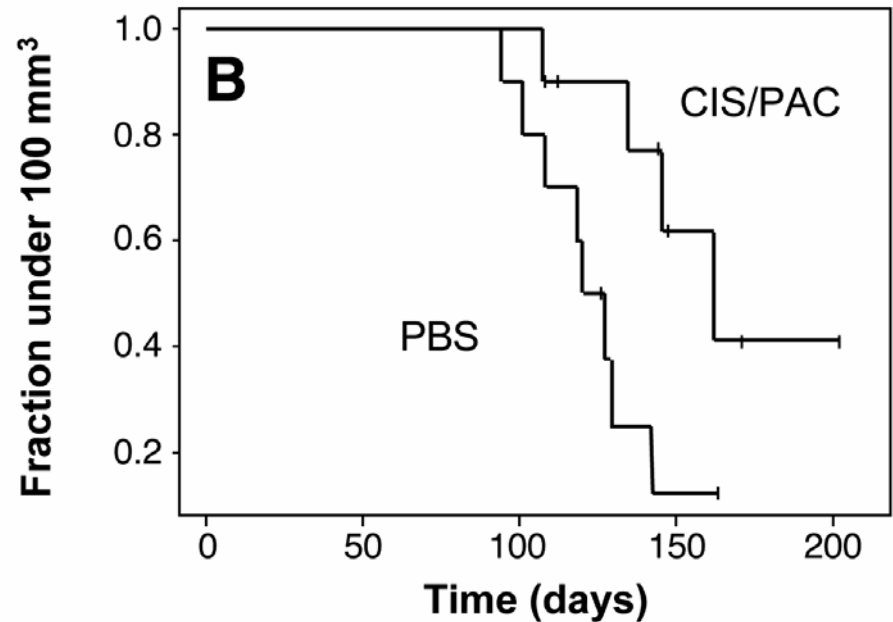
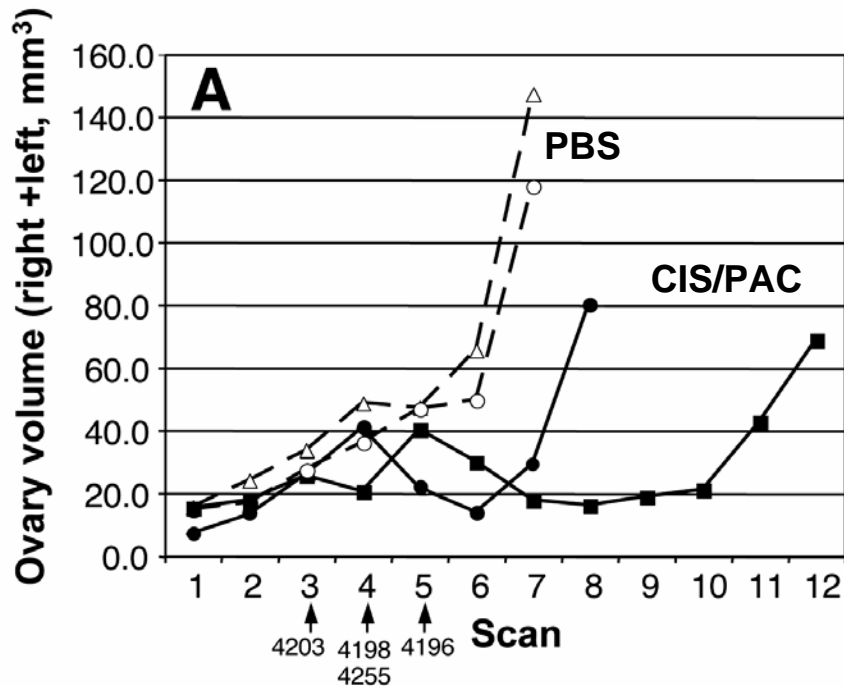
C57Bl/6



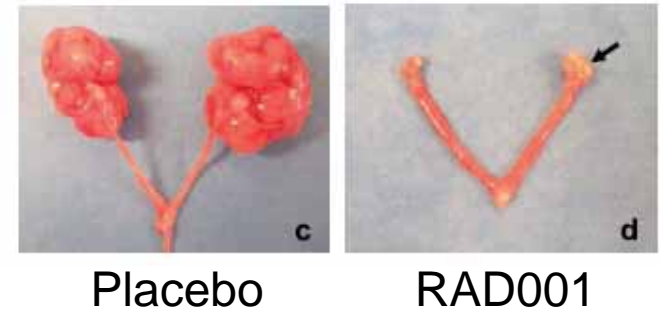
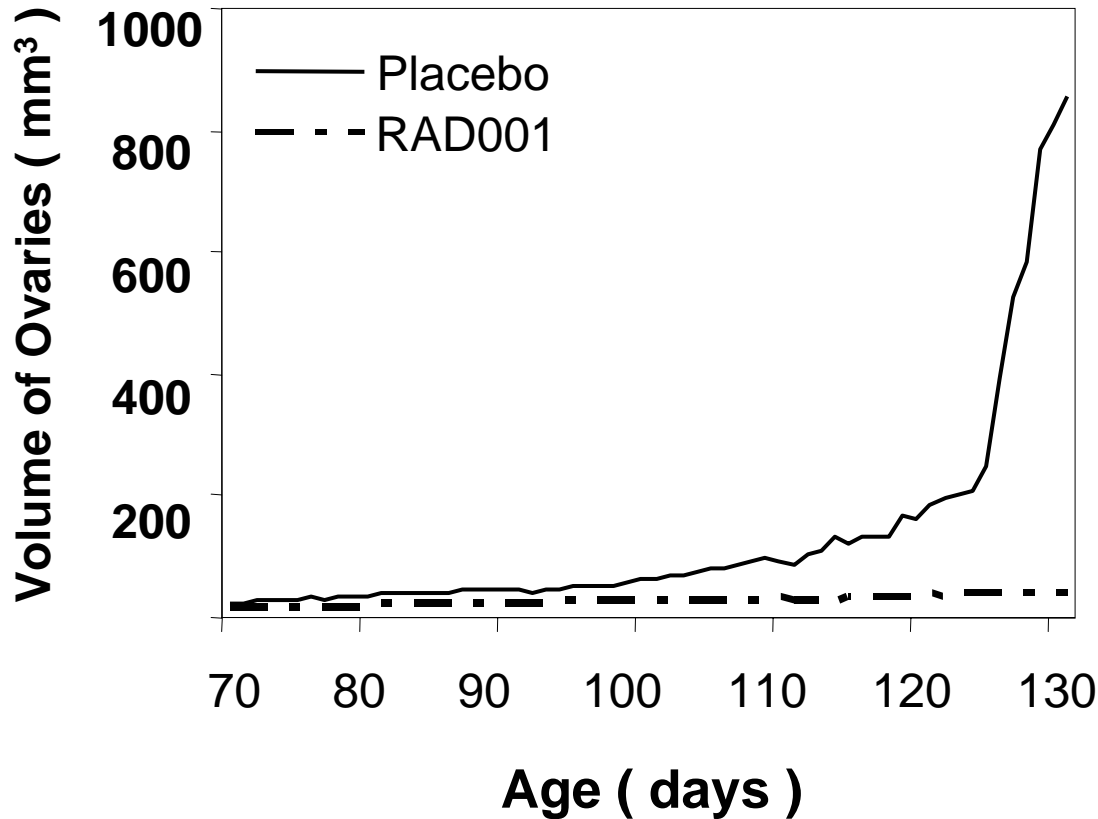
TgMISIIR-TAg



Tumor regression and prolonged survival in drug-treated *TgMISIR-TAg* mice



RAD001 treatment delays tumor progression in TgMISIIR-TAg mice



Clinical trial:
SWOG

Mabuchi et al., Cancer Research, 67:2408-2413, 2007. RAD001 (Everolimus) delays tumor onset and progression in a transgenic mouse model of ovarian cancer

TgMISIIR-TAg mice as pre-clinical model of EOC

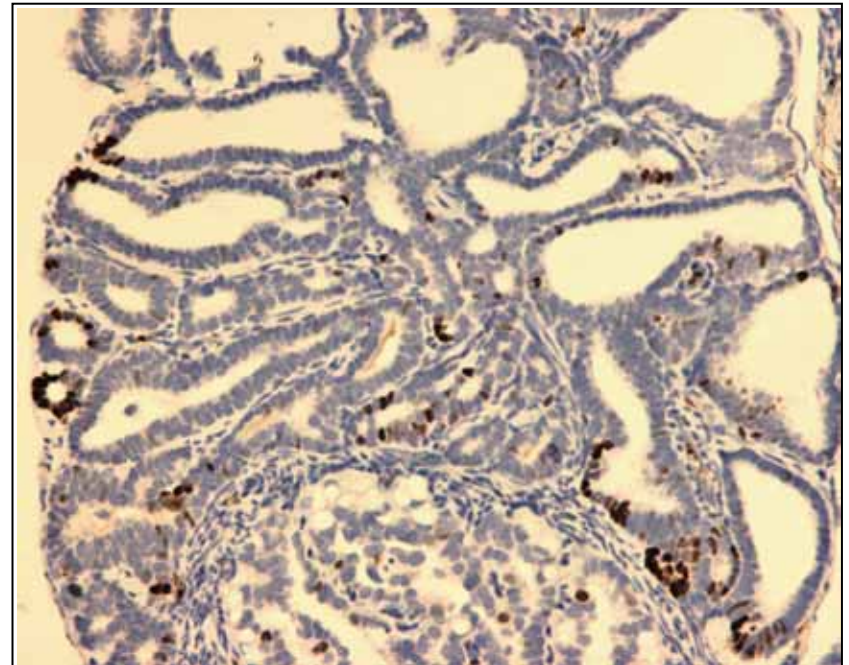
- Spontaneous disease
- 100% penetrance
- Quantitative analysis by MRI
 - Longitudinal measurement of tumor growth
 - Response to chemotherapy- stasis vs. regression
 - Imaging time ~20 min., 3 mice/hour
- Statistical analyses
 - n = 20 animals/arm to detect 25% difference with 80% power and 5% type I error
- Pre-clinical studies can inform clinical trial design
 - Example RAD001 (Everolimus)

Syngeneic model of EOC:
Allograft MOVCAR cells in TgMISIIR-
TAg-Low mice

Goal: Develop an *in vivo* model system
amenable to modulation of gene expression to
study proteins involved in EOC

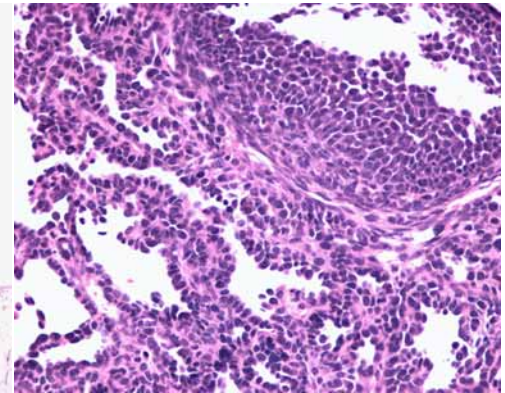
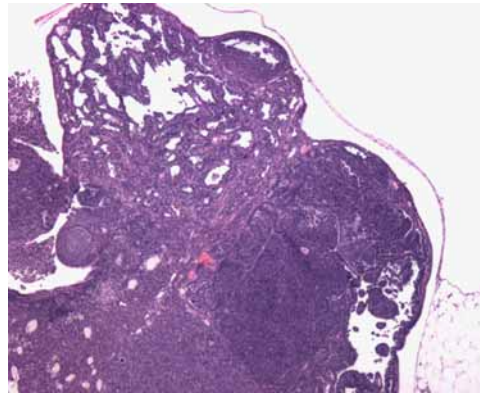
TgMISIIR-TAg-Low mice

- TAg expression in FT
- Mice **do not** develop tumors
- Pure C57Bl/6
(backcrossed >9 gen.)
- Syngeneic recipients
for MOVCAR cell lines
(TAg tolerized)

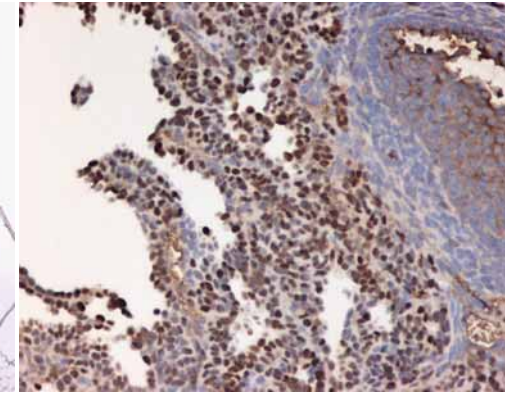
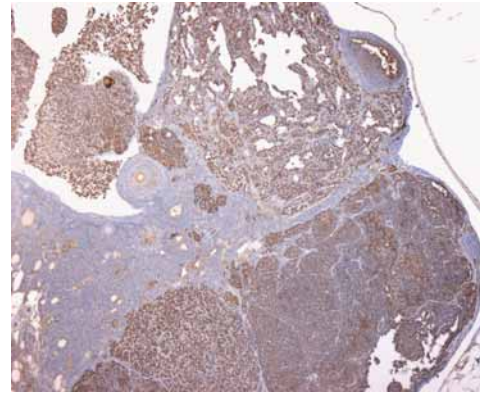


IHC TAg

MOVCAR TgMISIIR-TAg-Low allografts



H&E



TAg

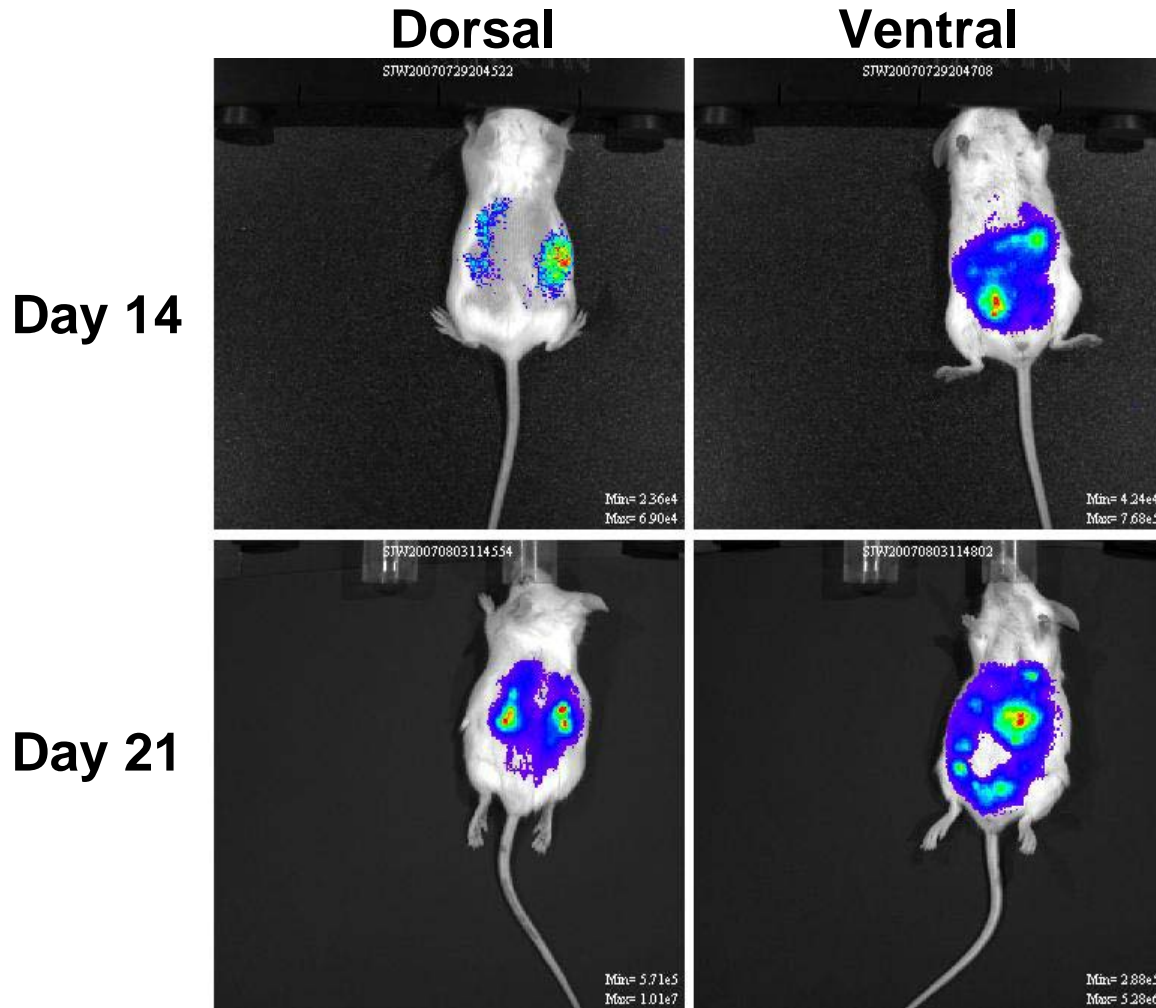
- Ascites
- Serous histology maintained
- Tumors home to ovary

Bioluminescent Imaging (BLI)

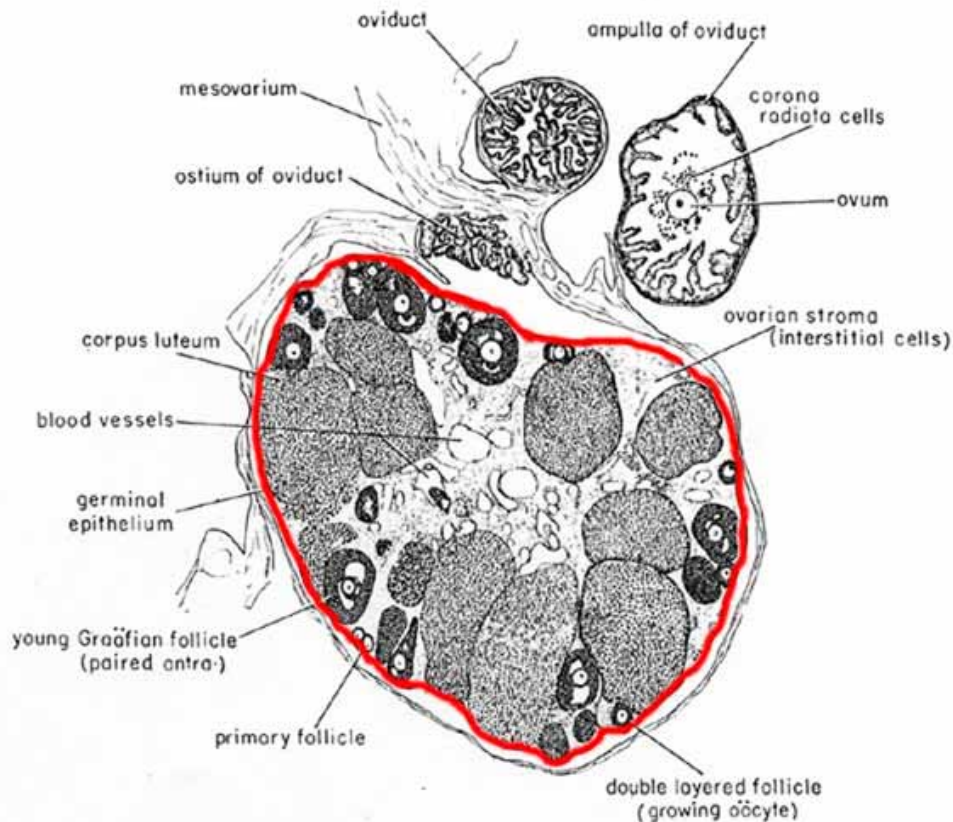
Goal: *In vivo* detection and quantitation of tumor formation in TgMISIIR-TAg-Low mice injected with MOVCAR cells

- Increased throughput
- Coordinated start/finish

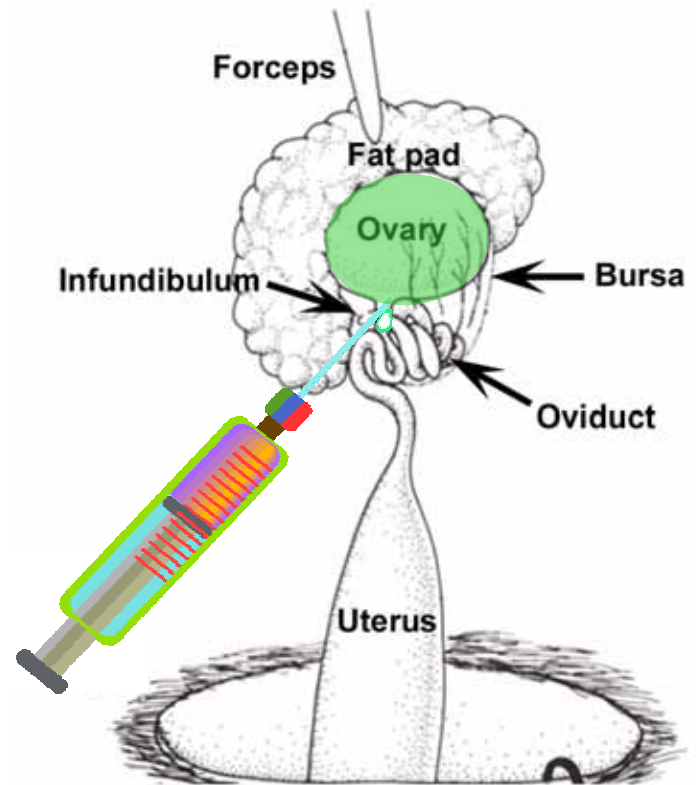
Detection of i.p. tumor growth by BLI



Orthotopic implantation of ovarian tumor cells: Intrabursal injection



MOUSE OVARY POST-OVULATION
SHOWING MANY CORPORA LUTEA

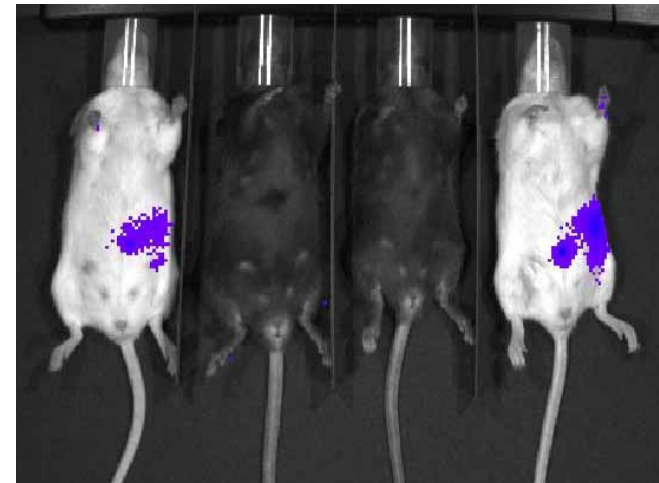
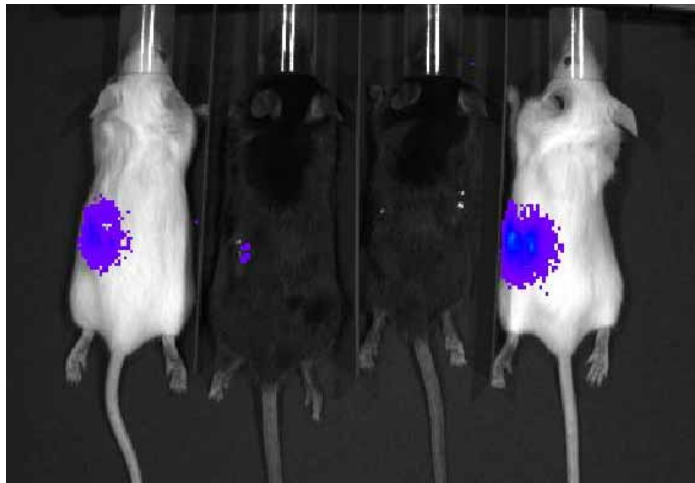


Detection of orthotopic tumor growth by BLI

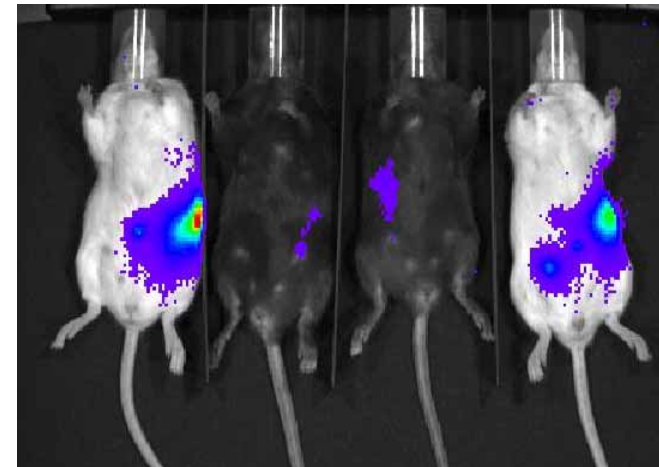
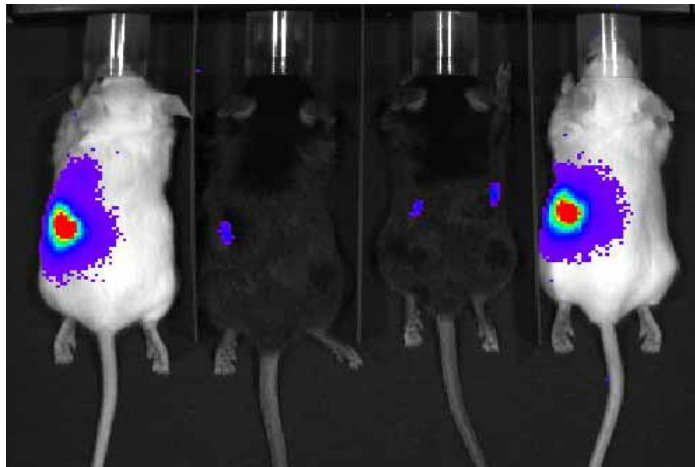
Dorsal

Ventral

Day 2



Day 11



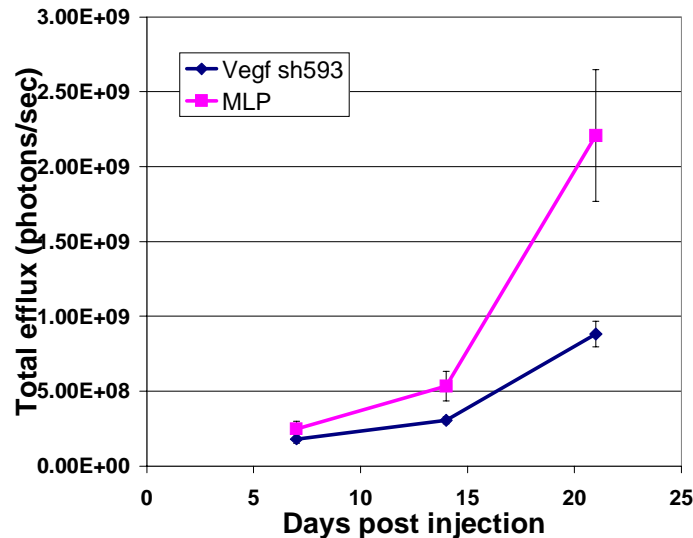
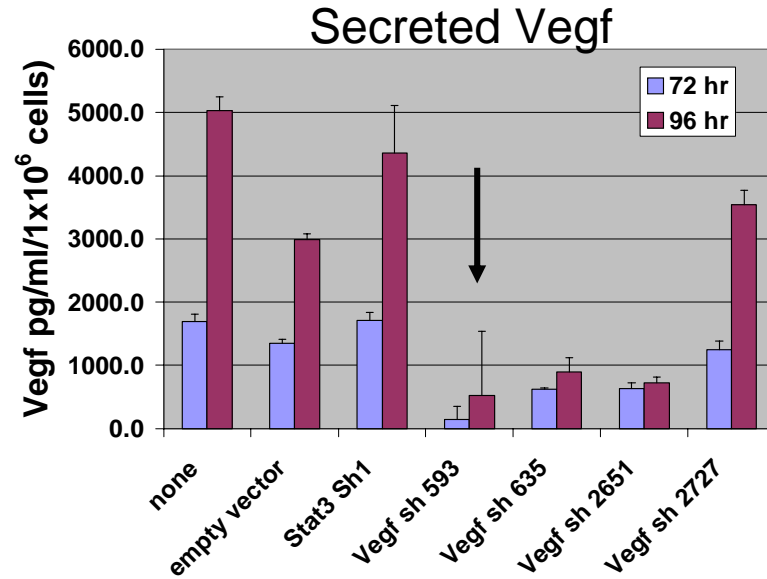
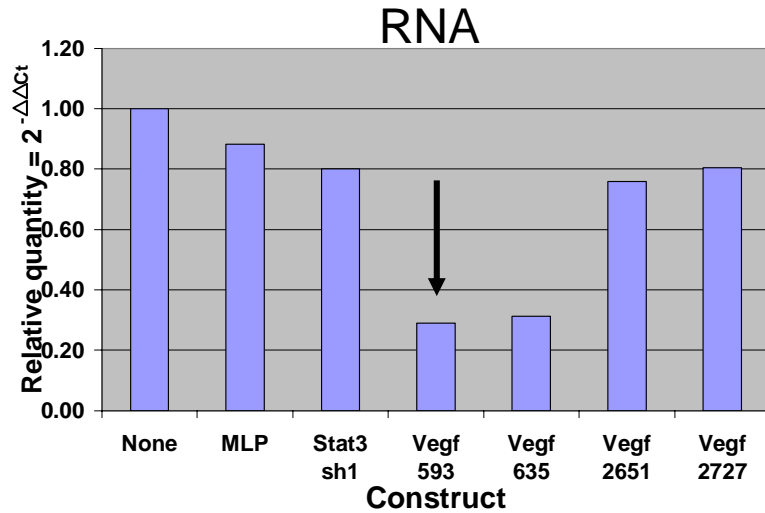
L L Bi L

L L Bi L

RNAi with short hairpin RNAs

Goal: Stable expression of shRNA in MOVCAR cell lines for *in vivo* studies in mice

RNAi Vegf – retroviral shRNA



In vivo

MOVCAR-Luc cell allografts in TgMISIIR-TAg-Low as model of EOC

- Modulation of gene expression
 - Enforced expression or RNAi
- Therapeutics
- Flexible combinations
 - Multiple pharmacologic/biologic agents
 - Gene expression modulation + pharmacologic/biologic agents
- Contemporaneous start
- Quantitative analysis by BLI
 - Longitudinal measurement of tumor growth
 - Response to chemotherapy- stasis vs. regression
 - Increased throughput - ~25 mice/hour
- Statistical analyses
 - n = 20 animals/arm (80% power and 4.8% type I error)

Conclusions

- Transgenic TgMISIIR-TAg mice develop EOC and can be used in conjunction with MRI for pre-clinical testing of therapeutic agents
- TgMISIIR-TAg-Low mice do not develop EOC can be used as syngeneic tumor allograft recipients
 - Tumor growth in can be measured *in vivo* using BLI
 - Tumor biology can be studied *in vitro* and *in vivo* by gene expression modulation (enforced expression or RNAi)
 - Flexible means to evaluate targeted therapies

Acknowledgments

Contributors:

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Vy Do, PhD

Laura Bickel

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Jack Zilfou, PhD

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Harvey Hensley, PhD

Quantitative Real Time PCR Facility

Emmanuelle Nicolas, PhD

Biostatistics Facility

Sam Litwin, PhD

Laboratory Animal Facility

Research Histopathology

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DOD - OCRP