臨床醫師為何要進修

Cheng-Huang Shen .Ph.D

2016.05.26

需克服問題

業務-生理

醫糾-心理

探討...

對於科學了解只僅有存在在大學領域與醫學臨床的認知

無法了解較深入探討文章

臨床統計

無壓力 ----原地踏步

學校-整體規畫 由深入淺

生化 分生 細生 免疫.....

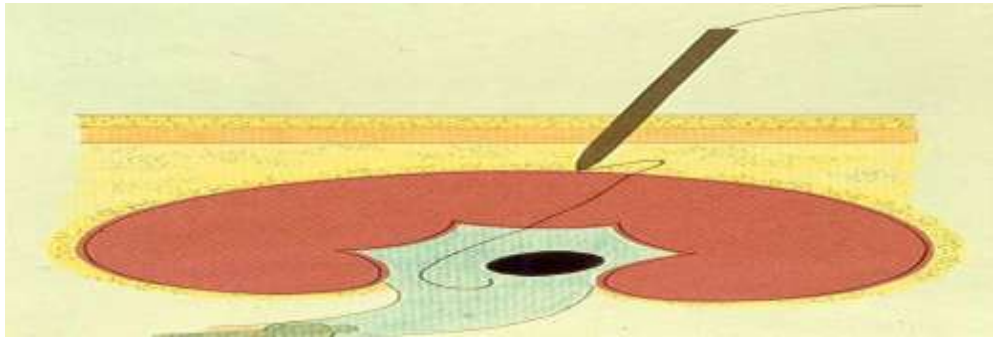
MP34-13

Cheng-Huang Shen, Ming-Chin Cheng, Chang-Te Lin, Yeong-Chin Jou, Pi-Che Chen, Chih-Yu Yang

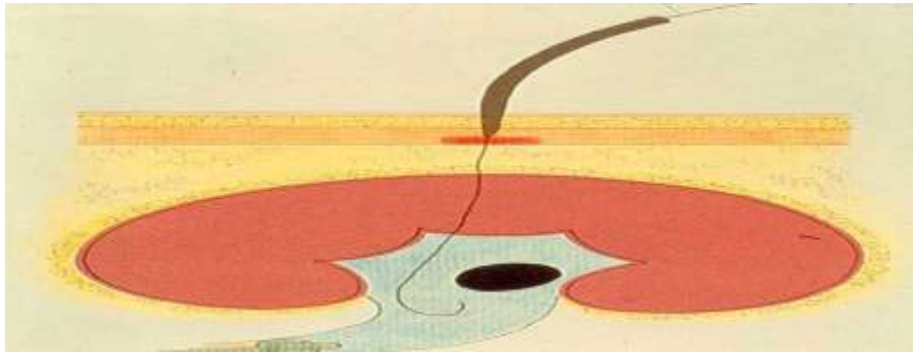
Divisions of Urology, Department of Surgery, Chia-Yi Christian Hospital Taiwan

Percutaneous Nephrolithotomy: A Single Institute Experience of 1003 Cases

•Failure to construct the access tract

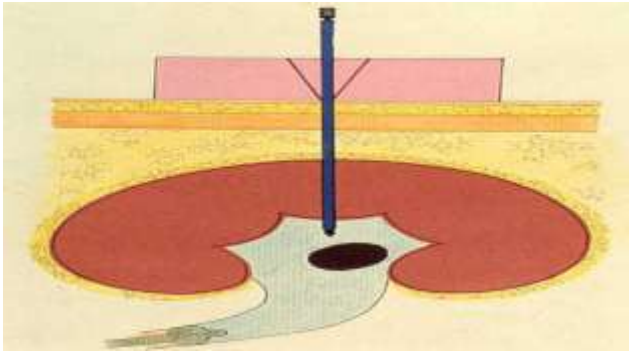


Guide wire kinking

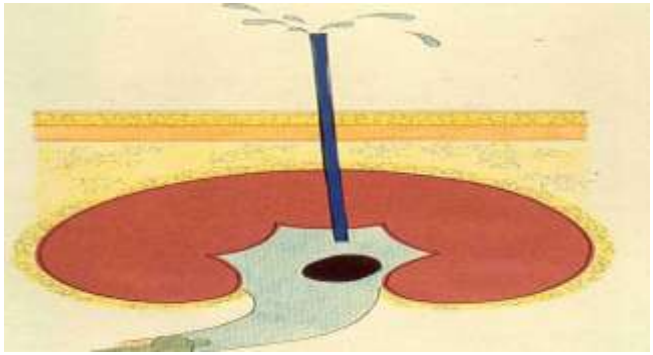


Tough fascia

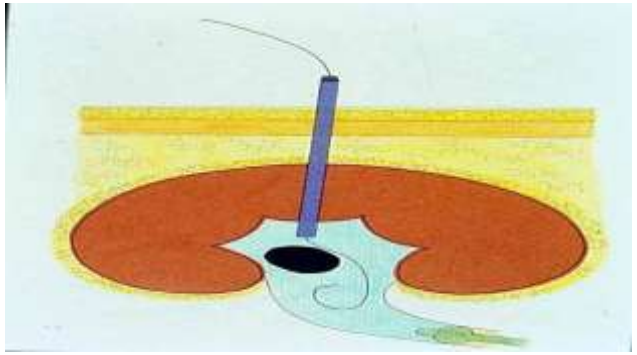
•Sequential dilation



Echo guide puncture the collecting system



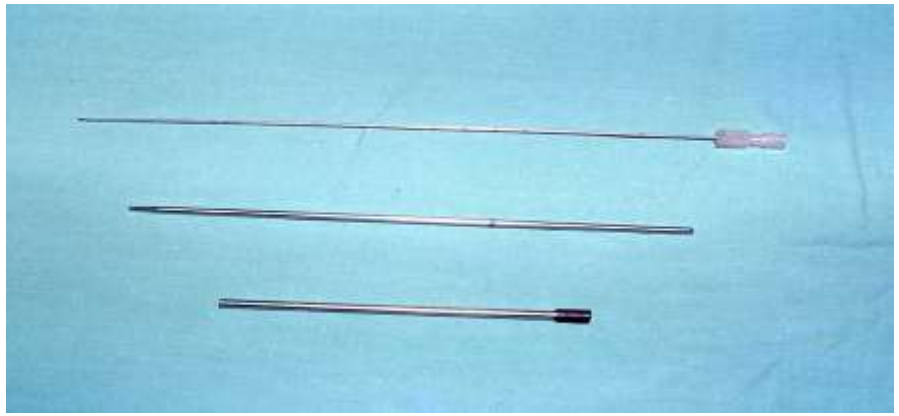
Blue urine will be noted if puncture needle sheath was in collecting system

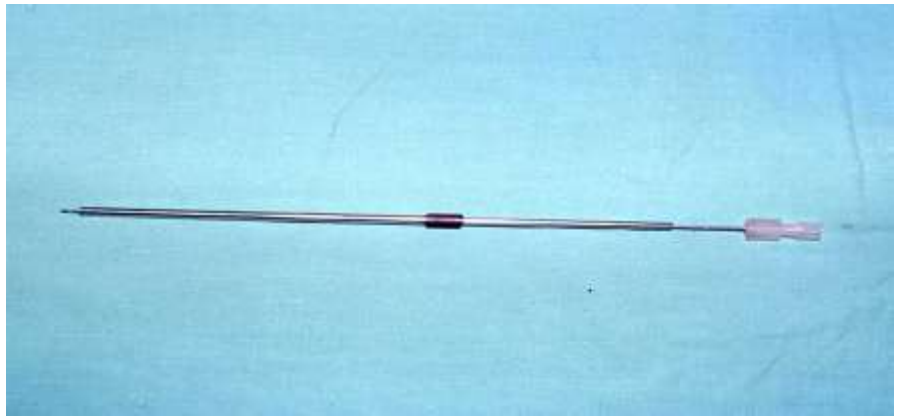


Dilation to 28-30 Fr.

Innovative concept (1)

Improved initial dilator

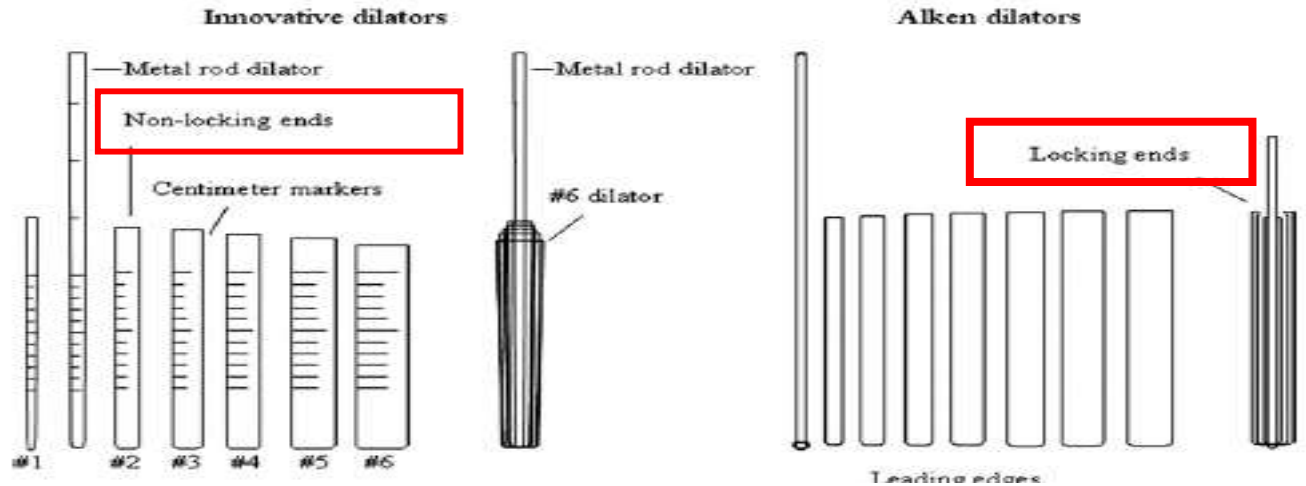




Innovative concept(2)

Table 1. Specifications of the innovative dilators

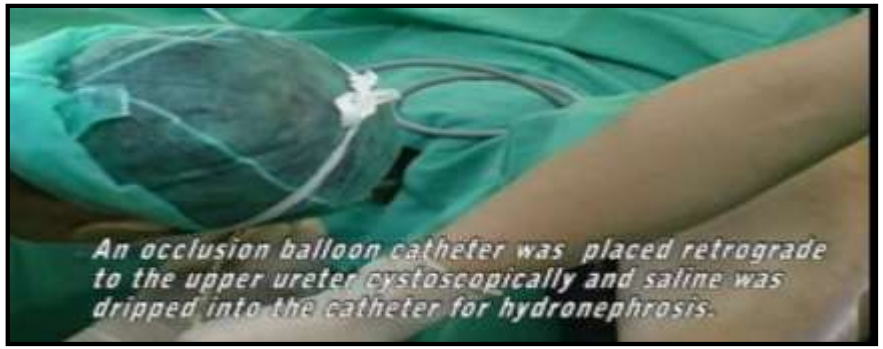
Variable	Leading-Edge Inner Diameter (F)	Nonleading Edge Outer Diameter (F)	Length (cm)	Comment
Puncture needle	4.5	4.5	30	
No. 1 dilator	5	9.5	20	
Metal rod dilator	9.5	10	34	
No. 2 metal dilator	11	14	19	
No. 3 metal dilator	16	19	18.5	
No. 4 metal dilator	21	24	18	
No. 5 metal dilator	25	28	17.5	Used most often
No. 6 metal dilator	31	34	16.5	Rarely used



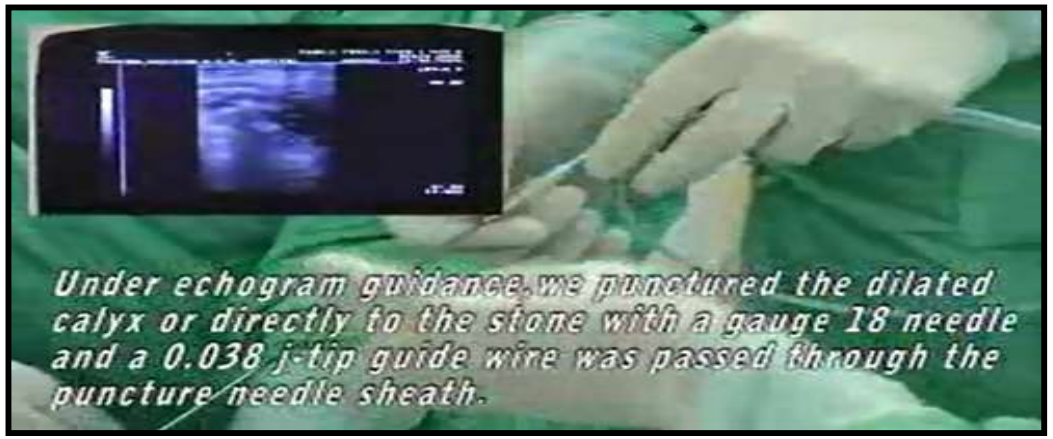
Innovative metal dilators

PCNL procedure

Innovative Metal Dilators For Percutaneous Nephrostomy Tract:Report on 546 Cases （UROLGY 2007, 70：418-421）



PCNL


Under echogram guidance,we punctured the dilated calyx or directly to the stone with a gauge 18 needle and a 0.038 j-tip guide wire was passed through the puncture needle sheath.


Sequential dilation began with a pencil-shaped metal dilator,which was passed through the puncture needle sheath till the tip.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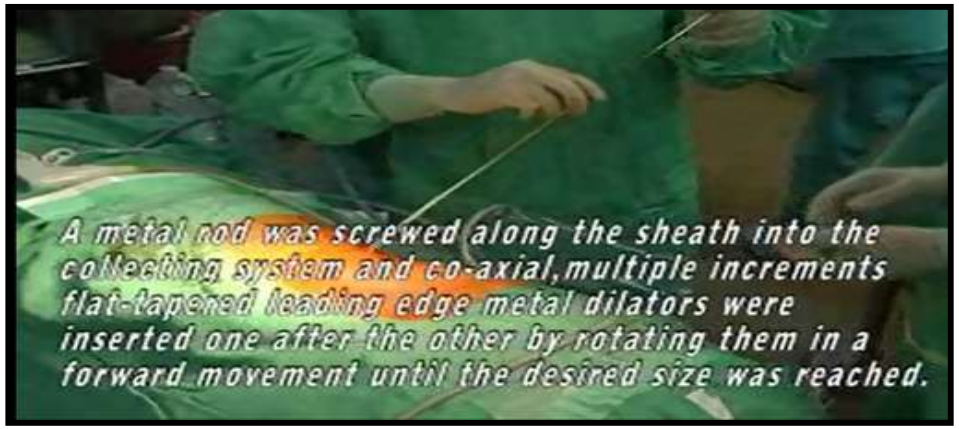
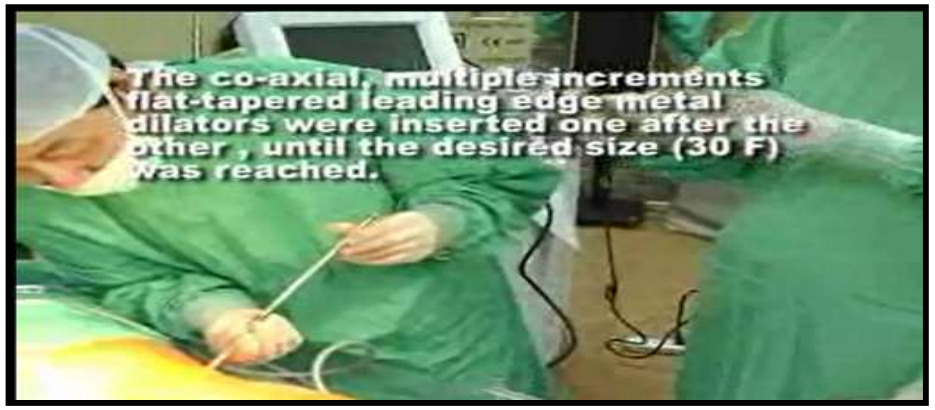
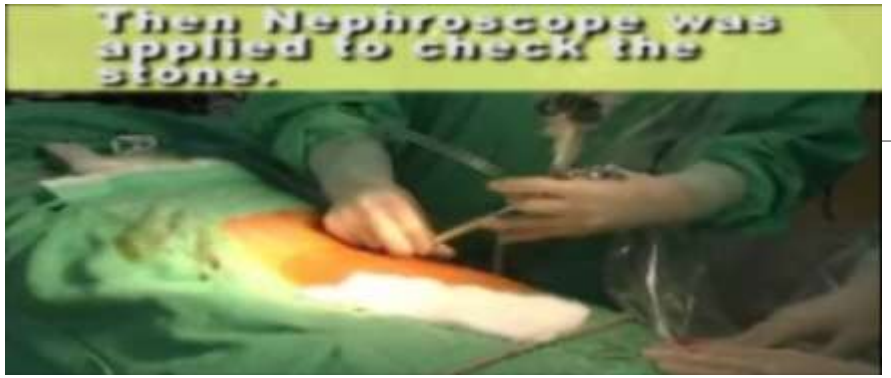
PCNL


A metal rod was screwed along the sheath into the collecting system and co-axial,multiple increments flat-tapered leading edge metal dilators were inserted one after the other by rotating them in a forward movement until the desired size was reached.


A 6.5 Fr ureteroscope was applied to check the track when dilated to 12 Fr and adjusted the dilator to a better position if necessary.


The co-axial, multiple increments flat-tapered leading edge metal dilators were inserted one after the other , until the desired size (30 F) was reached.

PCNL


Then Nephroscope was
applied to check the
stone.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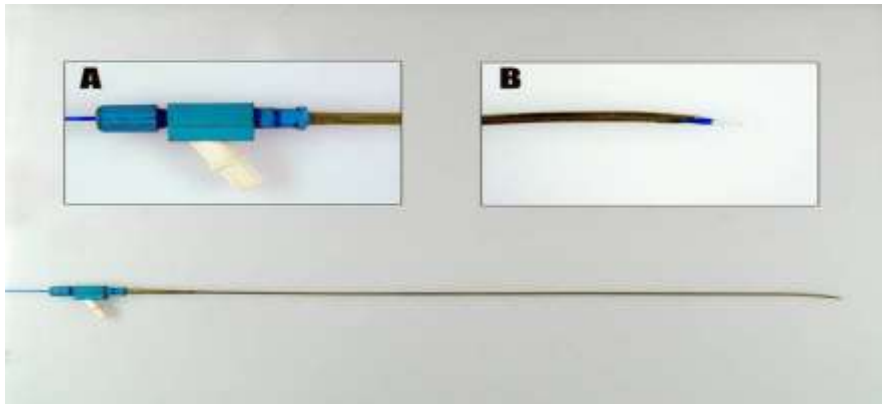
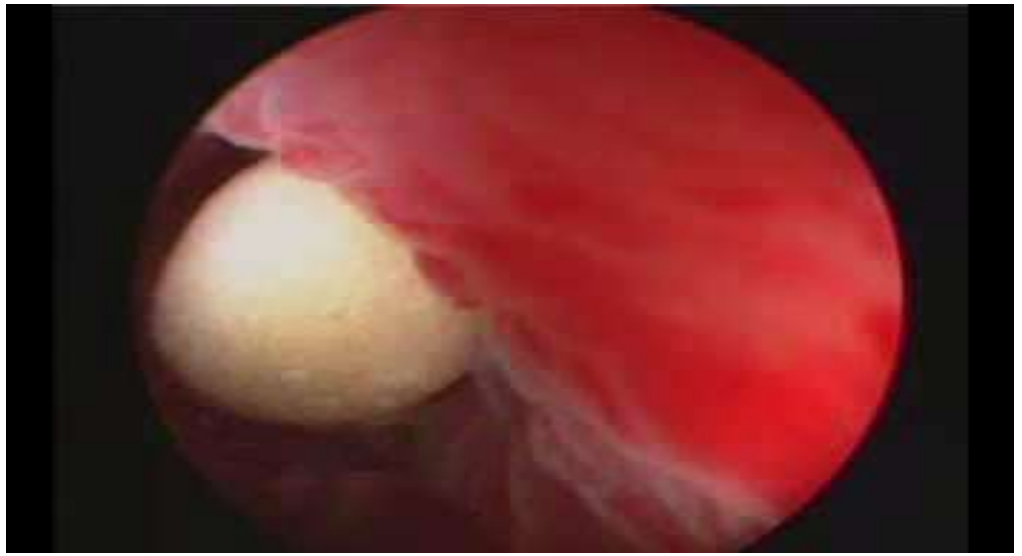
stone.

Innovative concept(3)

- High-power Holmium: Yttrium -Aluminum-Garnet Laser for percutaneous Treatment of Large Renal Stones (Urology 2007 69:22-25
- **Laser Fiber Guider**



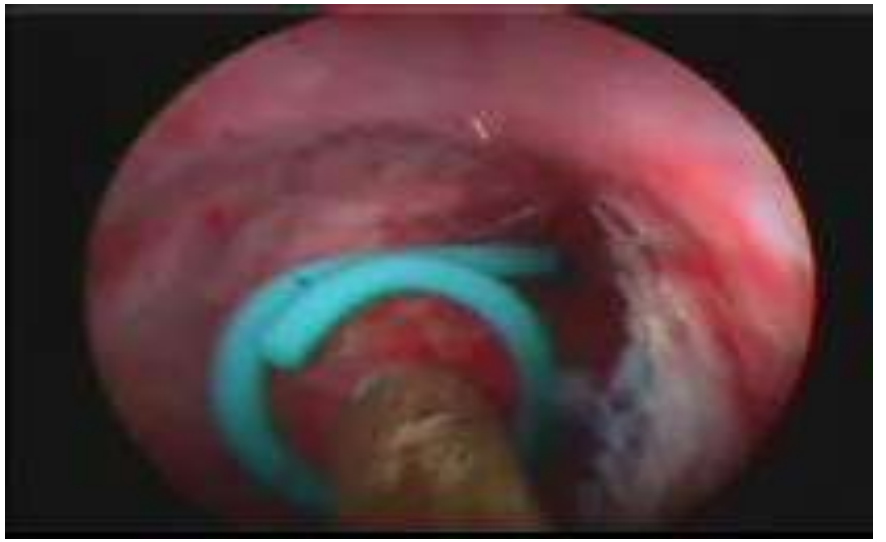


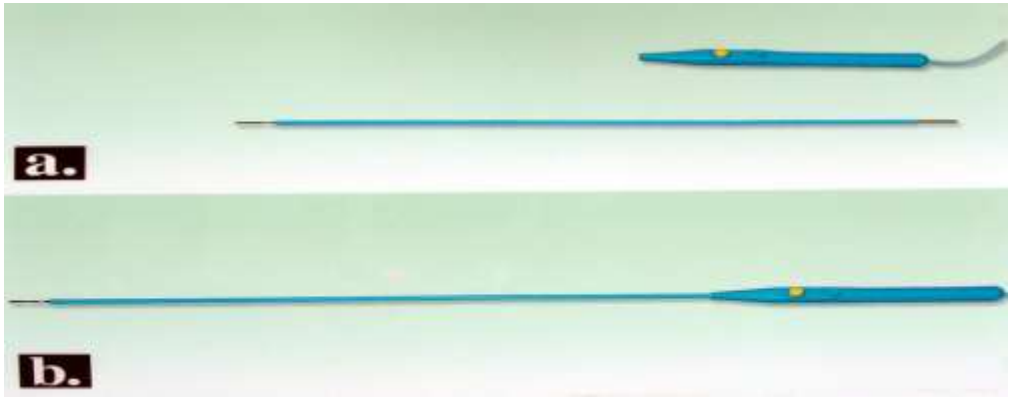
Innovative concept(4)

☛Electrocauterization for hemostasis:

- ☞ Electrocauterization of Bleeding Points for Percutaneous Nephrolithotomy. (UROLOGY 2004,64:443-447)
- ☞ Nephrostomy Tube-free Percutaneous Nephrolithotomy for patients with Large Stones and Staghorn Stones(UROLGY 2006 67：30-34)

  Cauterization of access tract for nephrostomy tube-free Percutaneous Nephrolithotomy(J Endourol.2004,18:547-549)
- ☞ Tubeless Percutaneous Nephrolithotomy for Geriatric Patients Urol Int,(2008, accepted, in press)





Results

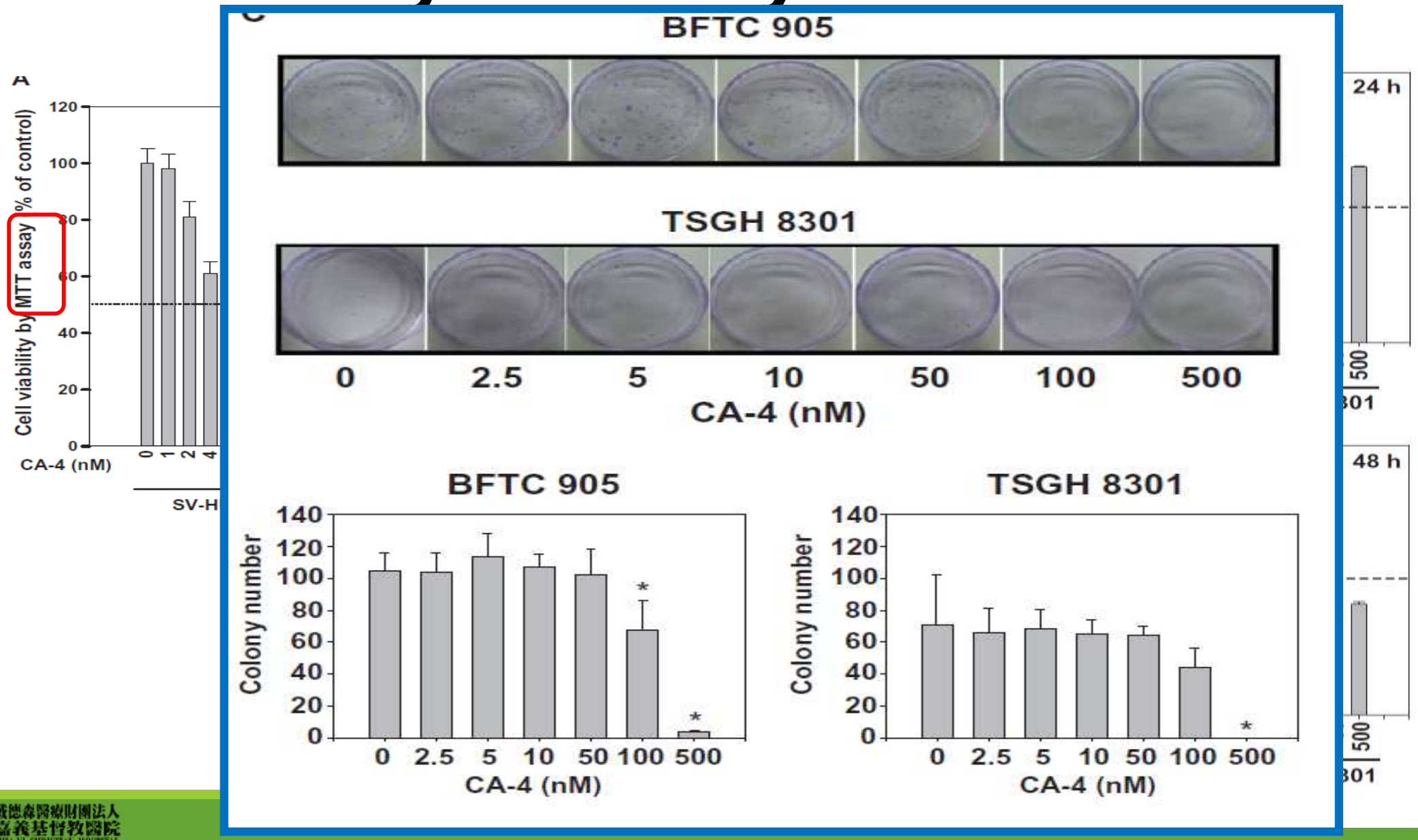
Results of 1003 patients receiving PCNL, with innovative metal dilators used to create percutaneous nephrolithotomy tract

Results:

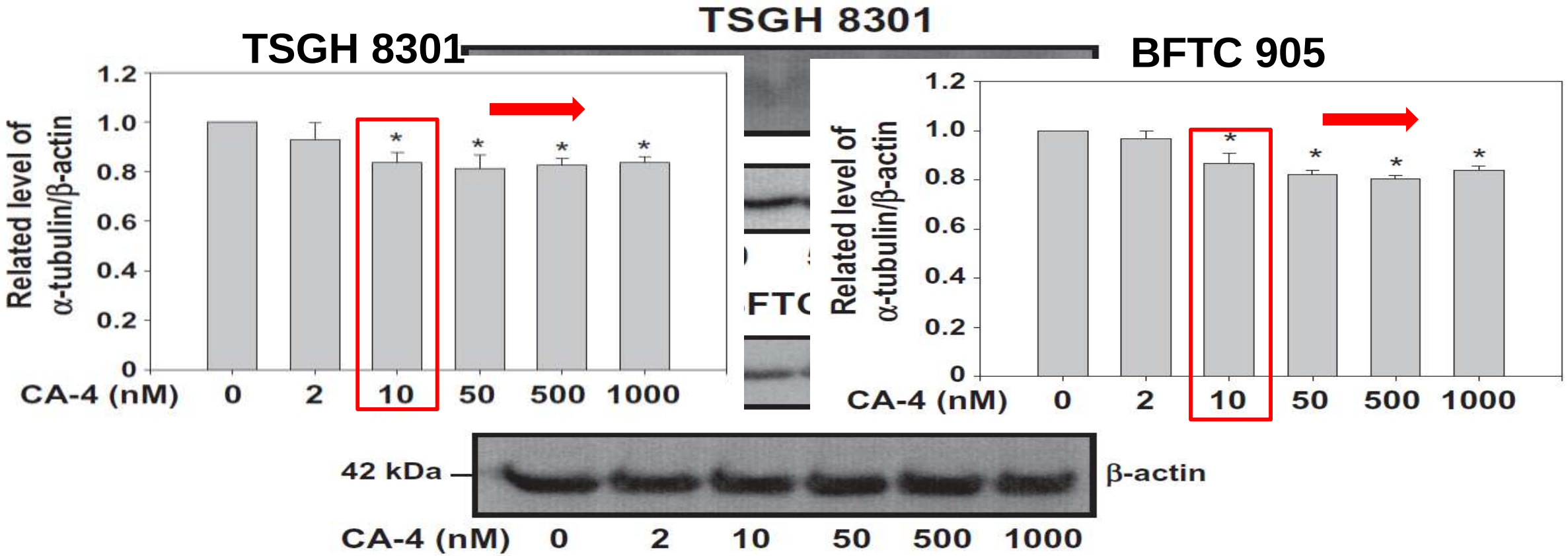
- **mean stone burden - 1150.6 mm2.**
- **average operation time - 88 minutes.**
- **the mean hospital stay - 4 days.**
- **the initial stone free rate - 77.7%**
- **staghorn stone- 41.4%**
- **renal stone - 82.3%**
- **proximal ureteral stone - 98.0%**
- **combined renal and proximal ureteral stone- 79.8**

Combretastatin A-4 (CA-4) Inhibits Cell Growth and Metastasis in Bladder Cancer Cells and Retards Tumor Growth in a Murine Orthotopic Bladder Tumor Model

Cytotoxicity of CA-4

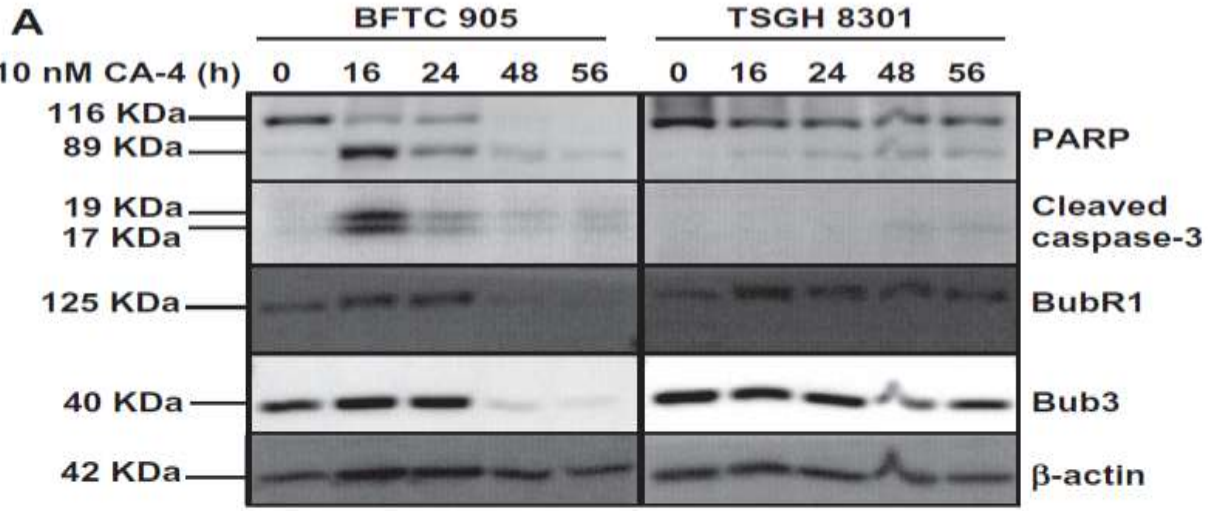


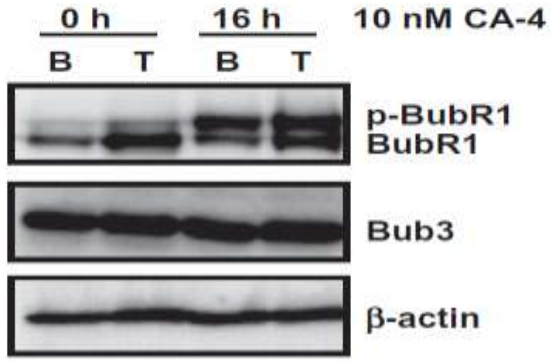
CA-4
Inhibit microtubule polymerization

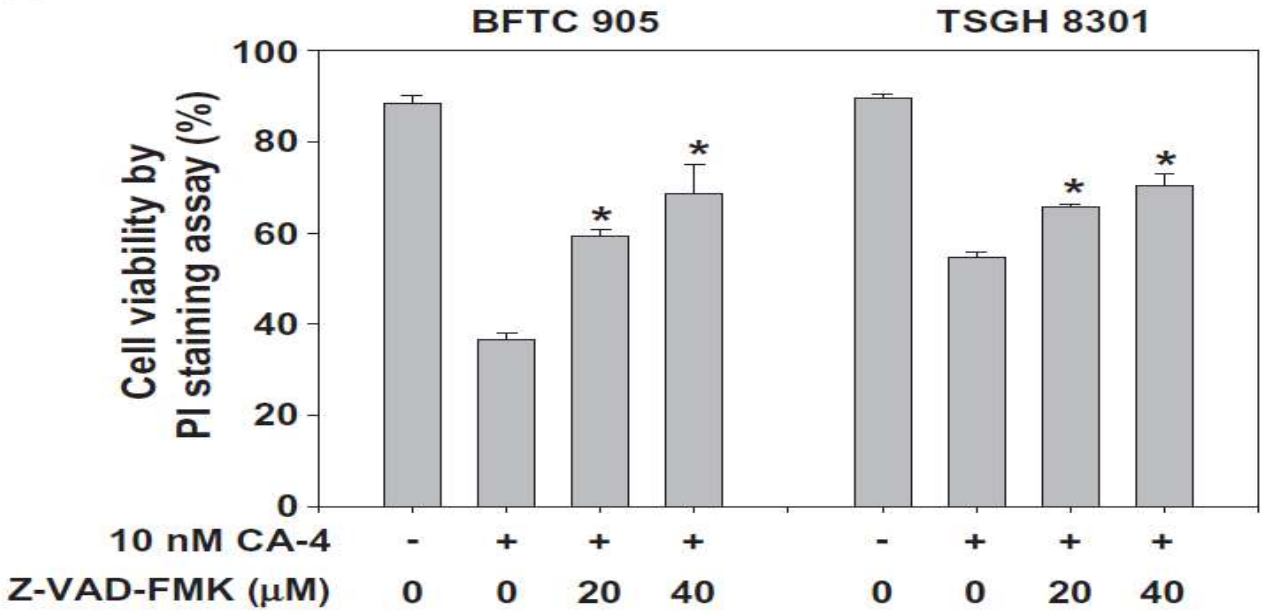


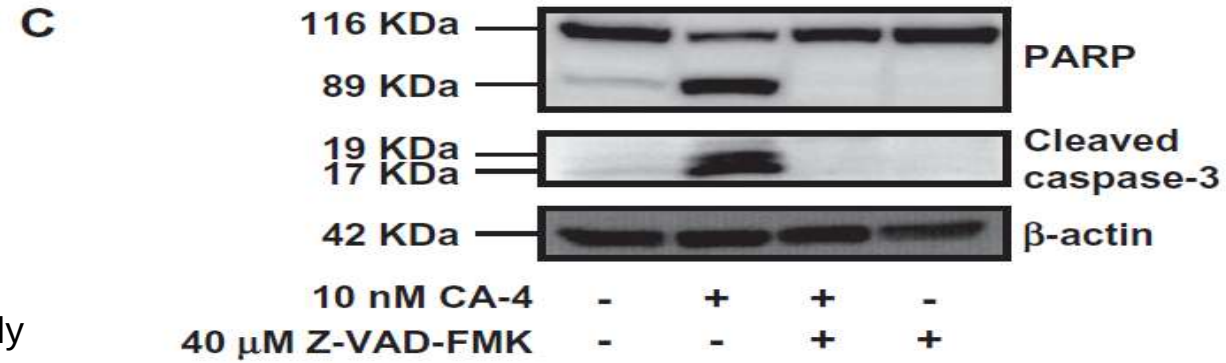
TSGH 8301, BFTC 905: bladder cancer cell lines

CA-4 Induces Apoptosis



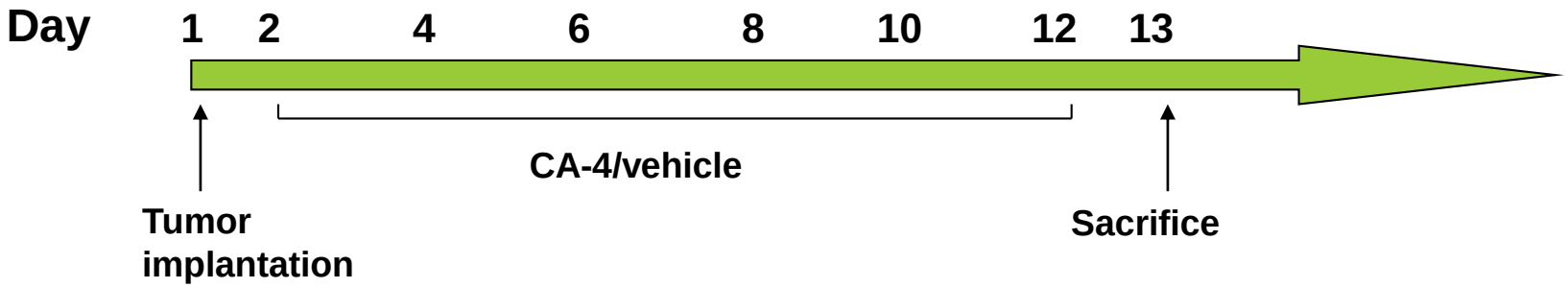






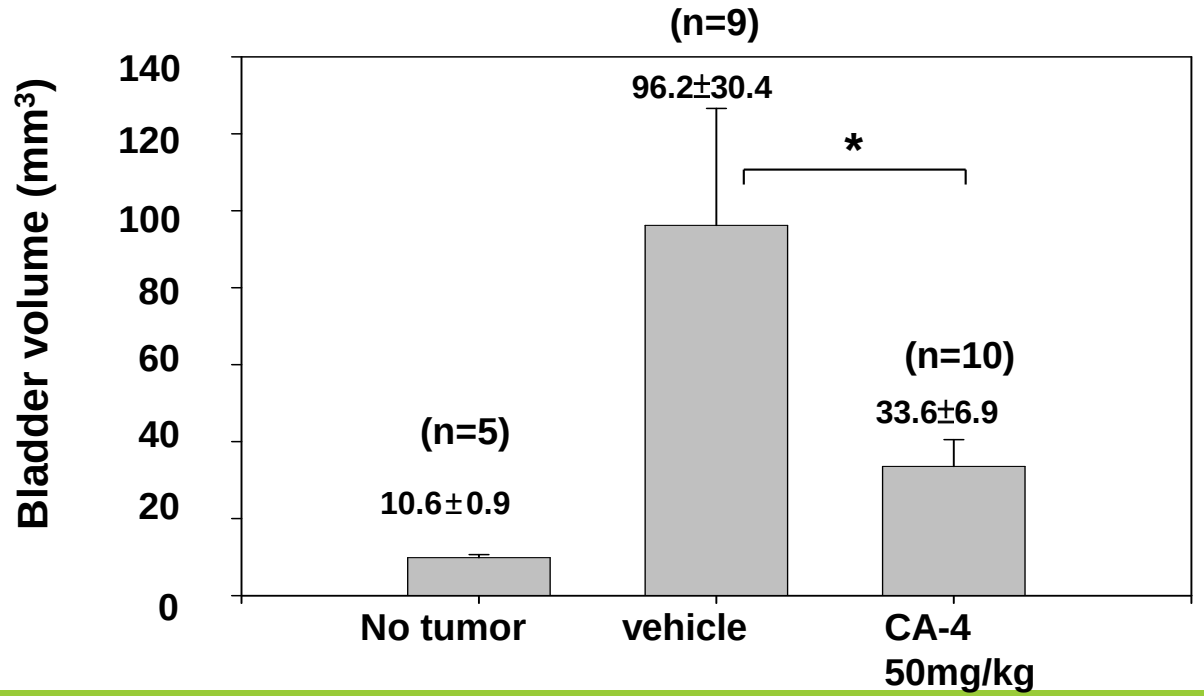
Bub3 :involved with the regulation of the Spindle Assembly Checkpoint (SAC)

Effect of CA-4 on murine orthotopic bladder tumor model



C57BL/6 female mice

bladder volume = (length×width²)/2



Conclusion

- 80% of all TCC initially develop as superficial papillary carcinoma
  - usually managed with transurethral resection, followed by intravesical chemotherapy.
  - The recurrent rate after intravesical chemotherapy is still high
  - we want to develop **new agents** to improve the intravesical chemotherapy

- **CA-4**
  - induces bladder cancer cell death
  - through apoptosis and mitotic catastrophe
  - inhibits cell migration *in vitro.*
  - *when applied by* the intravesical route, retards bladder tumour growth *in vivo.*

Investigation of developing a gene delivery vector using the human JC virus-like particle to inhibit human urinary bladder carcinoma growth

SPEAKER ：沈正煌

DATE：NOV. 18, 2014

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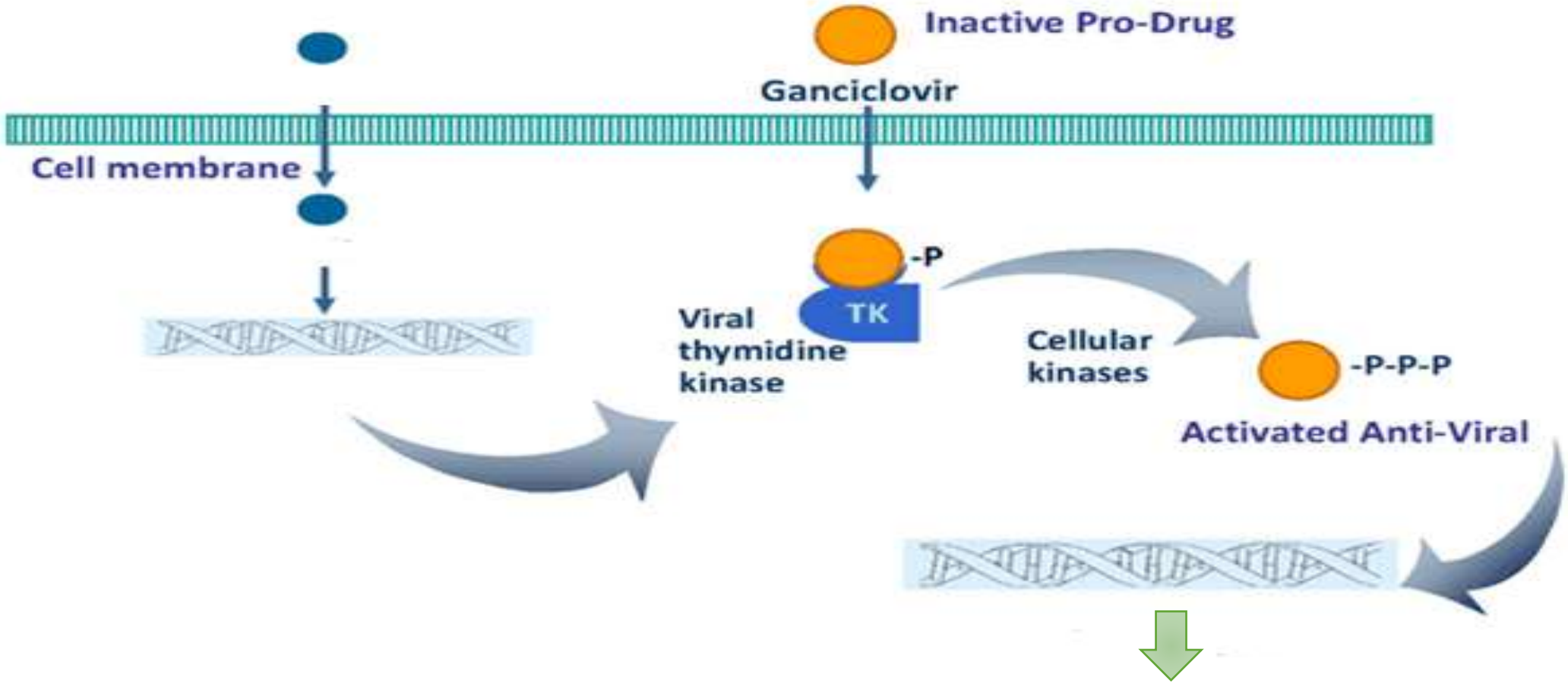
陳學毅 碩士

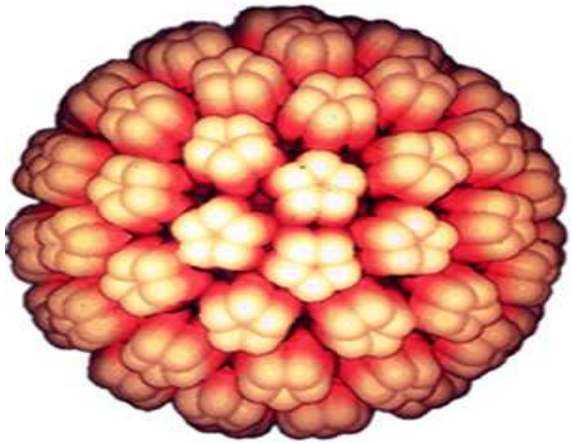
小兒科
趙崇男醫師

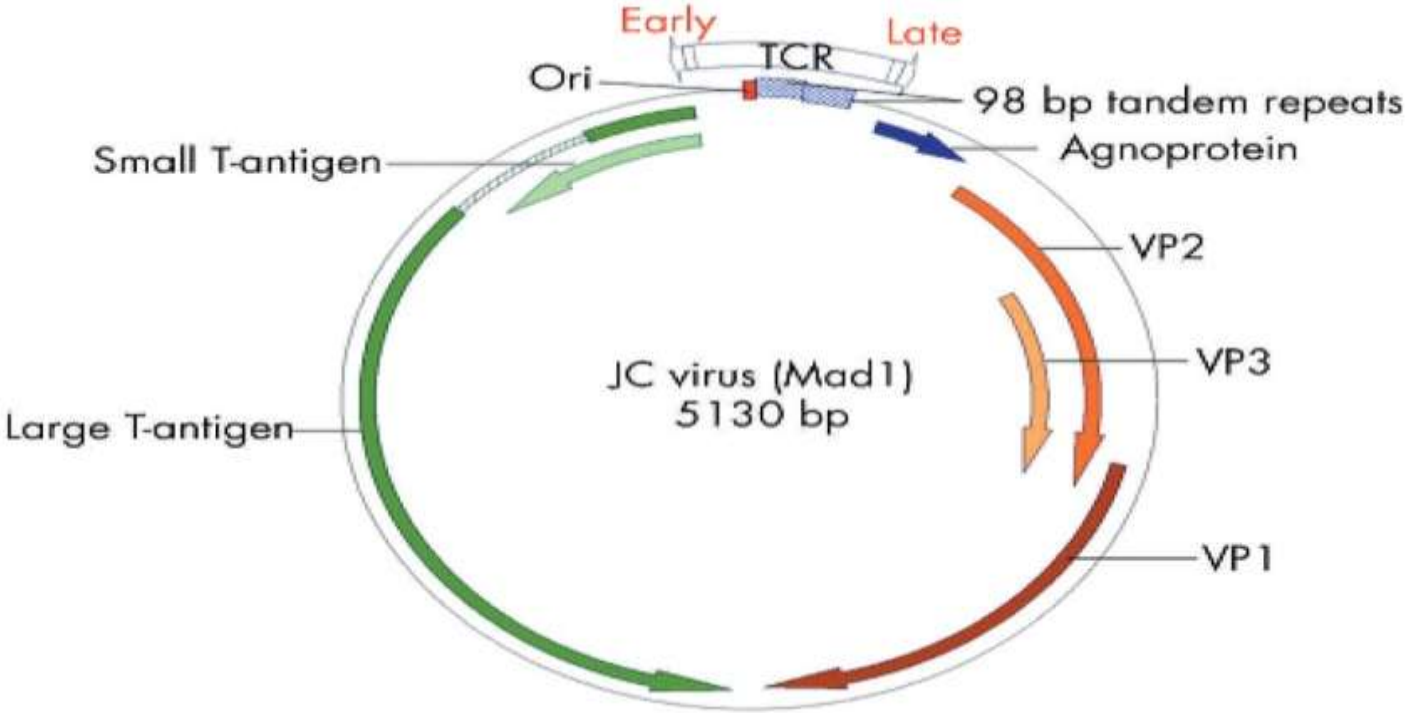


Cell death

http://www.takara-bio.com/medi_e/gene.html

JC virus





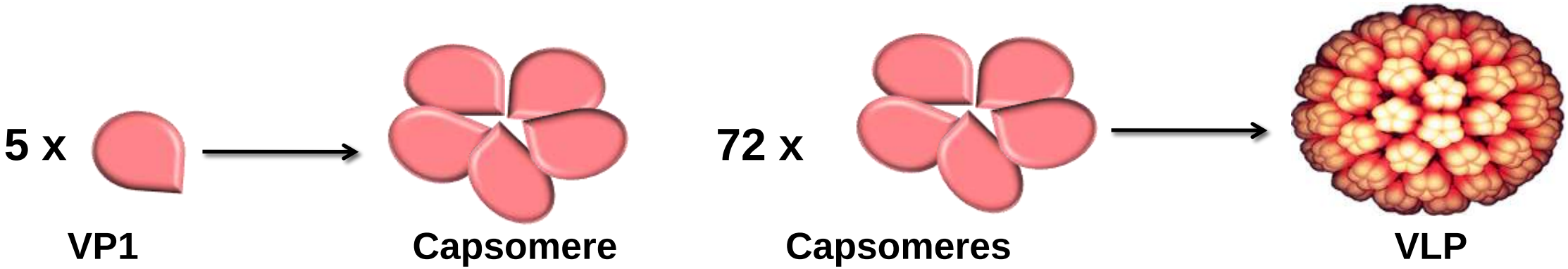
Early region encodes
large tumor (LT) antigens
small tumor (st) antigens

Late region encodes
agnoprotein
structure capsid proteins
(VP1, VP2 ,VP3)

Virus-like particle

The recombinant VP1 protein is able to self assemble to form a virus-like particle (VLP) in *E. coli*.

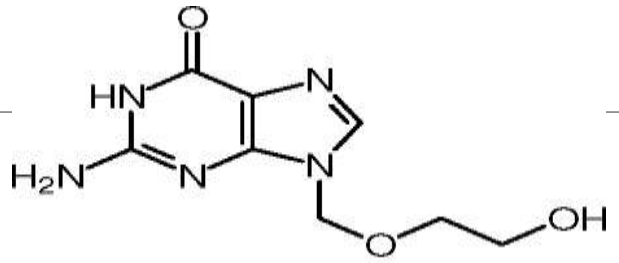
Ou, W. C. *et al. J Gen Virol,* 1999



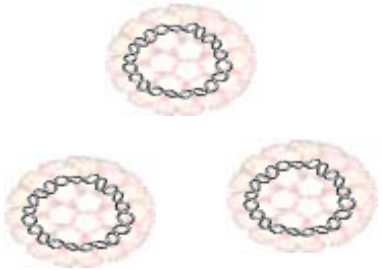
The characteristics of the VLP, such as its typical morphology, its binding to cells, internalization and its transportation to nucleus, are similar to that of native JCV virions.

Goldmann C *et al. J Virol,* 1999

Aim

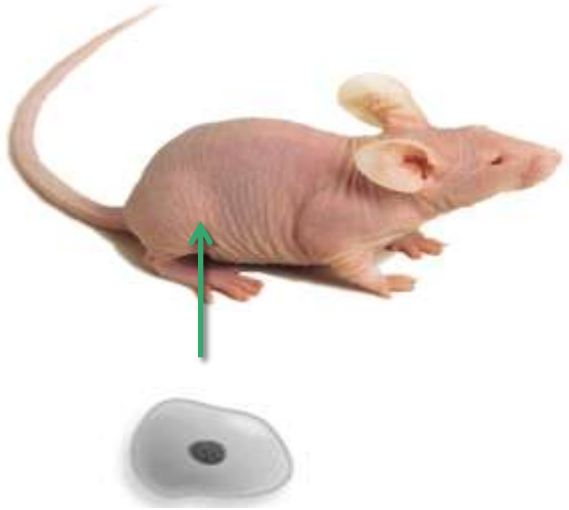


Acyclovir (ACV)



pEGFP-N3-VLP or tk-VLP

?

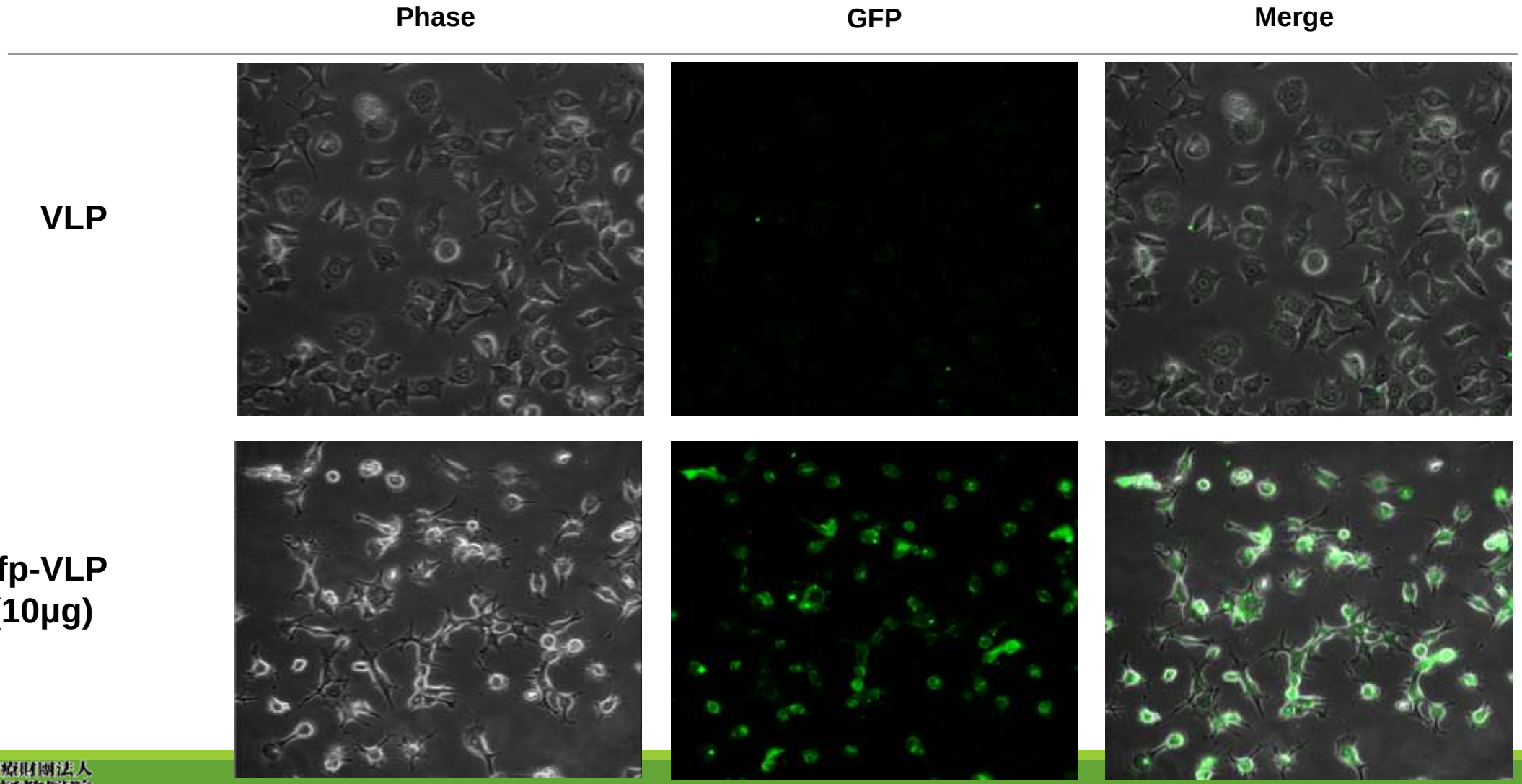


Bladder cancer

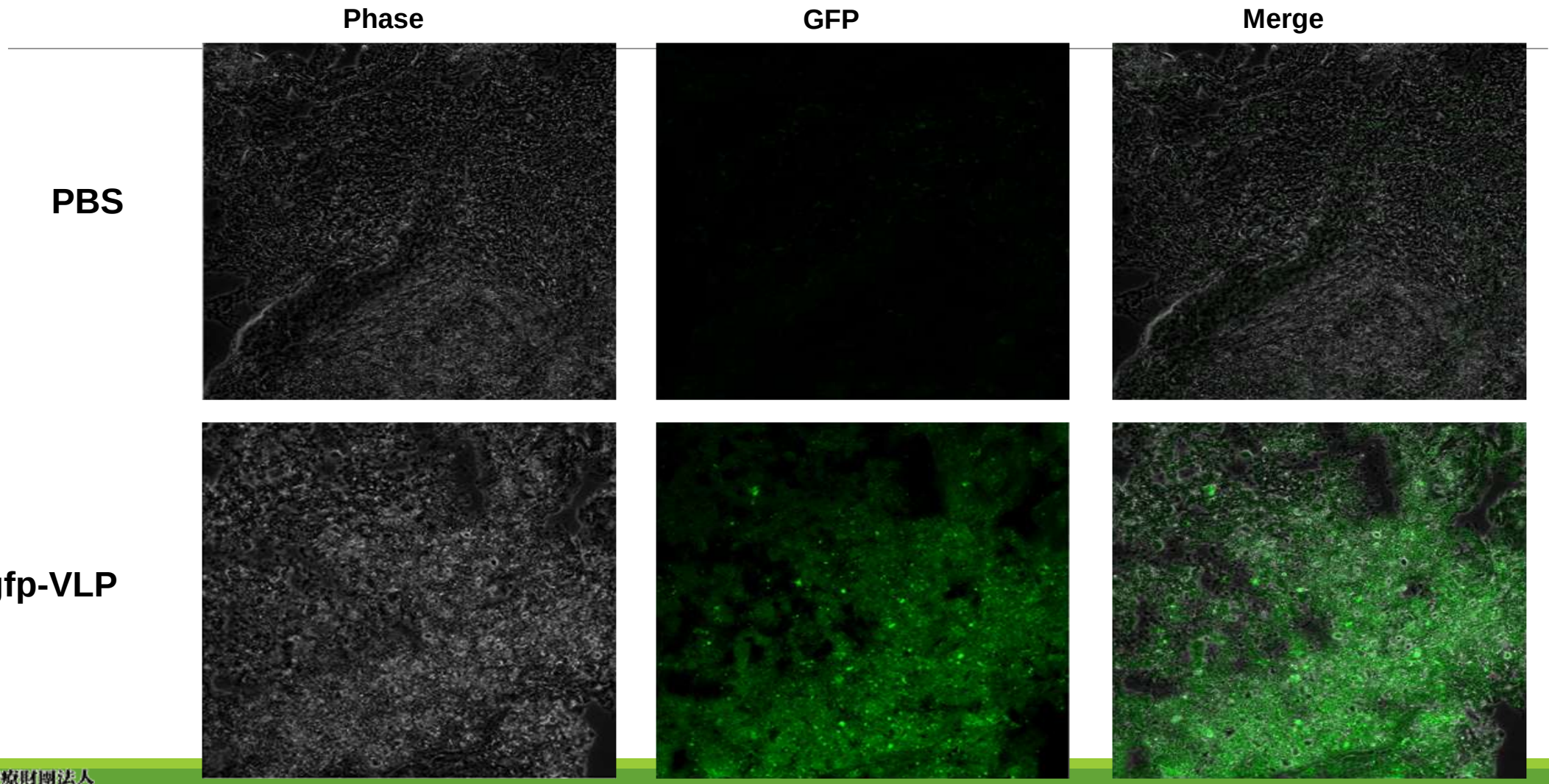
Cell death

Tumor regression

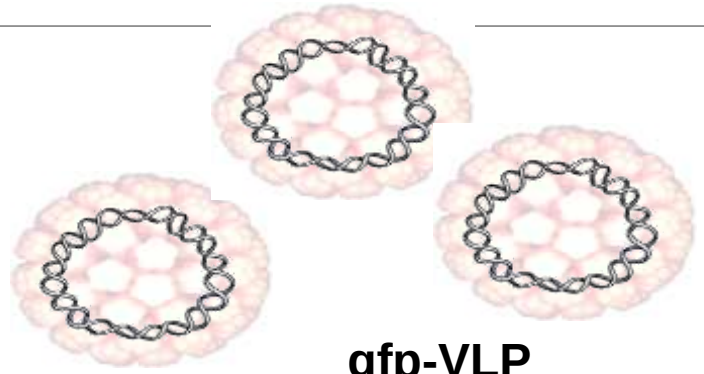
JCV VLP can package and transduce the pEGFP plasmid into TSGH 8301



Gene transduction using gfp-VLP in mice

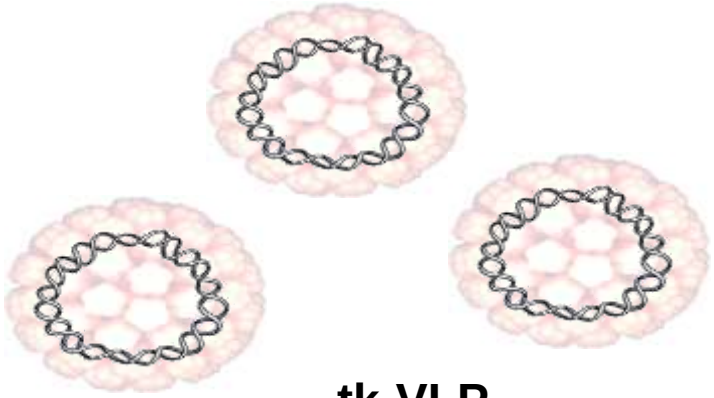


Animal model

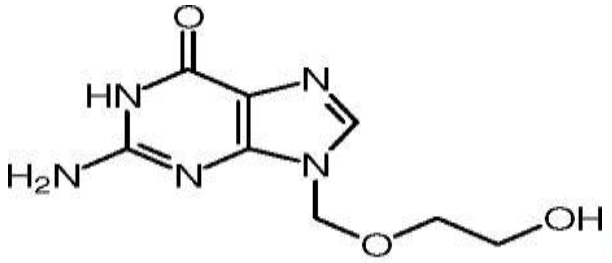


gfp-VLP

HT-1197 tumor



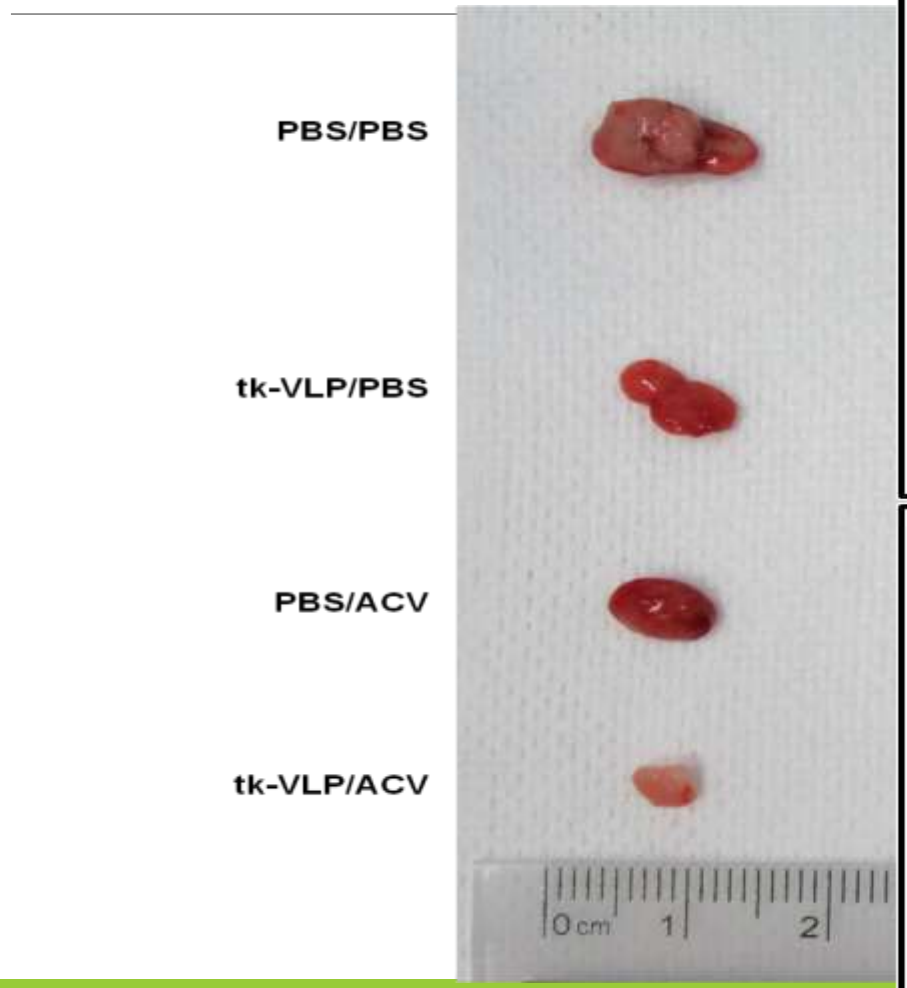
tk-VLP

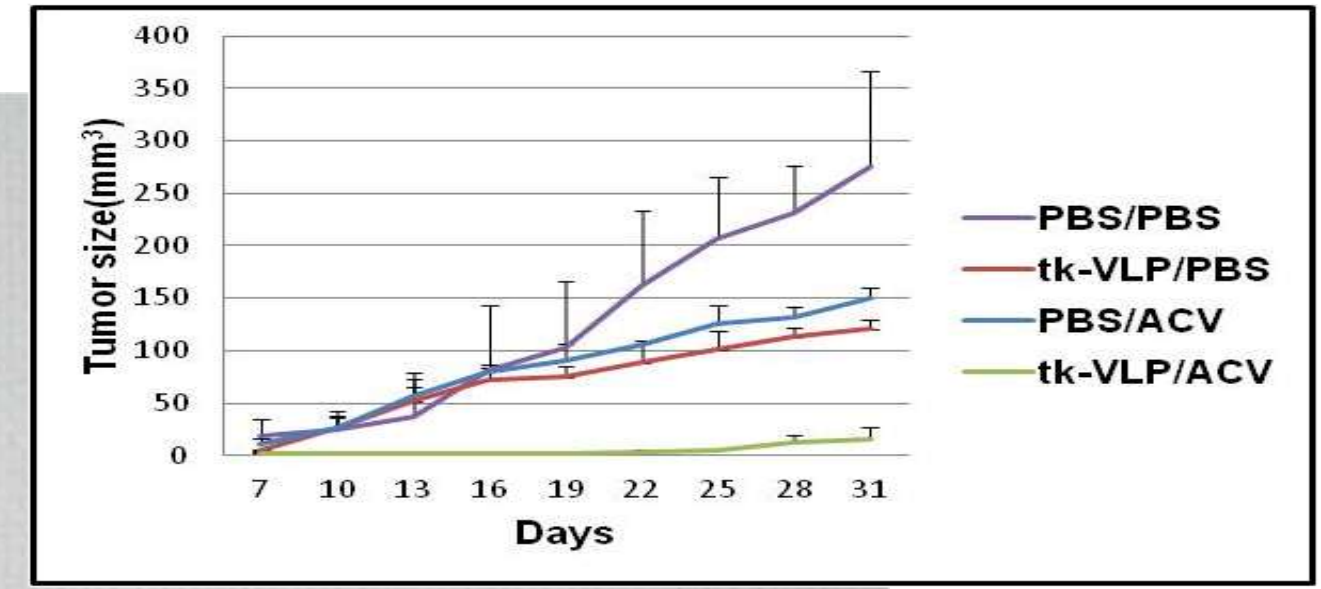


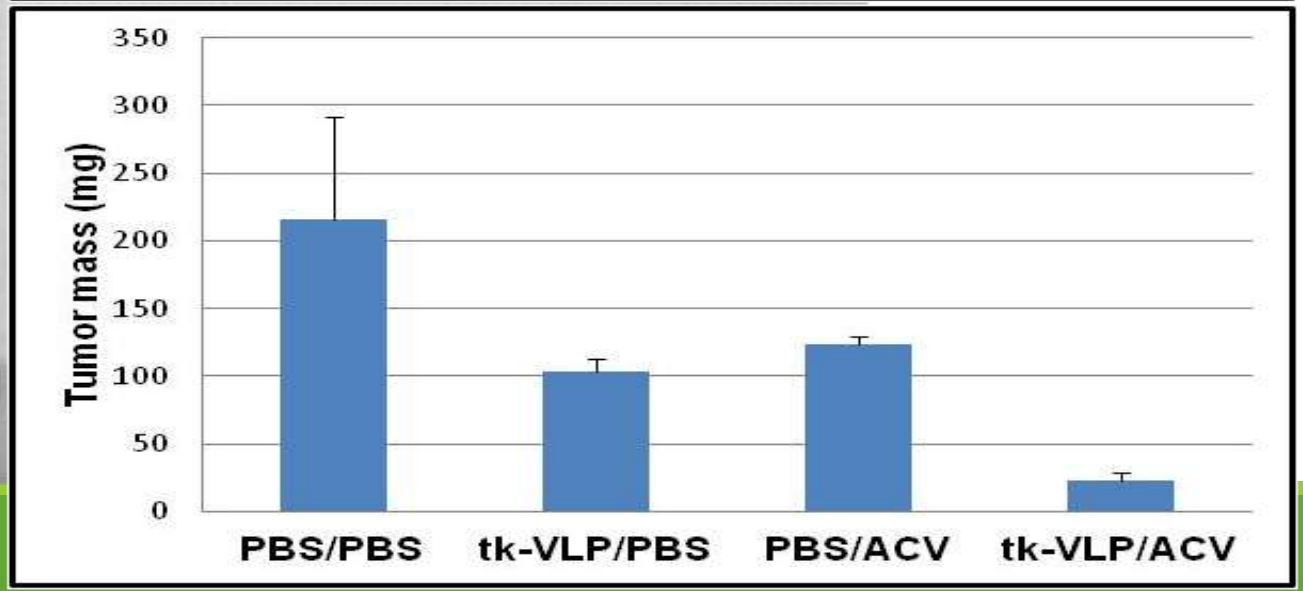
Acyclovir (ACV)

HT-1197 tumor

Inhibition of tumor nodule growth by tk-VLP

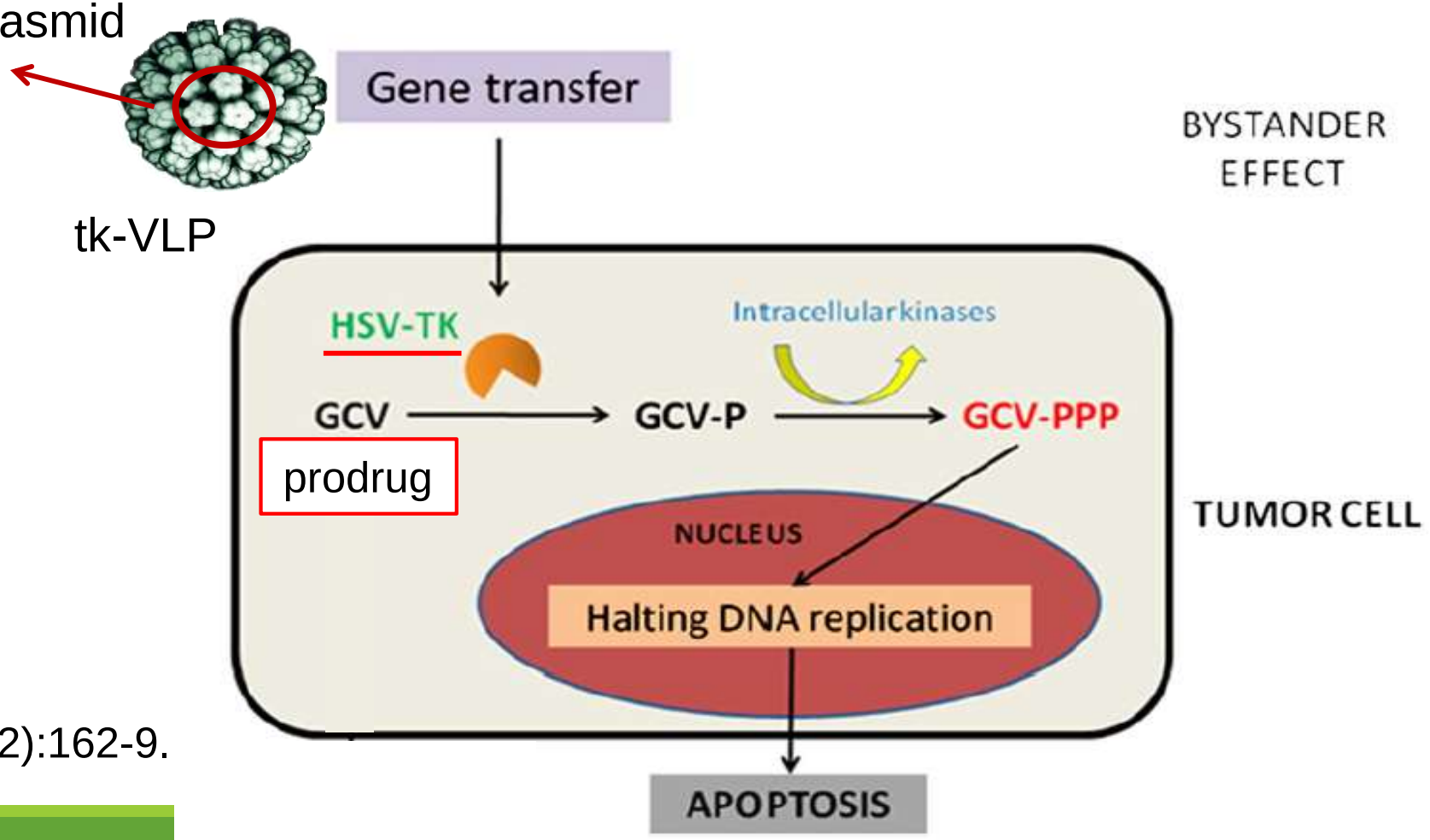






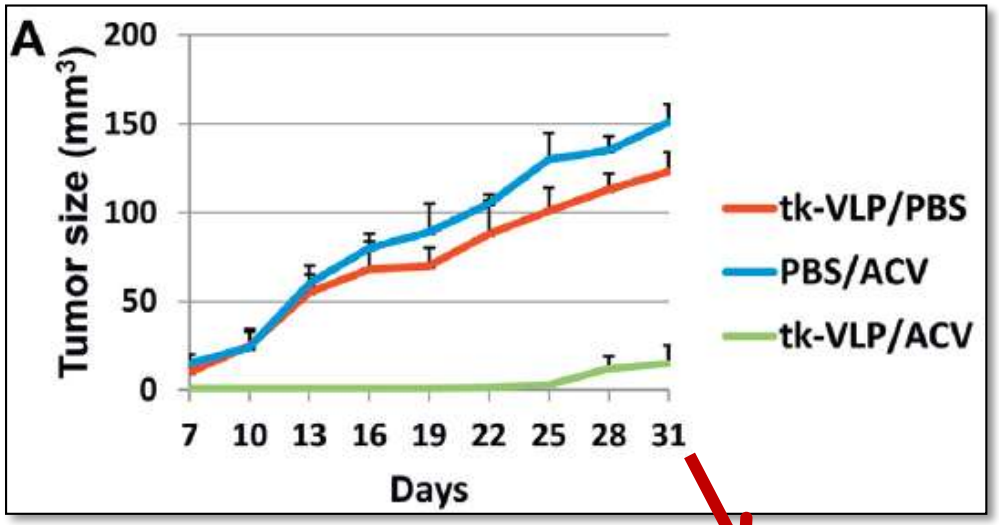
The HSV-tk suicide gene could be delivered in bladder tumor cells via JCPyV VLPs.

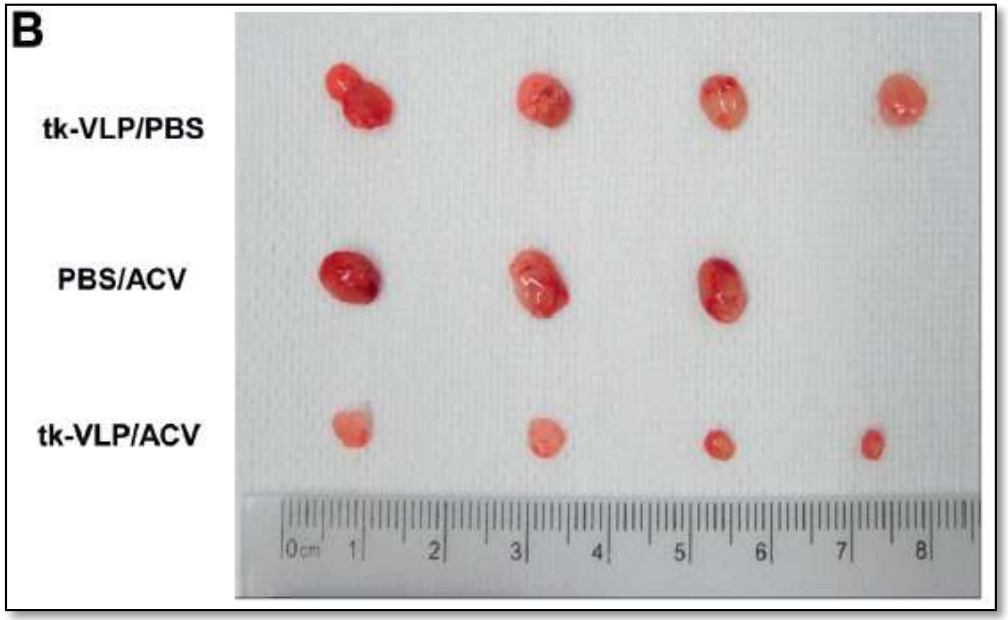
TK gene expression plasmid

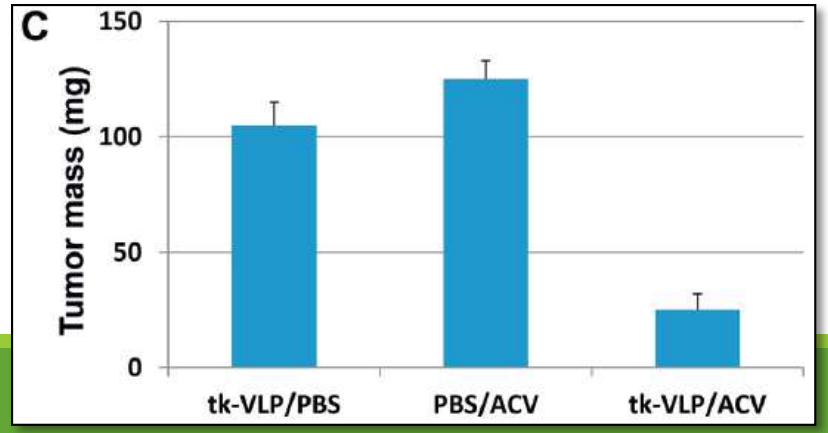


Gene. 2013 Aug 10;525(2):162-9.

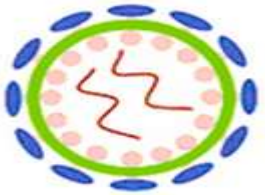
The tk suicide gene delivered by JCPyV VLPs could effectively inhibit the growth of bladder tumors in vivo





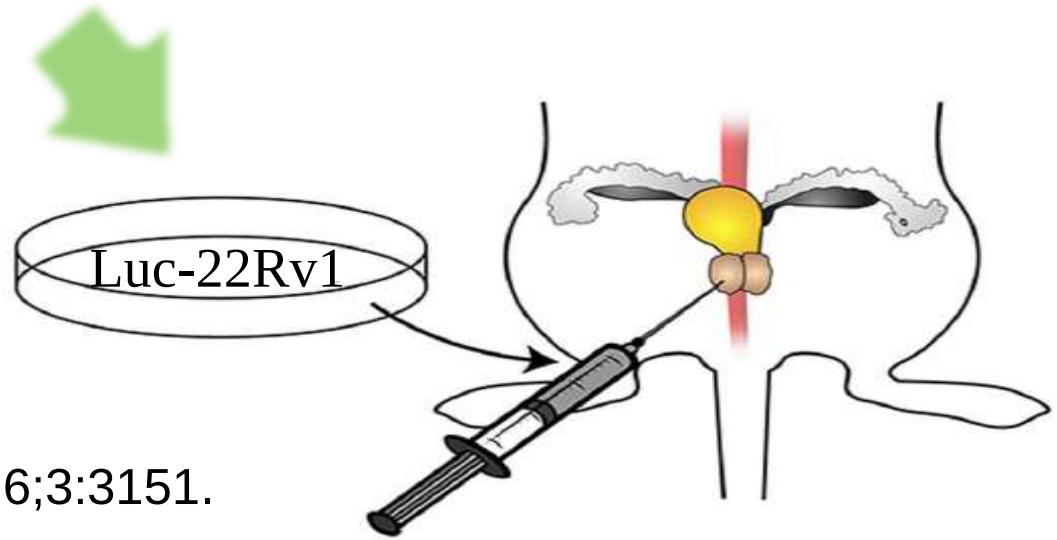


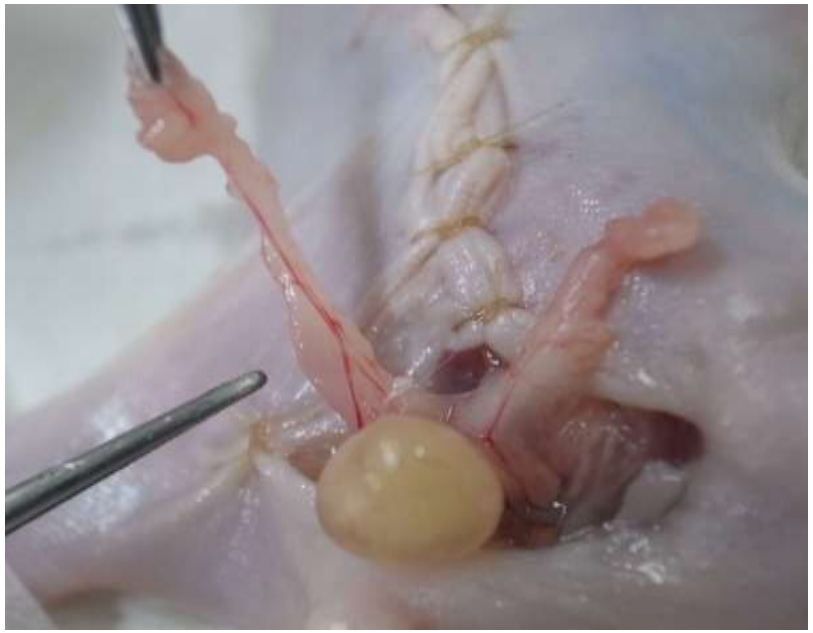
Development of an orthotopic human prostate tumor model



Lentivirus

carrying Luciferase transgene



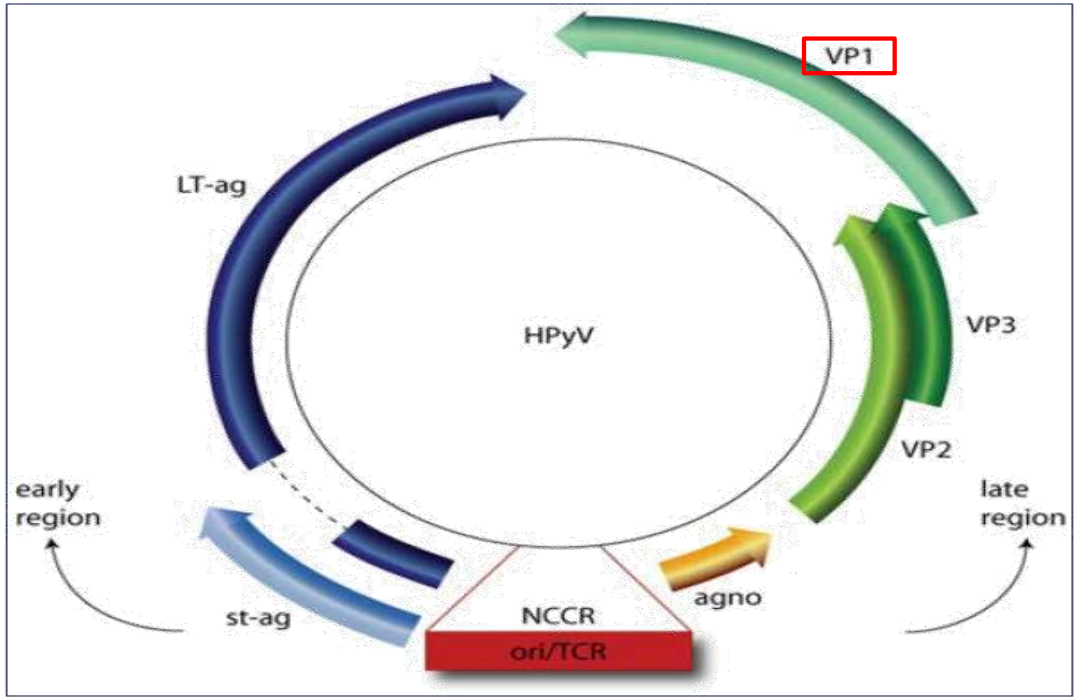


Sci Rep. 2013 Nov 6;3:3151.

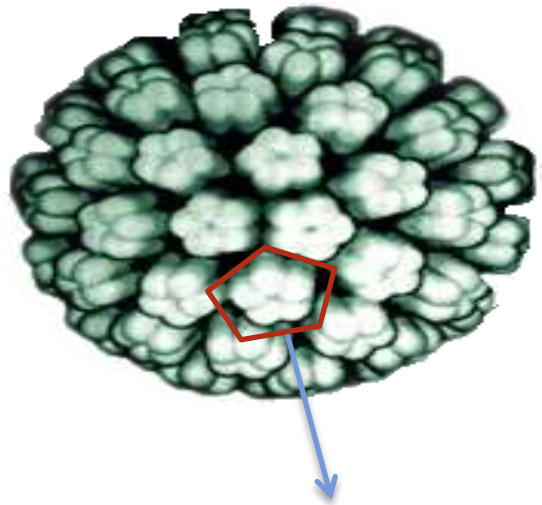
http://www.cellbiolabs.com/lentivirus-associated-p24-elisa-kit

JC polyomavirus (JCPyV)

JCPyV, a small nonenveloped DNA virus that infects humans.



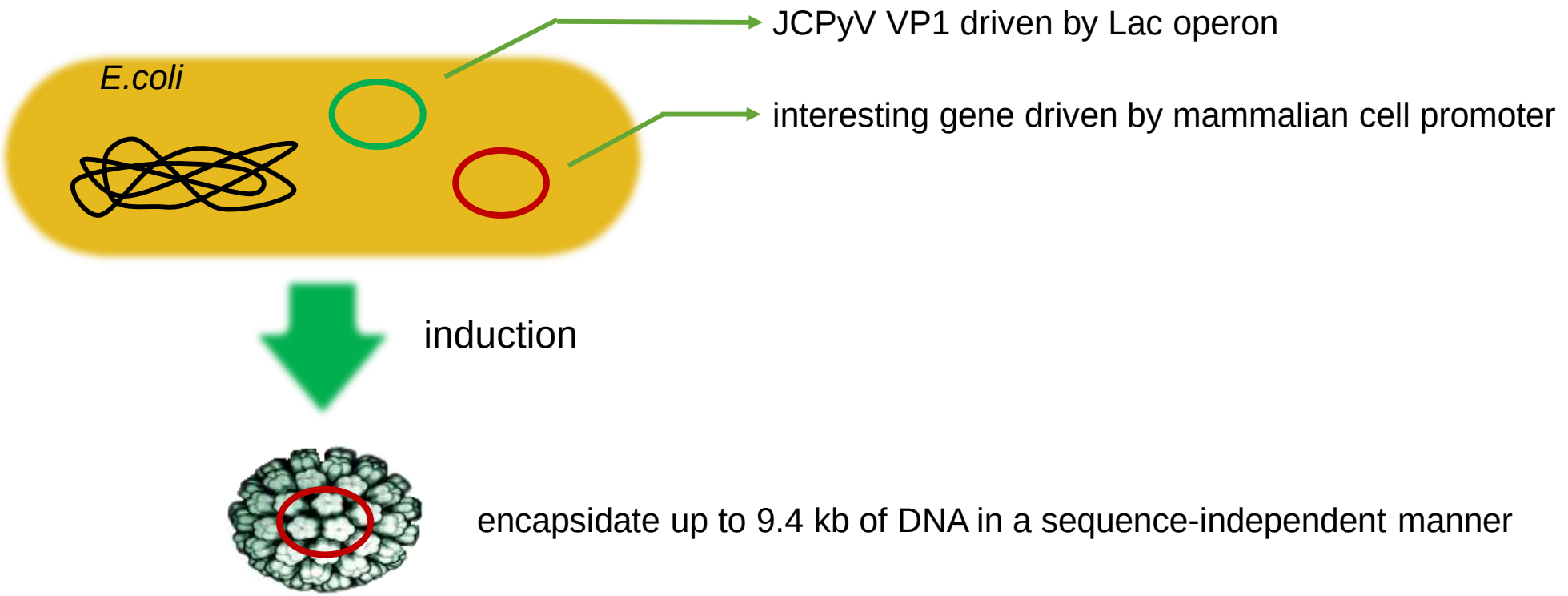
J Dtsch Dermatol Ges. 2008 Sep;6(9):704-8.



A capsomere of 5 VP1+VP2 or VP3

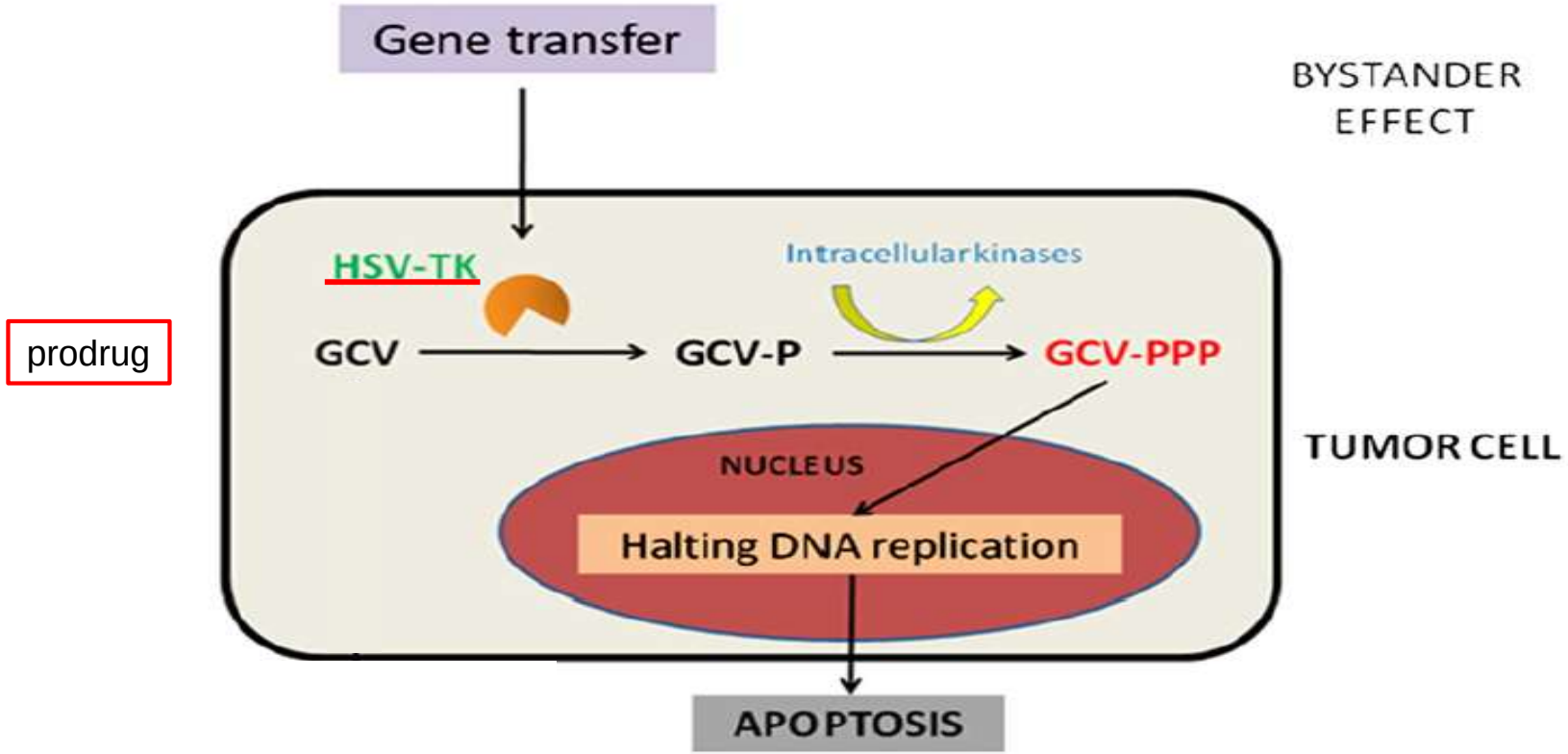
(83%)

JCPyV VP1 alone can self-assemble into virus-like particles (VLPs) and package interesting gene simultaneously in *E.coli*



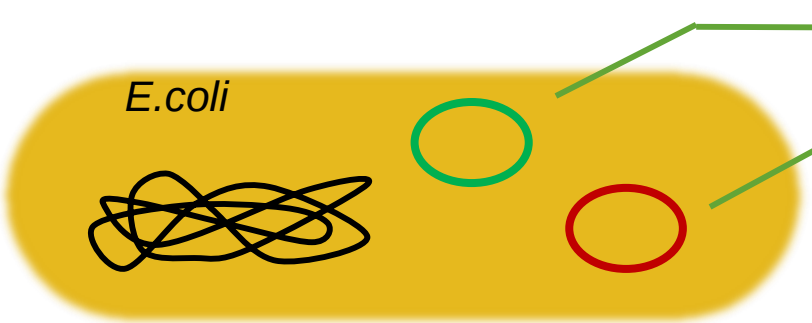
J Gen Virol. 1999 Jan;80 (Pt 1):39-46.

Herpes simplex virus thymidine kinase (HSV-tk), a suicide gene



Gene. 2013 Aug 10;525(2):162-9.

JCPyV VLPs will package with tk gene in *E. coli*



JCPyV VP1 driven by Lac operon

tk gene driven by CMV promoter (pUMVC1-tk)

induction

tk-VLP

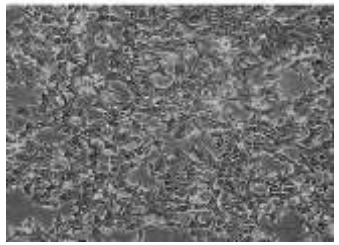
Using JCPyV VLPs for delivery of the tk gene to inhibit prostate tumor growth in culture and in an animal model.

The cytotoxicity of this tk-VLP/GCV system will be assessed using Cell Counting Kit-8

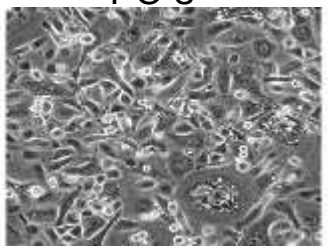
tk-VLP

GCV

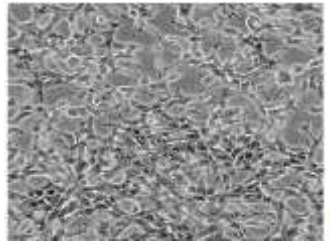
22Rv1



PC-3



LNCaP



ATCC

1. Add CCK-8 solution

2. Incubate for 1-4 hours

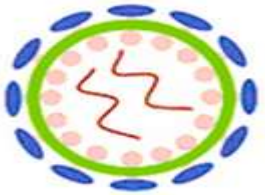
3. Measure O.D. at 450 nm

DOJINDO

Welcome to Dojindo Europe!

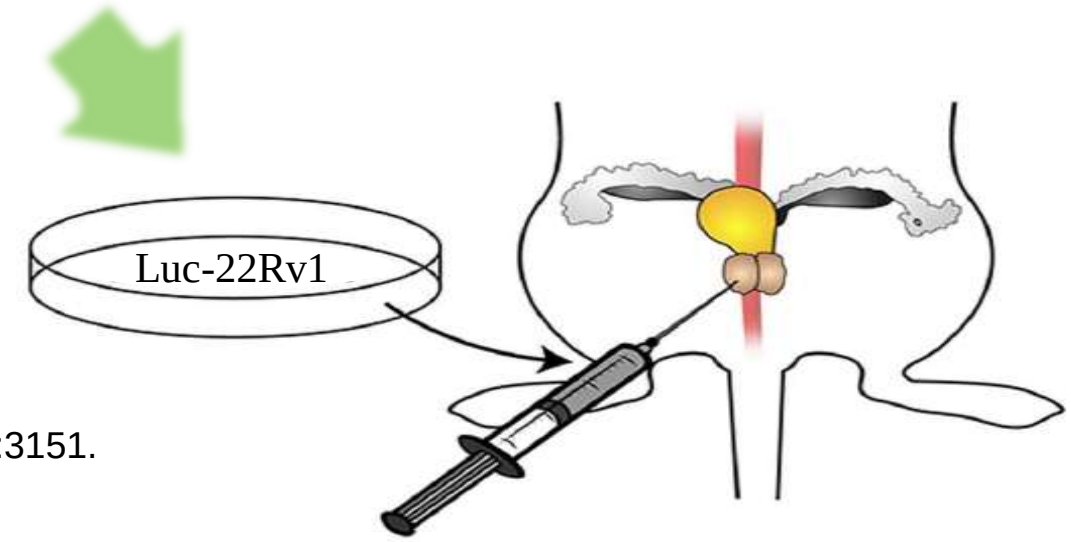
http://www.atcc.org/ (ATCC)
http://www.dojindo.eu.com/store/p/456-Cell-Counting-Kit-8.aspx

Development of an orthotopic human prostate tumor model



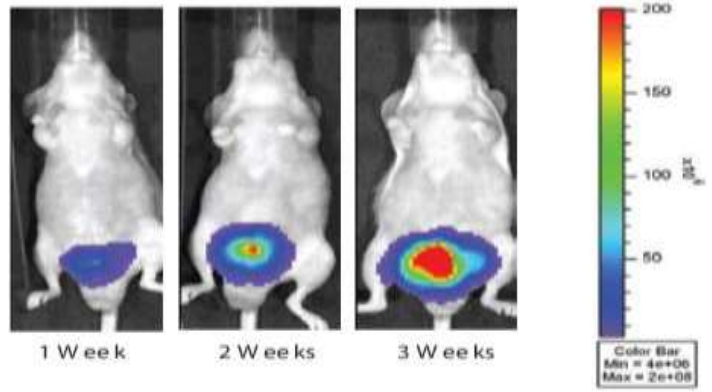
Lentivirus

carrying Luciferase transgene

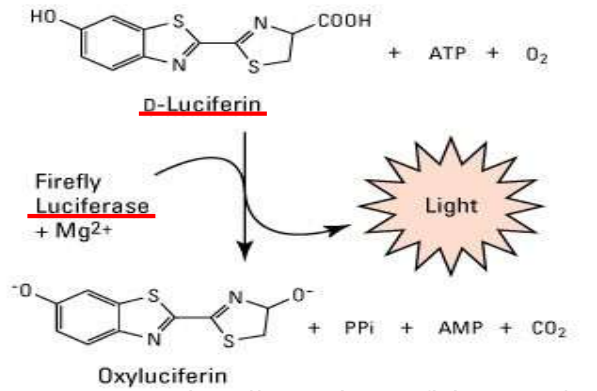


Sci Rep. 2013 Nov 6;3:3151.

http://www.cellbiolabs.com/lentivirus-associated-p24-elisa-kit

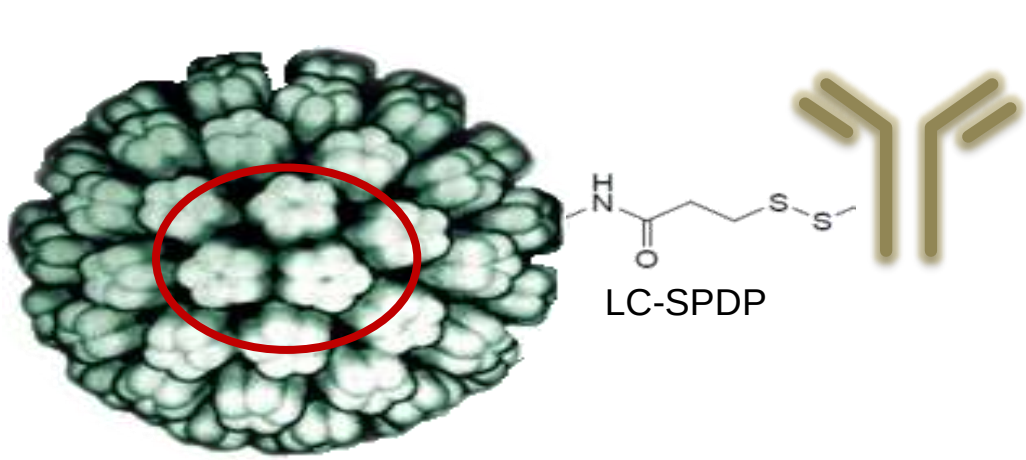


http://www.molecularimaging.com/imaging/optical-imaging



http://www.thermofisher.com/tw/

Antibody-guided JCPyV VLP for specific targeting of prostate cancer cells



The goals of this proposal

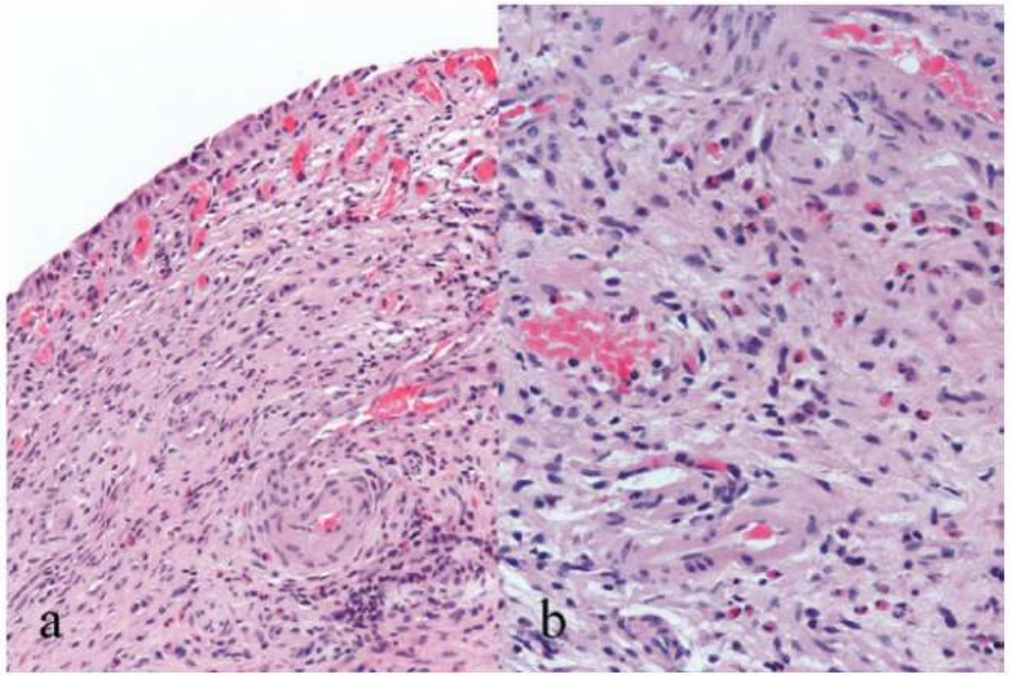
I. Examining the effectiveness of JCPyV VLPs in delivering therapeutic genes to human prostate cancer cells as a potential non–androgen deprivation therapy.

II. Examining the tissue specificity and effectiveness of JCPyV VLPs carrying reporter and therapeutic genes driven by a prostate-specific promoter.

III. Testing an antibody-guided JCPyV VLP for specific targeting of prostate cancer cells.

Ketamine-Associated Cystitis

- ketamine consumption and the occurrence of urinary symptoms and urinary tract injuries was first published in 2007.
- The authors describe the severe genitourinary symptoms experienced by 9 patients with chronic recreational ketamine use



Shahani R.et al. Urology. 2007 May;69(5):810-2.

Hong Kong Med J. 2007 Aug;13(4):311-3. Epub 2007 Jun 21.

'Street ketamine'-associated bladder dysfunction: a report of ten cases.

Chu PS, Kwok SC, Lam KM, Chu TY, Chan SW, Man CW, Ma WK, Chui KL, Yiu MK, Chan YC, Tse ML, Lau FL.

Department of Surgery, Tuen Mun Hospital, Hong Kong. peggychului@gmail.com

TABLE. Physical characteristics, clinical and radiological features of the 10 patients

Patient No.	Sex/age (years)	Date of presentation	Duration of taking ketamine (years)	ALP/ALT* (U/L)	Serum creatinine (μmol/L)	USG† kidney
1	F/25	Nov 2000	1	382/129	400	B hydro
2	M/30	Jun 2006	2	142/27	220	B hydro
3	M/30	Sep 2006	4	413/83	177	B hydro
4‡	M/25	Jan 2007	Unknown	558/407	99	B hydro
5‡	F/22	Jan 2007	Unknown	164/74	46	B hydro
6	M/25	Feb 2007	2	114/114	85	Normal
7	M/26	Mar 2007	Unknown	124/242	95	B hydro
8§	M/20	Mar 2007	Unknown	107/40	75	Normal
9§	M/21	Mar 2007	1	624/1141	237	B hydro
10	F/26	Apr 2007	1	229/48	100	B hydro

* ALP denotes alkaline phosphatase (reference range, 46-127 U/L); and ALT alanine aminotransferase (reference range, 10-57 U/L)

† USG denotes ultrasonography, and B hydro bilateral hydronephrosis

‡ Patients 4 and 5 are a couple; patient 5 became a ketamine abuser after marrying patient 4

§ Patient 8 was prescribed cimetidine while patient 9 was prescribed omeprazole for epigastric pain

Hong Kong Med J. 2007 Aug;13(4):311-3. Epub 2007 Jun 21.

'Street ketamine'-associated bladder dysfunction: a report of ten cases.

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FIG 2. Video-urodynamic study of patient 2 with markedly contracted and trabeculated bladder

FIG 4. Right antegrade nephrostogram of patient 2 showing complete obstruction just below the pelvic-ureteric junction level

Ketamine

- Ketamine was synthesized in 1962, which was initially introduced into clinical use as dissociative anesthesia in 1970.

  Clin. Pharmacol. Ther. (1965)6: 279-291.
  Handb. Exp. Pharmacol. (2008): 313-333.

- Ketamine is similar pharmacologically to phencyclidine (PCP or 'Angel Dust').

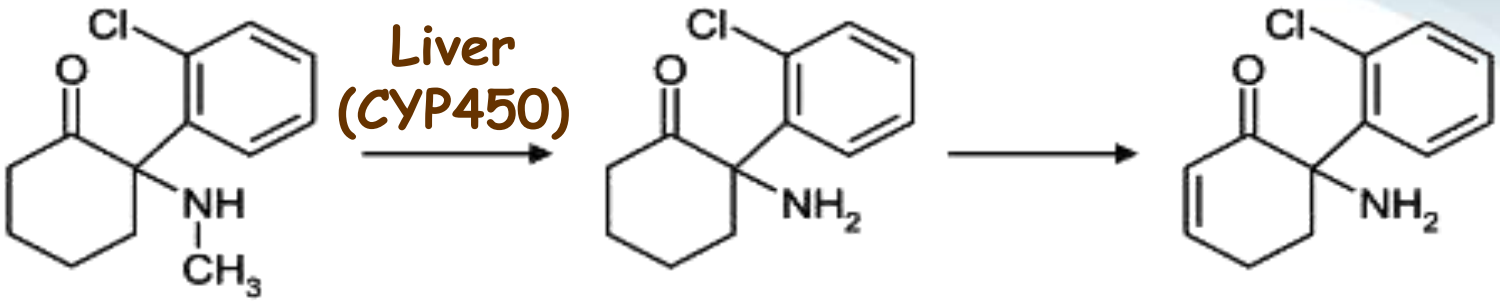
  J. Pain. Symptom. Manag. (2011)41: 640-649.

- It is a noncompetitive N-methyl-D-aspartic (NMDA) acid receptor antagonist used as a short-acting general anesthesia in both human and veterinary settings. In human, ketamine is used for pediatric anesthesia.

  Minerva. Anesthesiol. (2003)69: 468-471.

Ketamine

Ketamine	:	Norketamine	:	Dehydronorketamine
1	:	1.3	:	2.8



林惠茹，慈濟大學醫學研究所博士論文，中華民國九十六年一月

- Ketamine induces anesthesia with the advantage of bronchodilation and no cardiovascular depression.

Anesth. Prog. (1992)39: 61-68.

- It's clinical use may be limited by side-effect of hallucination. These side-effect of hallucination and "K-hole" state may be exploited by individuals using ketamine as a recreational drug.

Handb. Exp. Pharmacol. (2008)313-333.

Summary of patient details and clinical presentation of ketamine abuse

Case	Age/sex	Route	Dosage (per day, in g)	Time to symptom onset (months)	Blood urea nitrogen/creatinine (mg/dL)	Hydronephrosis	Bladder capacity (mL)	Hemorrhagic cystitis
1	26/M	Inhalation	1	12	16/1.1	Bilateral	<150	–
2	25/F	Inhalation	3–4	3	11/0.6	Unknown	Unknown	–
3	26/F	Inhalation	1	2	Unknown	Bilateral	<150	–
4	22/M	Inhalation	1	12	15/0.9	Normal	>150	–
5	21/M	Inhalation	0.3	6	9/0.7	Normal	<150	–
6	26/M	Inhalation	1–3	1	16/0.9	Unknown	>150	–
7	25/M	Inhalation	5	6	11/1.0	Bilateral	<150	+
8	19/F	Inhalation	1	4	10/0.9	Bilateral	<150	+
9	29/M	Inhalation	3–5	15	22/1.7	Bilateral	<150	–
10	23/F	Inhalation	2–3	24	7/0.5	Normal	<150	–
11	19/M	Inhalation	1–2	3–4	17/1.0	Normal	<150	–

- Inhalation range : 5 mg/kg/day ~ 83 mg/kg/day

International Journal of Urology (2009) 16, 826–829

Urinary toxicity use of ketamine

- The clinical syndromes include, severe frequency, urgency, dysuria, hematuria and remarkable reduced bladder volume.

  Urology. (2007)69: 810-2.

- Pathological changes with urothelial ulceration and eosinophil infiltration were also found in their bladders.

  Urology. (2007)69: 810-2.
  BJU Int. (2008)102: 1616–1622.

- Yew et al. (2009) and Cha et al. (2011) et al. have indicated that some similar clinical symptoms were also found in the mouse model which simulated ketamine abusers, but the precise mechanism of pathogenesis is still far from clear and required further investigation.

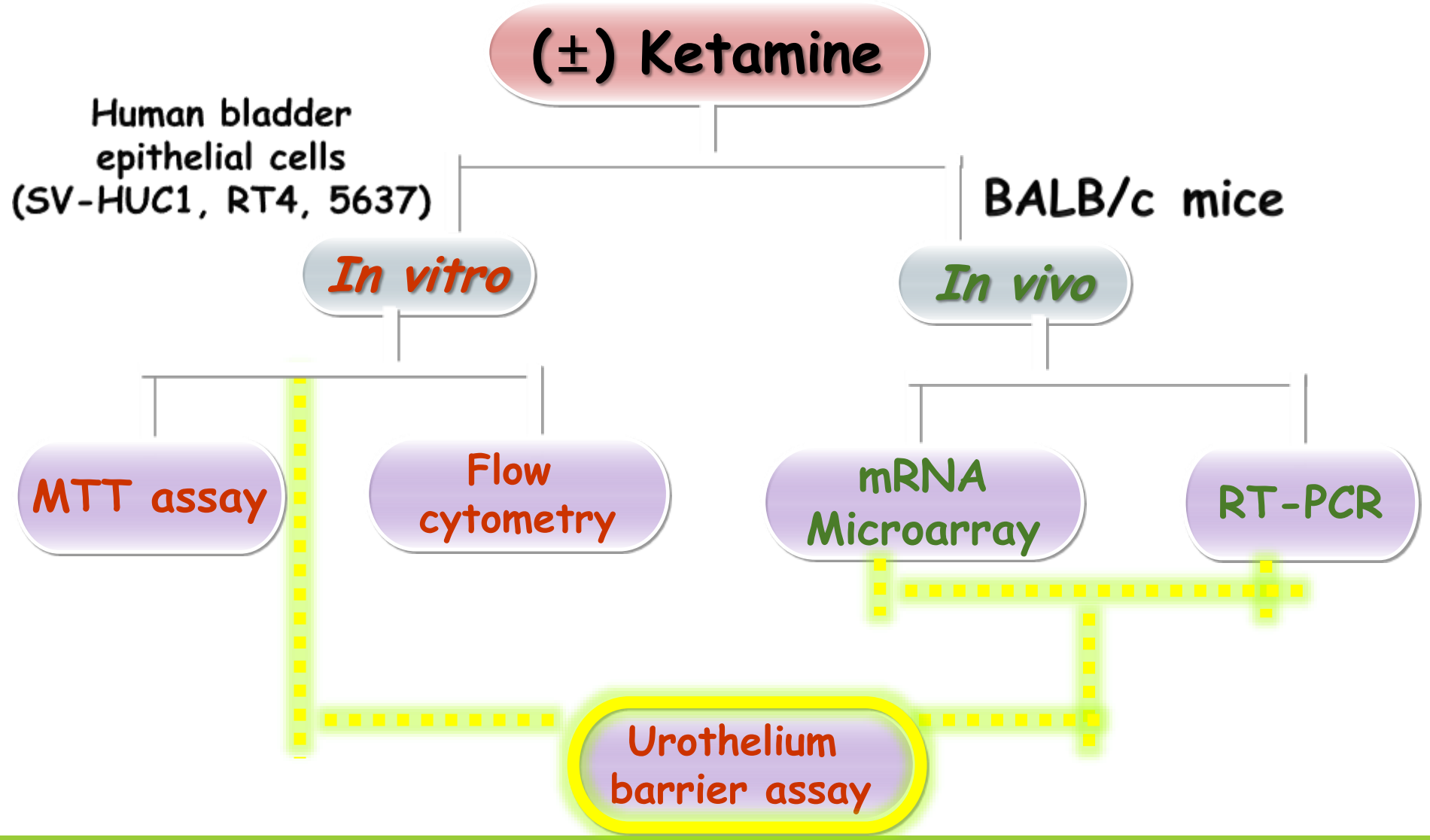
  Toxicol. Lett. (2009)191: 275–278.
  J. Urol. (2011)186: 1134-1141.

Aims

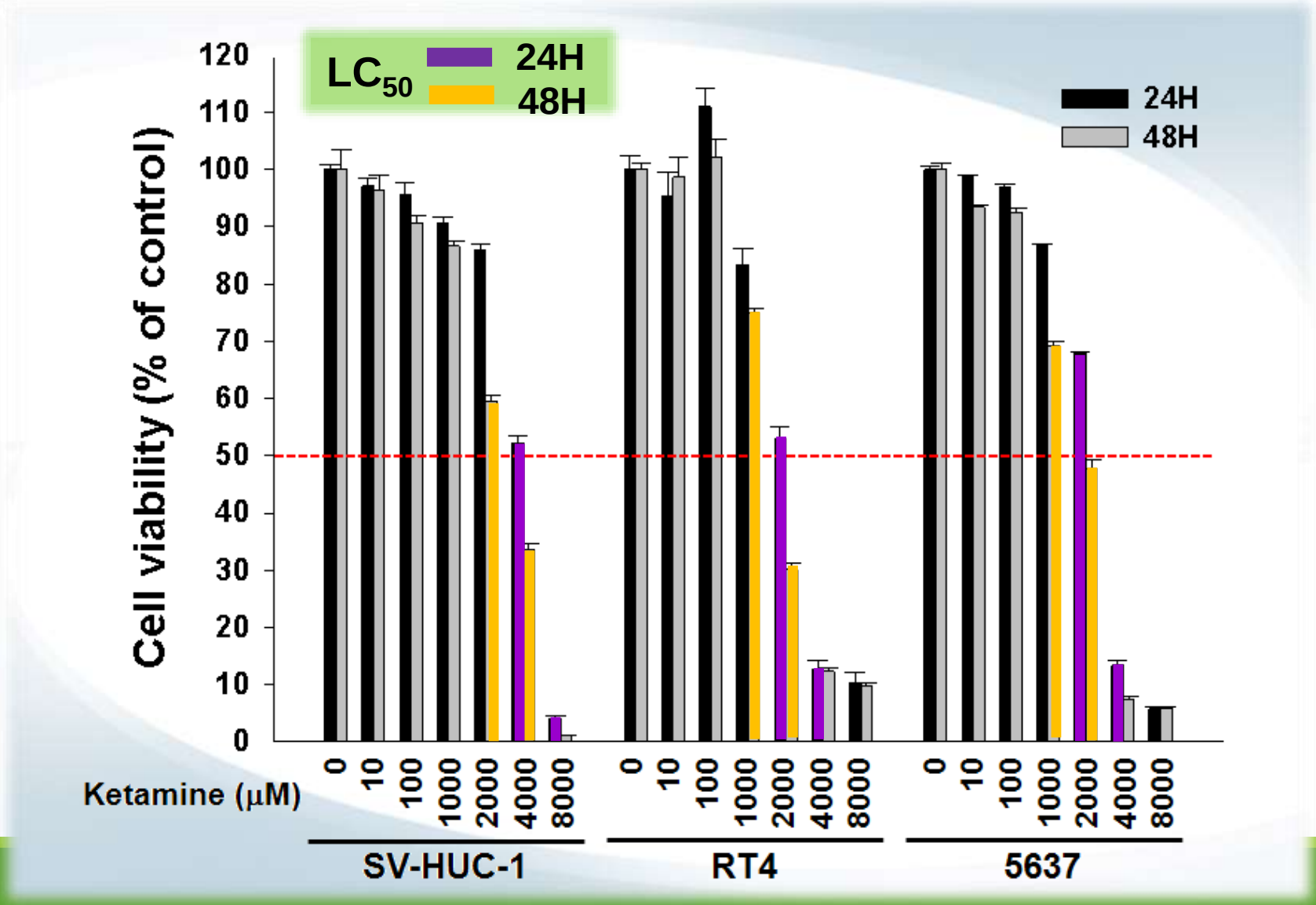
- *In vitro* study, the cell cytotoxicity and cell cycle change of human bladder cell lines by ketamine were studied.

- *In vivo* study, we established the Ketamine-abused animal model and observed gene expression change in mouse bladder tissue.

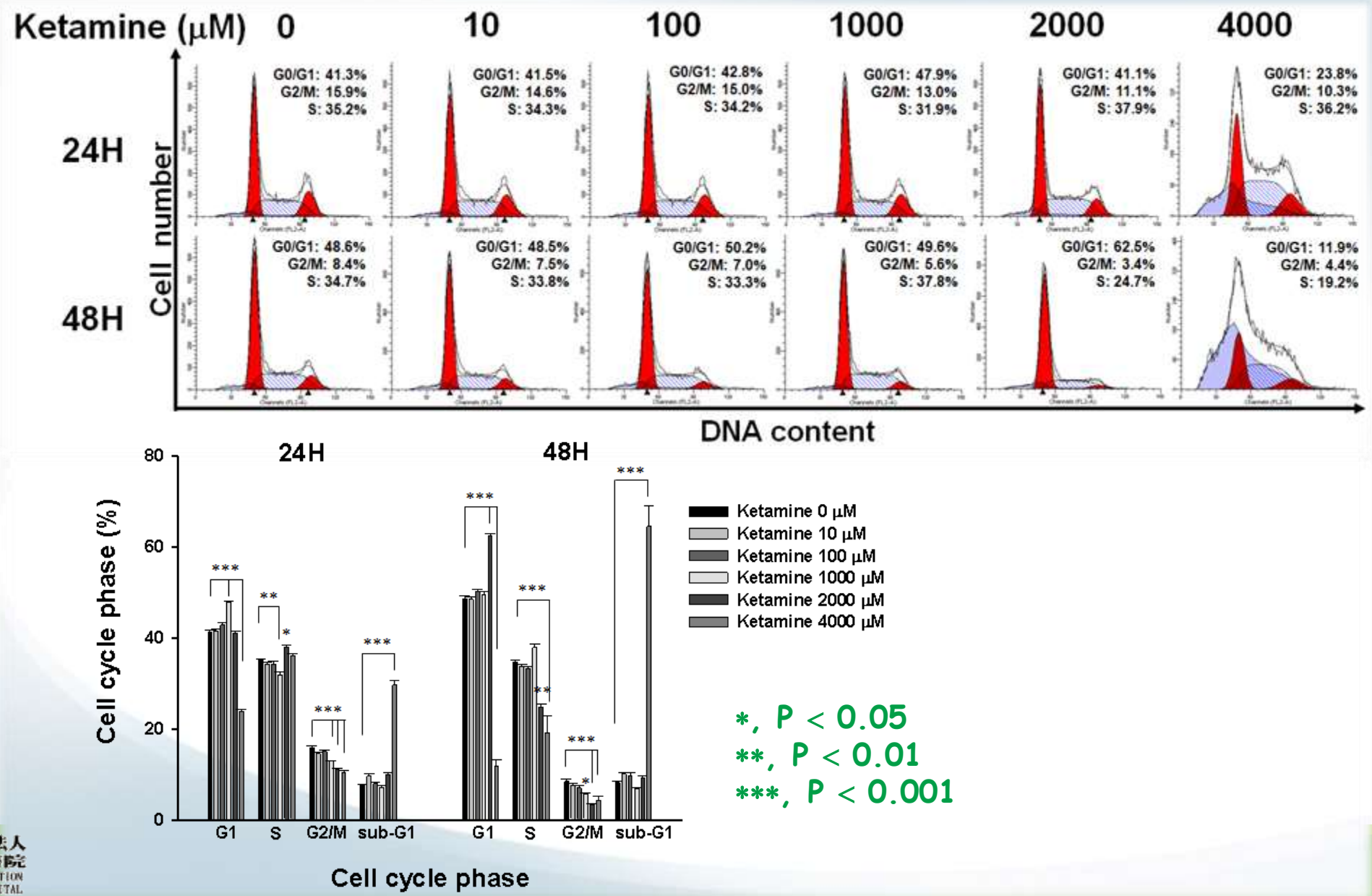
Materials and methods



Cytotoxicity of ketamine against two human bladder cancer cell lines and a human normal urothelium



Effect of ketamine on cell cycle distribution of 5637 cells



*, P < 0.05
**, P < 0.01
***, P < 0.001

Summary

- Ketamine has less cytotoxicity (LC_{50} > 1000 μM) in human urothelial cells, but ketamine in high concentration (> 1000 μM) significantly causes SV-HUC-1, RT4, 5637 cell arrest in G1 phase.
- Ketamine (> 2000 μM) increases sub-G1 level.

Flow chart

Observe the cytotoxicity and cell cycle change of urothelial cells by ketamine treatment

Observe the physiological and pathological changes in mouse after ketamine treatment

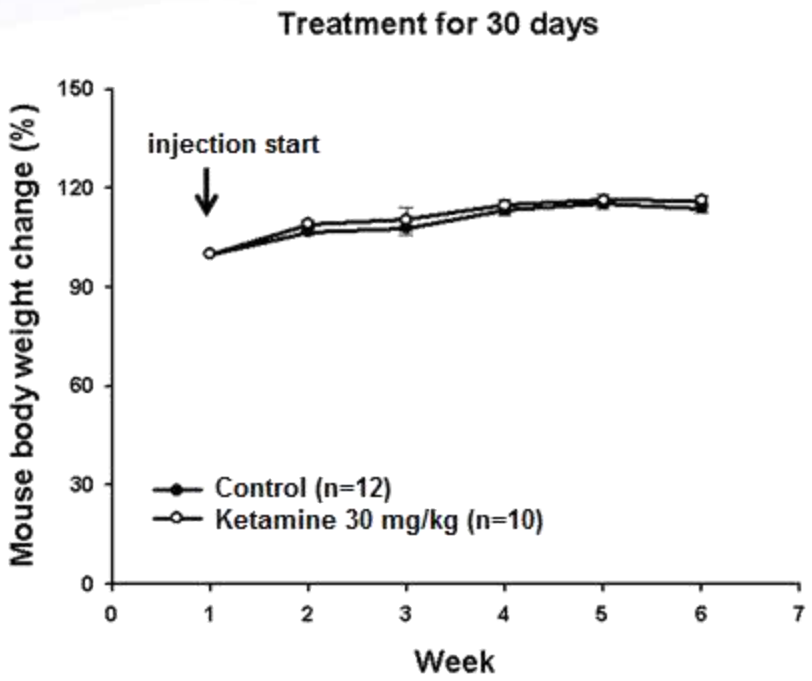
Observe the change of mRNA expression in mouse bladder tissue after ketamine treatment

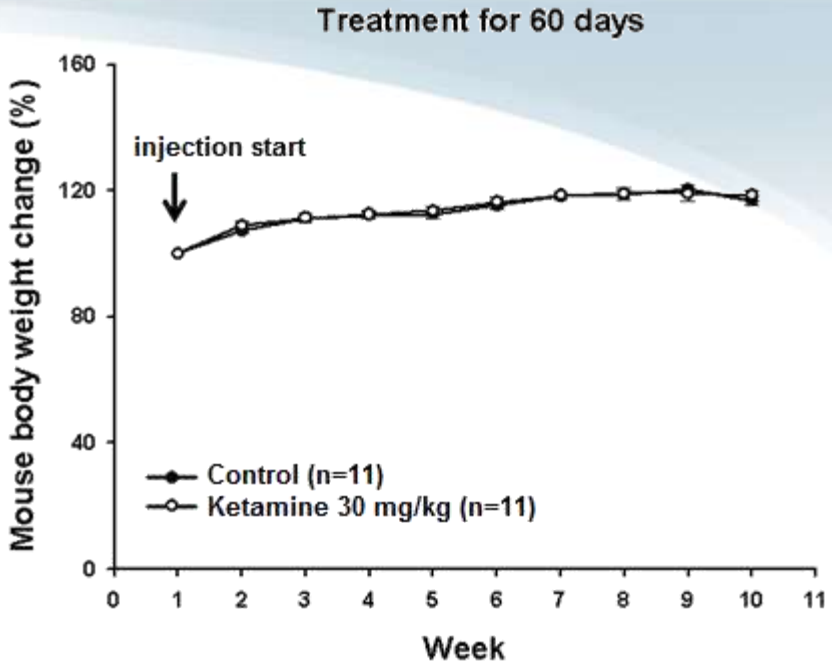
Epithelial cell adhesion

Bladder mucin protection

Urination

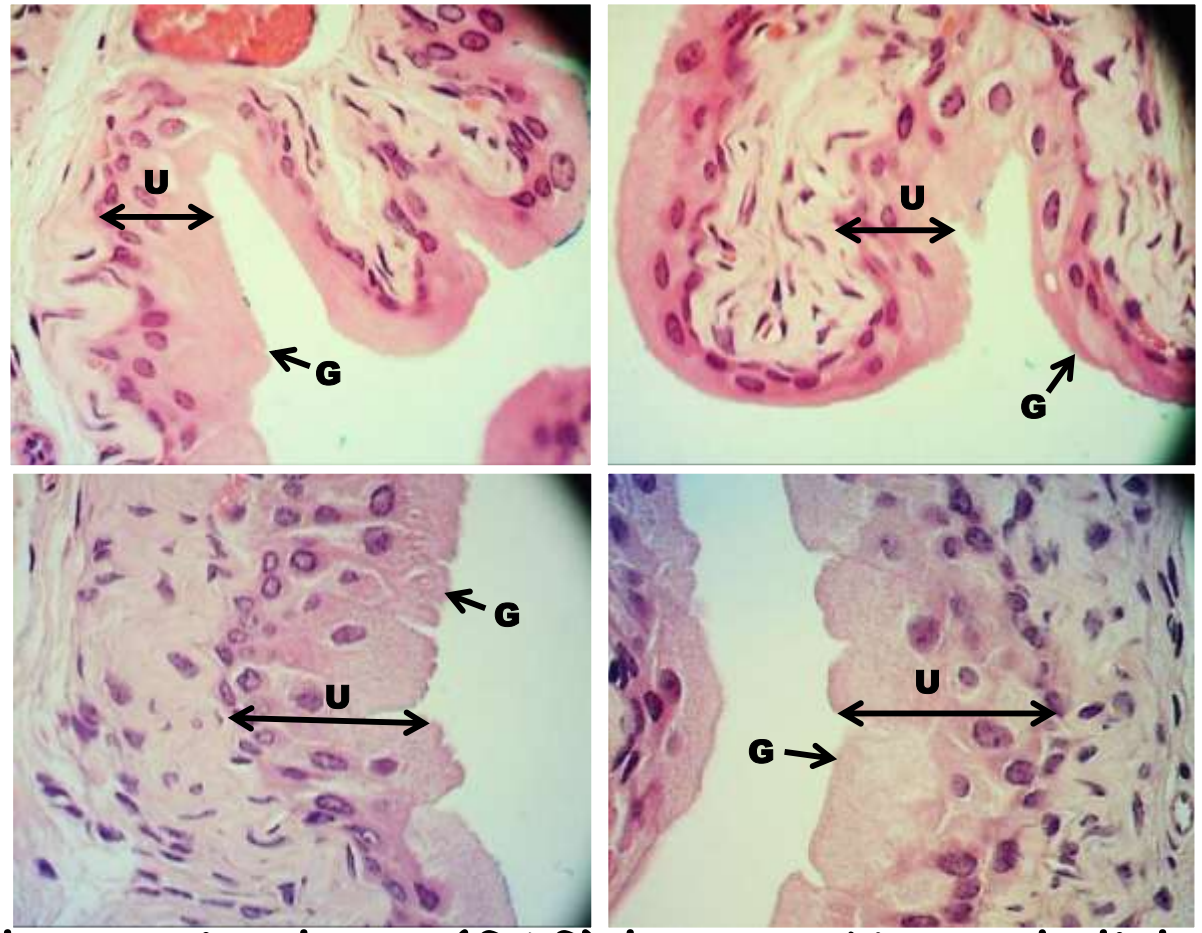
Change of mouse body weight





After 30 and 60 days treatment, there were no significant changes in mouse body weight between control and ketamine group.

Histology of mouse bladder tissue (HE stain - 400X)



G: glycosaminoglycan (GAG) layer, U: urothelial cells

Summary

- The morphology of urothelium between control and ketamine-treated group in histology has no significant difference.
- There is no inflammation reaction in mouse epithelial by ketamine treatment for 30 and 60 days.

Flow chart

Observe the cytotoxicity and cell cycle change of urothelial cells by ketamine treatment

Observe the physiological and pathological changes in mouse after ketamine treatment

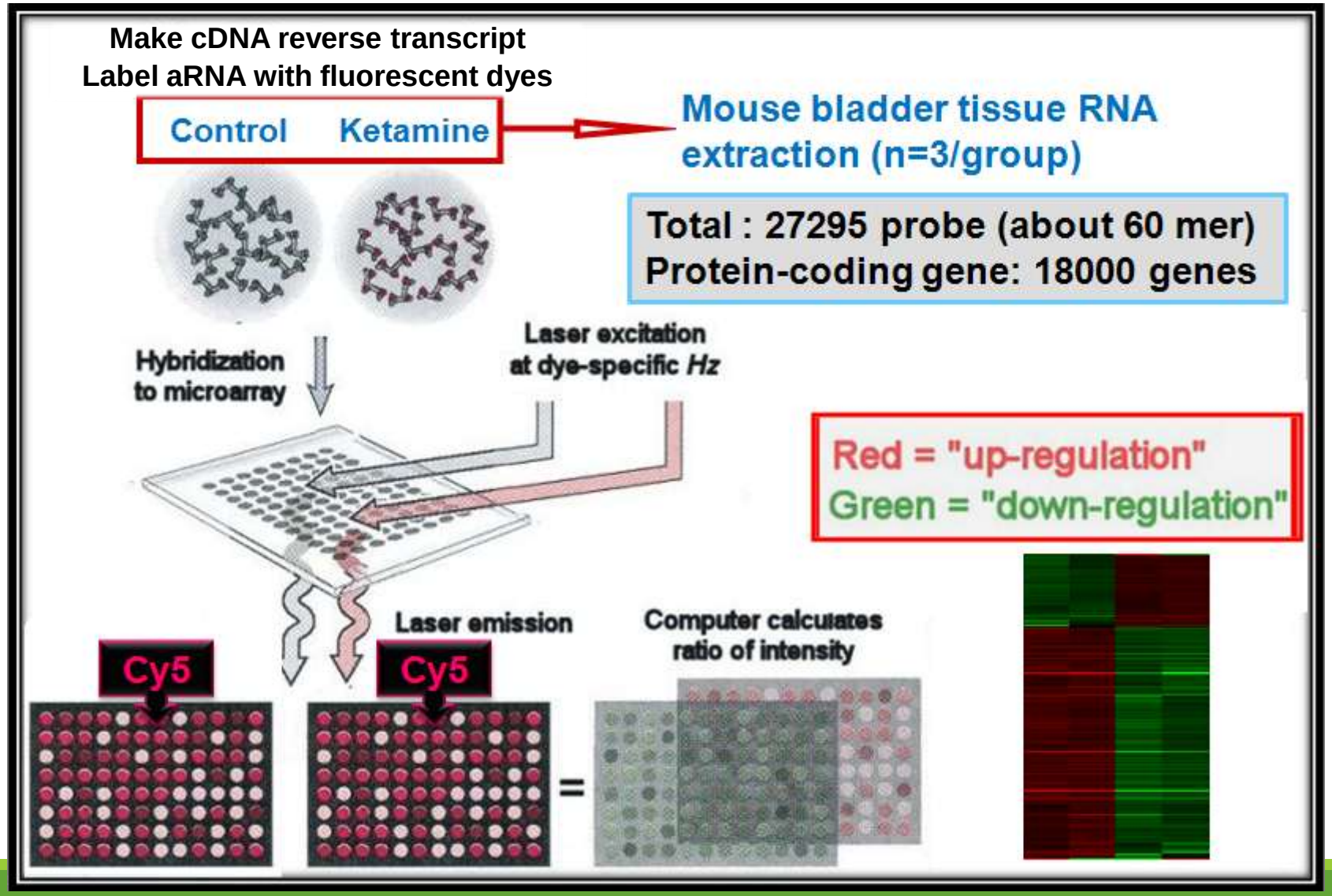
Observe the change of mRNA expression in mouse bladder tissue after ketamine treatment

Epithelial cell adhesion

Bladder mucin protection

Urination

cDNA microarray analysis from Phalanx Biotech Group



Genes selection procedure

Significant change (| log2 | > 1, p < 0.05, intensity ≧ 500) of genes selected from array data

Ongoing change of gene expression after ketamine treatment for 30 and 60 days

Clinical symptoms-related genes

Gene expression changes in mice after 30 mg/kg ketamine injection for 30 and 60 days

Accession number	Gene symbol	Gene name	Ratio of change (%)	
Up-regulated genes			30 days	60 days
NM_019662.2	Rrad	Ras-related associated with diabetes	140.6 # *	95.4 *
NM_205823.2	Tlr12	toll-like receptor 12	107.3 *	132.8 *
Down-regulated genes				
NM_001164724.1 NM_133775.2	Il33	interleukin 33	-49.7 # *	-67.6# *
NM_027961.1	Wfdc3	WAP four-disulfide core domain 3	-54.6 *	-88.7 *
NM_001163161.1 NM_010819.4	Clec4d	C-type lectin domain family 4, member d	-51.5 *	-67.0 *
NM_001044384.1 NM_011593.2	Timp1	tissue inhibitor of metalloproteinase 1	-61.9 # *	-76.8 # *
NM_008496.4	Lgals7	lectin, galactose binding, soluble 7	-78.9 *	-59.3 *
NM_029352.3	Dusp9	dual specificity phosphatase 9	-59.5 *	-53.6 *
NM_001195732.1	Fam150a	family with sequence similarity 150, member A	-49.6 *	-59.9 *
NM_016958.1	Krt14	keratin 14	-44.4#	-74.6# *

#, normalized intensity ≥ 500. *, $p < 0.05$, significant difference between vehicle and ketamine-treated mice. -, down-regulated.

Flow chart

Observe the cytotoxicity and cell cycle change of urothelial cells by ketamine treatment

Observe the physiological and pathological changes in mouse after ketamine treatment

Observe the change of mRNA expression in mouse bladder tissue after ketamine treatment

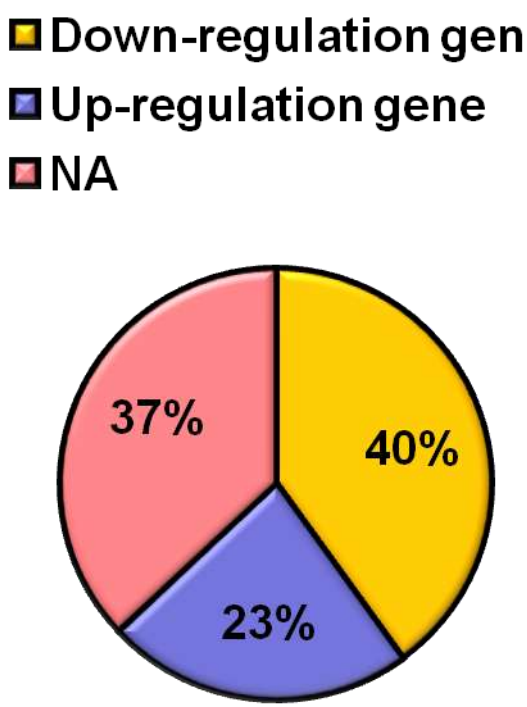
Epithelial cell adhesion

Bladder mucin protection

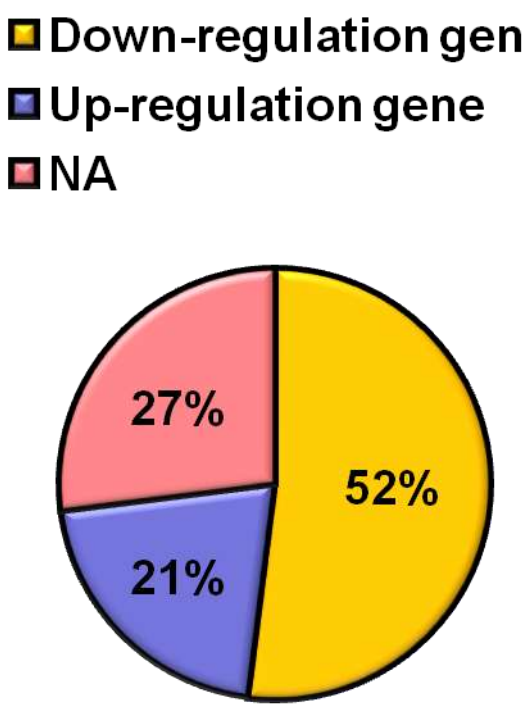
Urination

Percentage of change pattern in keratin family genes

Treatment for 30 days



Treatment for 60 days

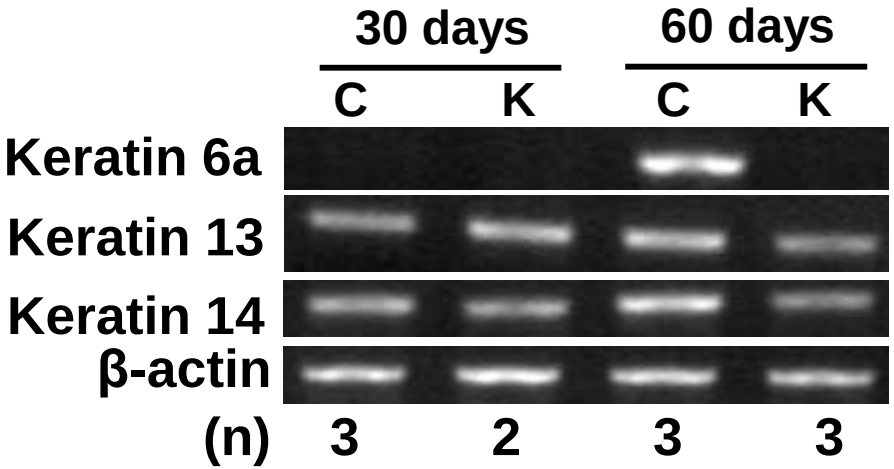


Top ten down-regulated keratin genes after 30 mg/kg ketamine injection for 30 and 60 days

Gene	AN [a]	Normalized intensity				Ratio of change (%)	
		30 days		60 days		(K-C) / C × 100%	
		C	K	C	K	30 days	60 days
Keratin 4	NM_008475.2	412.8	202.9	816.7	618.5	-50.8 *	-24.3
Keratin 5	NM_027011.2	6344.0	6327.0	9821.2	5129.2	-0.3	-47.8
Keratin 6a	NM_008476.3	42.6	24.3	985.4	46.0	-43.0	-95.3 *
Keratin 7	NM_033073.3	19855.3	21426.8	25146.2	18519.1	7.9	-26.4
Keratin 8	NM_031170.2	13729.3	15504.3	20223.6	13109.0	12.9	-35.2 *
Keratin 13	NM_010662.1	130.9	170.0	1165.9	181.6	29.9	-84.4 *
Keratin 14	NM_016958.1	2972.3	1652.2	6385.7	1621.5	-44.4 *	-74.6 *
Keratin 15	NM_008469.2	21919.7	18515.5	28069.2	20859.5	-15.5	-25.7
Keratin 19	NM_008471.2	9891.2	7572.7	15246.7	10048.5	-23.4 *	-34.1 *
Keratin 20	NM_023256.1	6661.3	9441.9	9028.3	7893.3	41.7 *	-12.6

a: is GenBank accession numbers. C: control group. K: ketamine group.

Analysis of three keratin genes mRNA by RT-PCR



Chip analysis result

Gene	Ratio of change (%)	
	30 days	60 days
Keratin 6a	- 43.0	- 95.3 *
Keratin 13	29.9	- 84.4 *
Keratin 14	- 44.4 *	- 74.6 *
β-actin	20.8	- 5.0

C : Control K: Ketamine -, Down-regulation *, $p < 0.05$

Flow chart

Observe the cytotoxicity and cell cycle change of urothelial cells by ketamine treatment

Observe the physiological and pathological changes in mouse after ketamine treatment

Observe the change of mRNA expression in mouse bladder tissue after ketamine treatment

Epithelial cell adhesion

Bladder mucin protection

Urination

Expression change of glucosaminoglycan (GAG) formation-related genes after 30 mg/kg ketamine injection for 30 and 60 days

Gene	AN [a]	Normalized intensity				Ratio of change (%)	
		30 days		60 days		(K-C) / C × 100%	
		C	K	C	K	30 days	60 days
Glucosaminyl (N-acetyl) transferase 1, core 2	NM_173442.3 NM_001136484.1 NM_010265.3	2800.1	1735.6	5114.6	2631.8	- 38.0 *	- 48.5 *
Glucosaminyl (N-acetyl) transferase 2, I-branching enzyme	NM_008105.2 NM_133219.1 NM_023887.3	2888.0	2899.1	4171.7	2404.1	0.4	- 42.4 *
Glucosaminyl (N-acetyl) transferase 3, mucin type	NM_028087.2	462.6	421.3	1258.2	555.1	- 8.9	- 55.9 *
Chondroitin sulfate proteoglycan 4	NM_139001.2	1786.9	2008.1	5063.5	2836.5	12.4	- 44.0 *
Chondroitin polymerizing factor 2	NM_133913.2	437.2	354.0	518.4	452.1	- 19.0	- 12.8

Flow chart

Observe the cytotoxicity and cell cycle change of urothelial cells by ketamine treatment

Observe the physiological and pathological changes in mouse after ketamine treatment

Observe the change of mRNA expression in mouse bladder tissue after ketamine treatment

Epithelial cell adhesion

Bladder mucin protection

Urination

Expression change of autonomic neurogenic receptor genes after 30 mg/kg ketamine injection for 30 and 60 days

Gene	AN [a]	Normalized intensity				Ratio of change (%)	
		30 days		60 days		(K-C) / C × 100%	
		C	K	C	K	30 days	60 days
Cholinergic receptor, muscarinic 2, cardiac	NM_203491.2	887.8	1832.1	546.4	319.8	106.4 *	- 41.5 *
Cholinergic receptor, muscarinic 3, cardiac	NM_033269.4	428.9	649.0	592.9	466.8	51.3 *	- 21.3

Flow chart

Observe the cytotoxicity and cell cycle change of urothelial cells by ketamine treatment

Observe the physiological and pathological changes in mouse after ketamine treatment

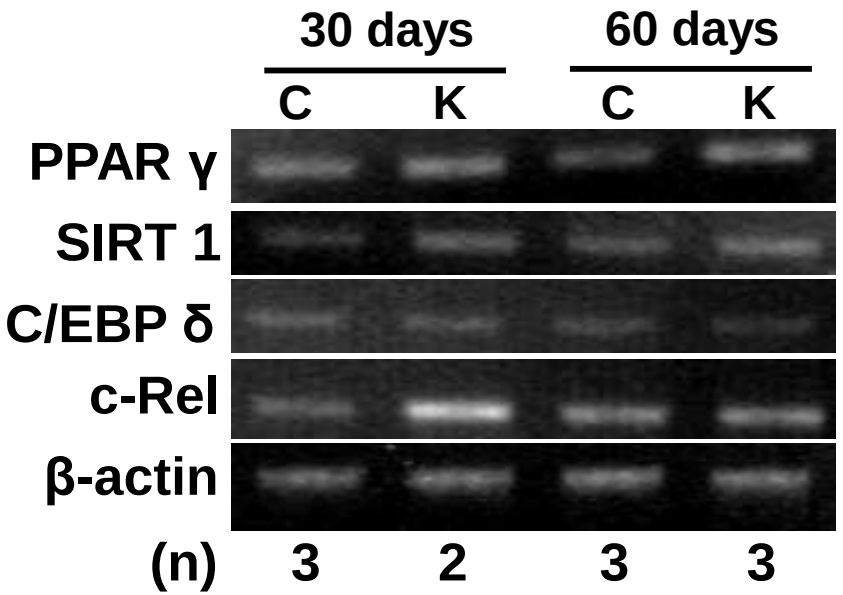
Observe the change of mRNA expression in mouse bladder tissue after ketamine treatment

Inflammation-related genes

Expression change of inflammation-related genes after 30 mg/kg ketamine injection for 30 and 60 days

Gene	AN [a]	Normalized intensity				Ratio of change (%)	
		30 days		60 days		(K-C) / C × 100%	
		C	K	C	K	30 days	60 days
Prostaglandin-endoperoxide synthase 2	NM_011198.3	301.0	265.6	167.7	106.5	- 11.8	- 36.5 *
Nitric oxide synthase 2, inducible	NM_010927.3	20.0	23.3	26.4	47.5	NA	79.9
Tumor necrosis factor	NM_013693.2	30.5	35.0	40.2	36.3	14.8	- 9.7 *
Interleukin 1 beta	NM_008361.3	115	97.7	109.6	133.6	- 15.04	21.9 *
Interleukin 6	NM_031168.1	11.5	5.0	7.9	9.5	NA	NA
Arachidonate 5-lipoxygenase	NM_009662.2	224.0	166.9	117.7	121.6	- 25.5 *	3.3

Analysis of four inflammation-related gene and others five genes mRNA by RT-PCR



Chip analysis result

Gene	Ratio of change (%)	
	30 days	60 days
IL-6	NA	NA
IL-10	1.8	10.4
COX-2	-11.8	-36.5 *
iNOS	NA	NA
PPAR γ	NA	NA
Sirt 1	14.4	29.0
C/EBP δ	5.1	- 33.5
c-Rel	47.4 *	NA
β-actin	20.8	- 5.0

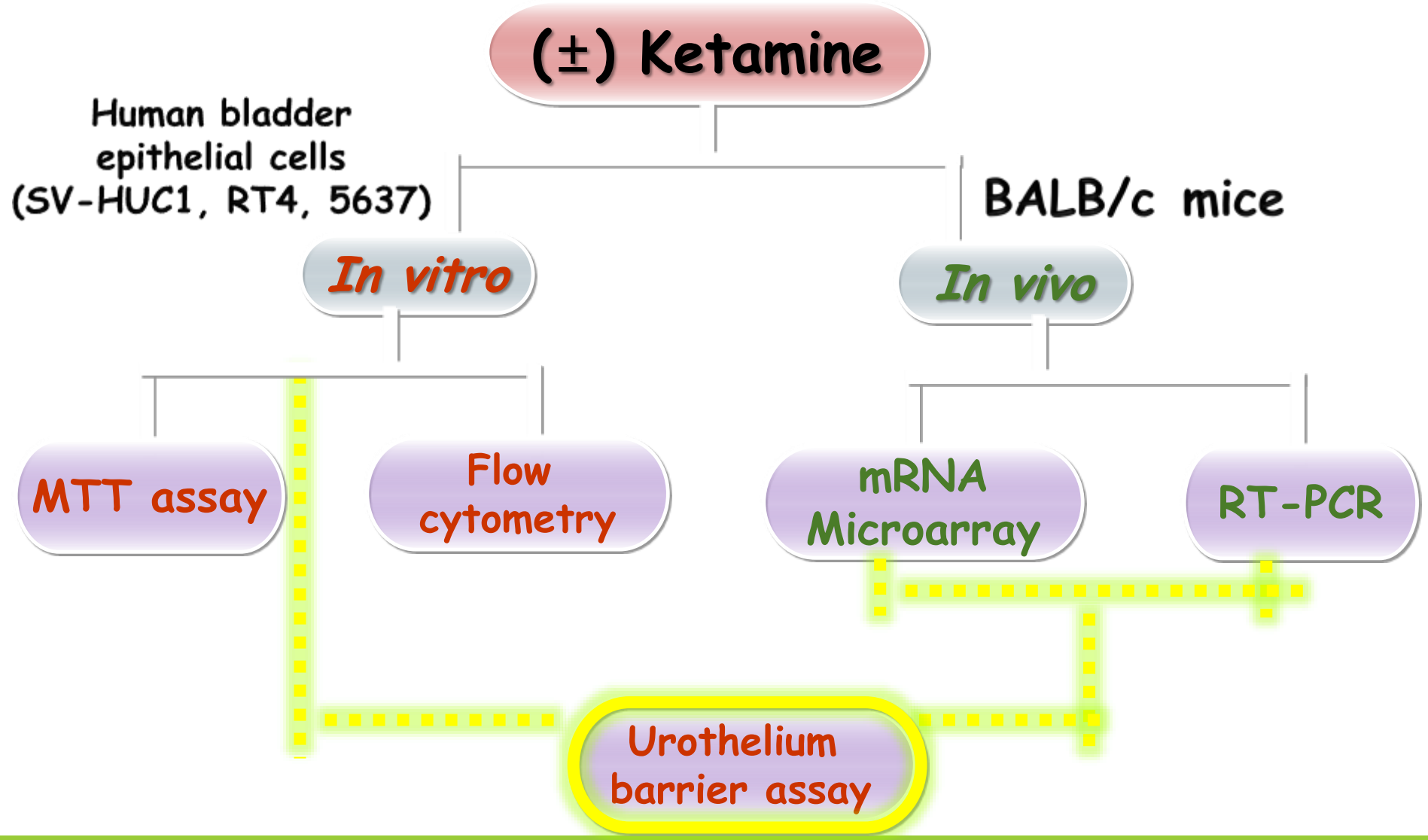
Summary

- Most of the bladder epithelium adhesion-relation genes (keratin family) and desmosome component genes are down-regulation in ketamine group.
- Most of the GAG or chondroitin synthesis genes are also down-regulation in ketamine group.
- But the uroplakin family genes are up-regulation in ketamine group.

Summary

- The autonomic neurogenic receptor genes which control urination have down-regulation tendency.
- The mRNA of inflammation-related genes (COX-2,iNOS, IL-6 and IL-10) are not induced.

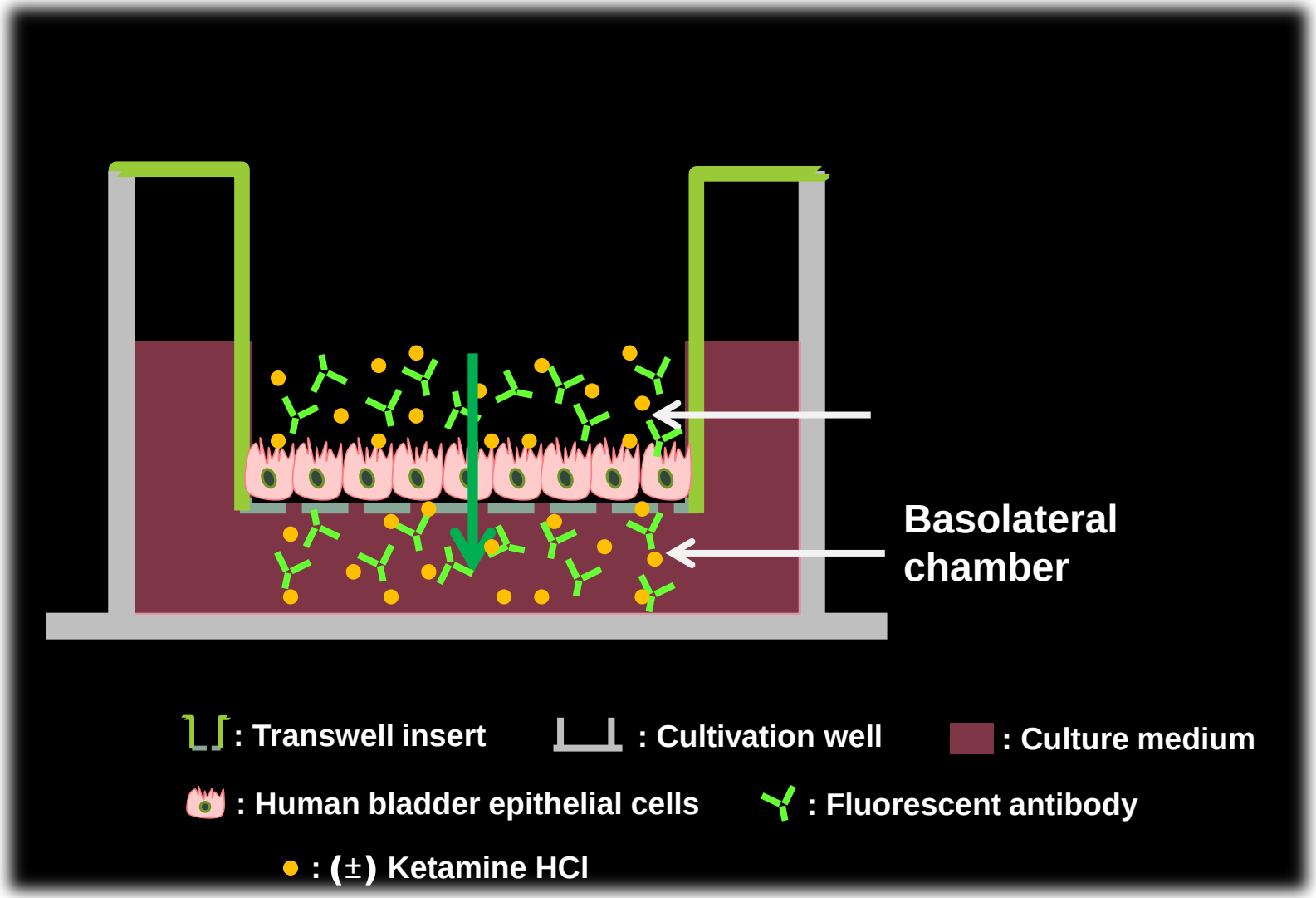
Materials and methods

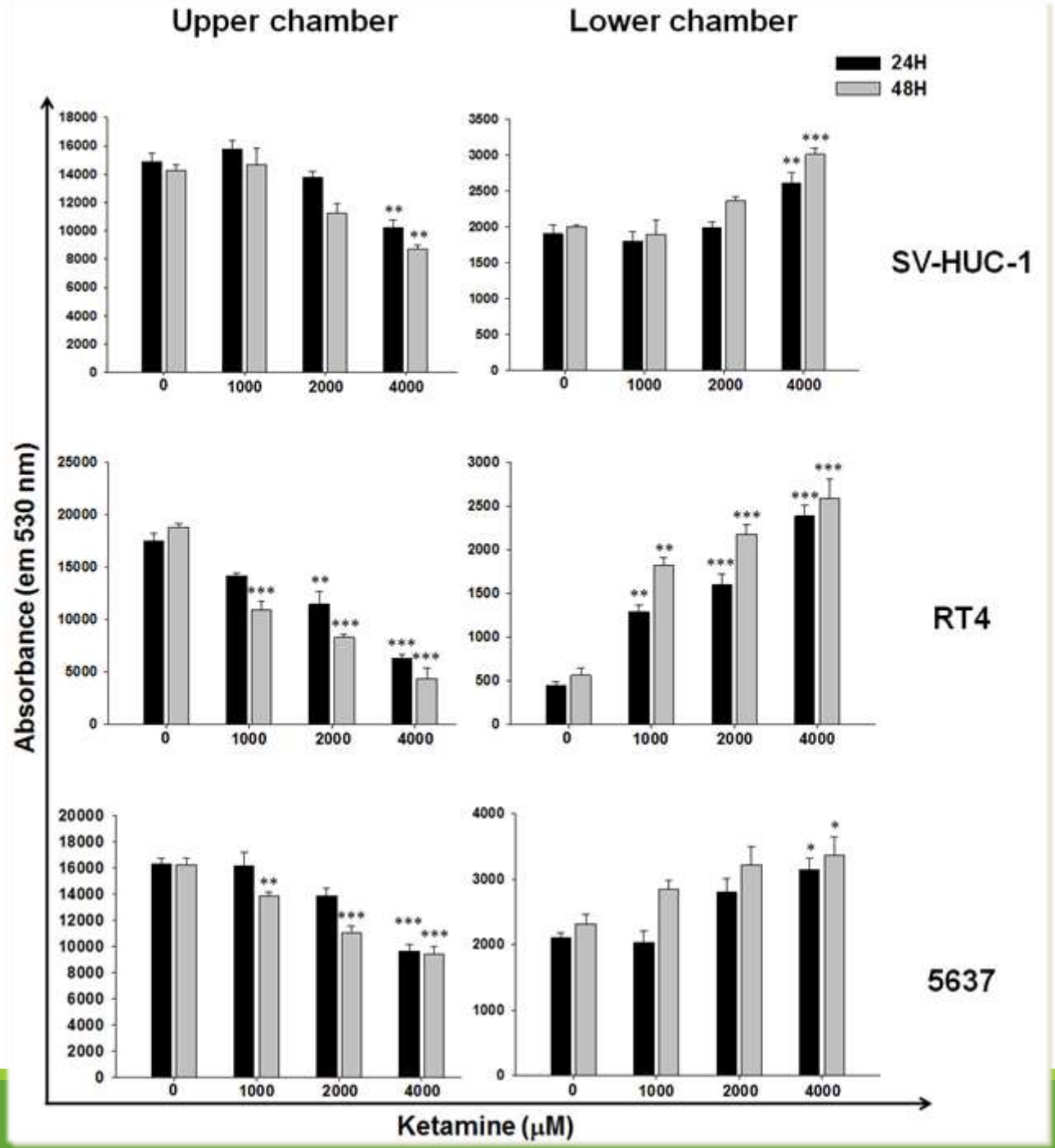


Flow chart

Observe the change of urothelium barrier permeability by ketamine treatment

Urothelium barrier assay




Upper chamber
Lower chamber
24H
48H
SV-HUC-1
RT4
5637
Absorbance (em 530 nm)
Ketamine (μM)
0
1000
2000
4000

Summary

- The results show that high dose of ketamine can significantly increase the permeability of urotheliums by green fluorescent antibody infiltration.

Conclusion

- *In vitro,* under high dose treatment (≧ 1000 μM), ketamine shows a significant cytotoxicity and the increase in urothelial permeability in three cell lines of human bladder urotheliums.

Conclusion

- *vivo*, there are no significant change after ketamine treatment under histological observation.
- The mouse whole gene chip assay shows
  - Keratin family genes down-regulation
  - Desmosome component genes down-regulation
  - Mucin synthesis genes down-regulation

Discussion

- In the previous study show that female C57BL/6 mice injected 100 mg/kg ketamine for 8 weeks and 16 weeks, they can observe mice weight gain slowed down, the thicker of bladder epithelium layer decreased and the inflammation happened in submucosa layer in ketamine group. However, they are not happened in our ketamine-abused mouse model. The differences may be associated with mouse strain, sex, ketamine dose and processing time.

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以上報告