EMEA/ICH WORKSHOP ON VIRAL/VECTOR SHEDDING

in conjunction with:

The XVth Annual Congress of the European Society of Gene and Cell Therapy

Vector Shedding and Biodistribution Following Intratumoral Delivery of Adenoviral *p53* Gene (ADVEXIN) in Patients with Advanced Non-small Cell Lung Cancer

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October 30, 2007
Rotterdam, The Netherlands

Okayama University Hospital
Center for
GENE&CELL
Therapy

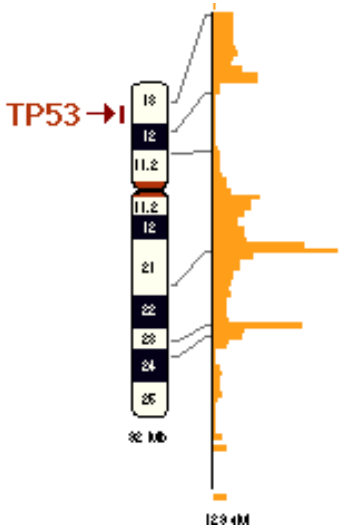
Human Gene Therapy Protocol

A Phase I Study of Adenovirus-Mediated Wild-Type *p53* Gene Transfer in Patients with Advanced Non-Small Cell Lung Cancer

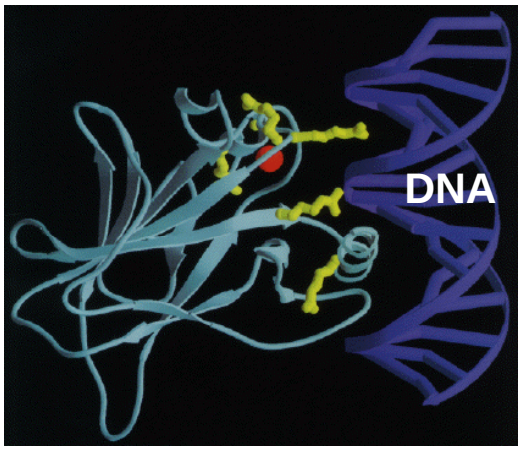
Okayama University Hospital

Tumor Suppressor *p53* Gene

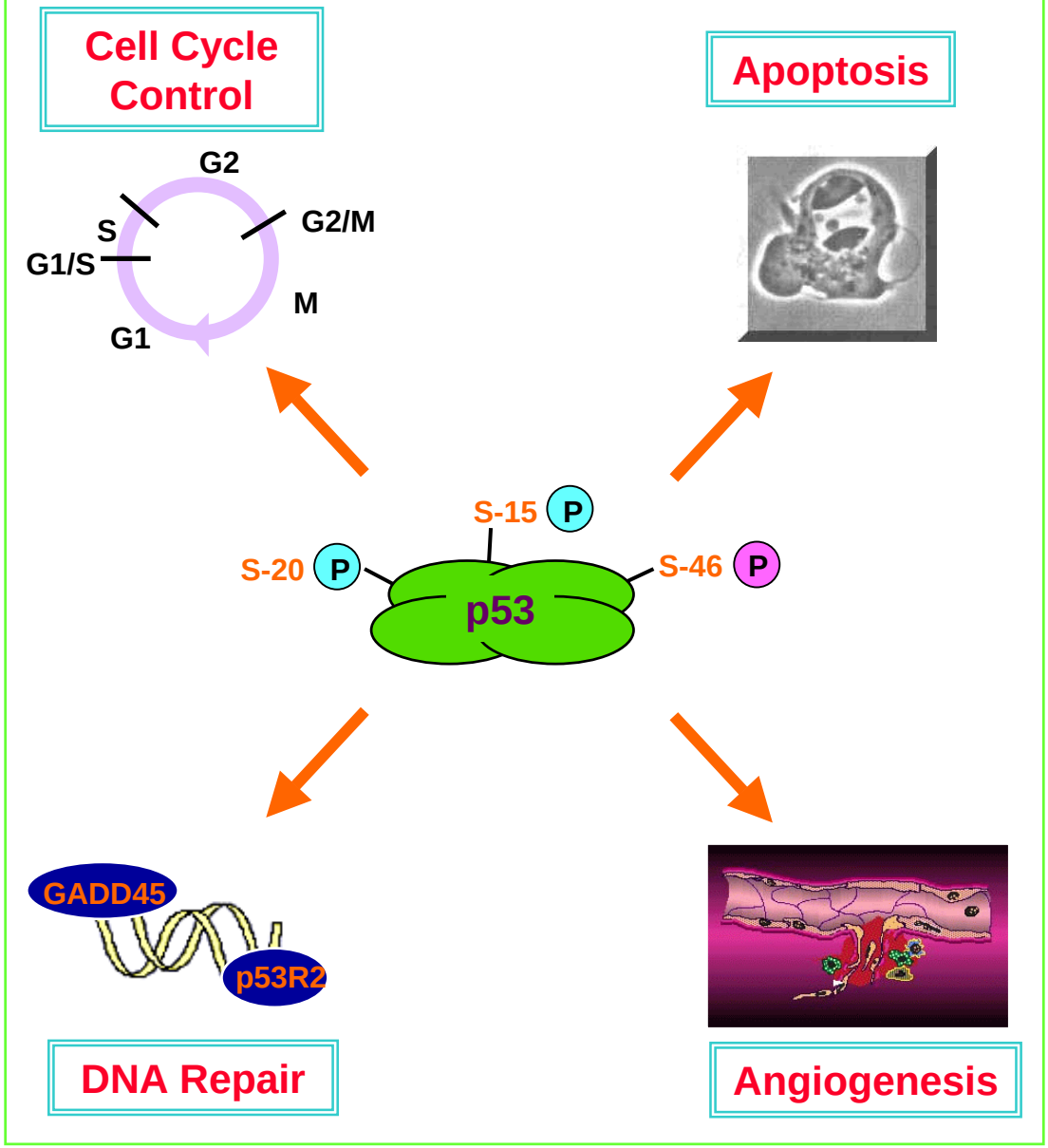
Chromosome 17



Transcriptional Factor

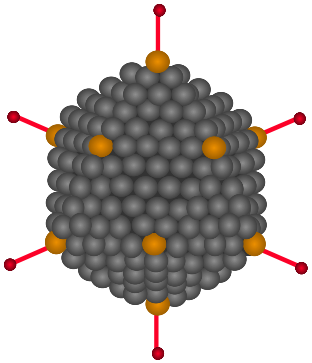


Cho Y, et al.: Science 265, 346-355 (1994)

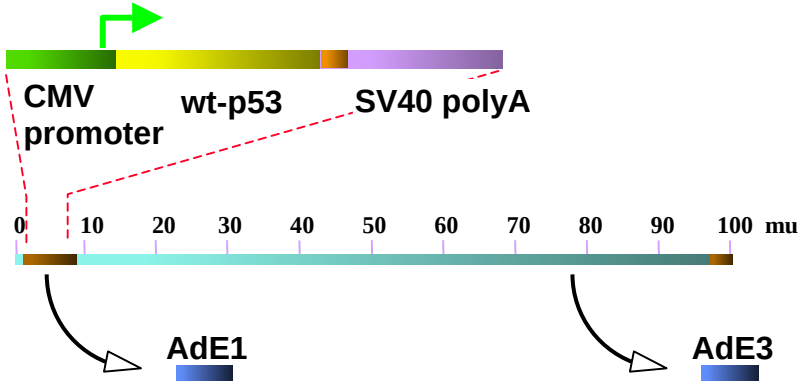


Adenoviral *p53* Gene Therapy for Non-Small Cell Lung Cancer

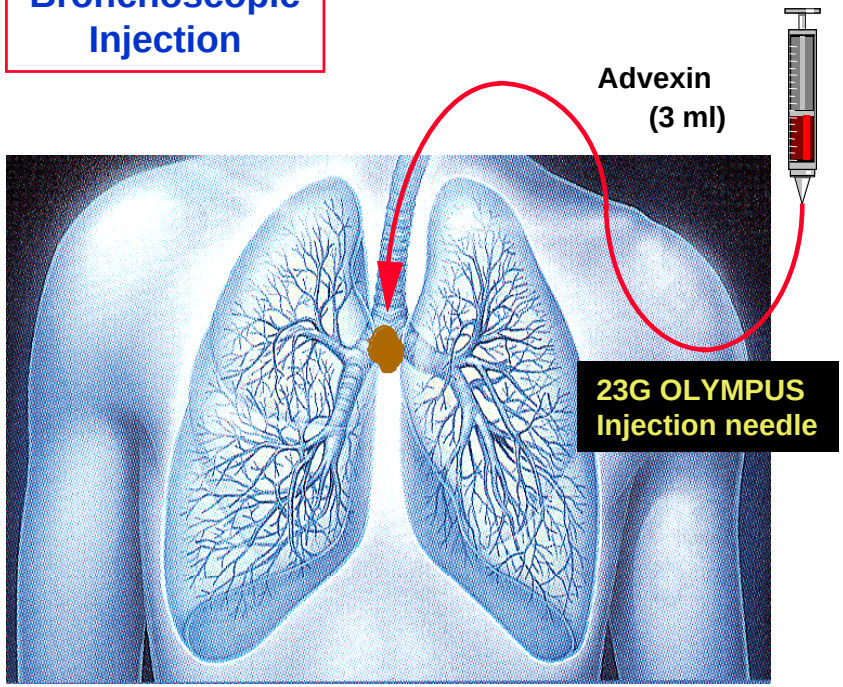
Ad5CMV-p53
Advexin®



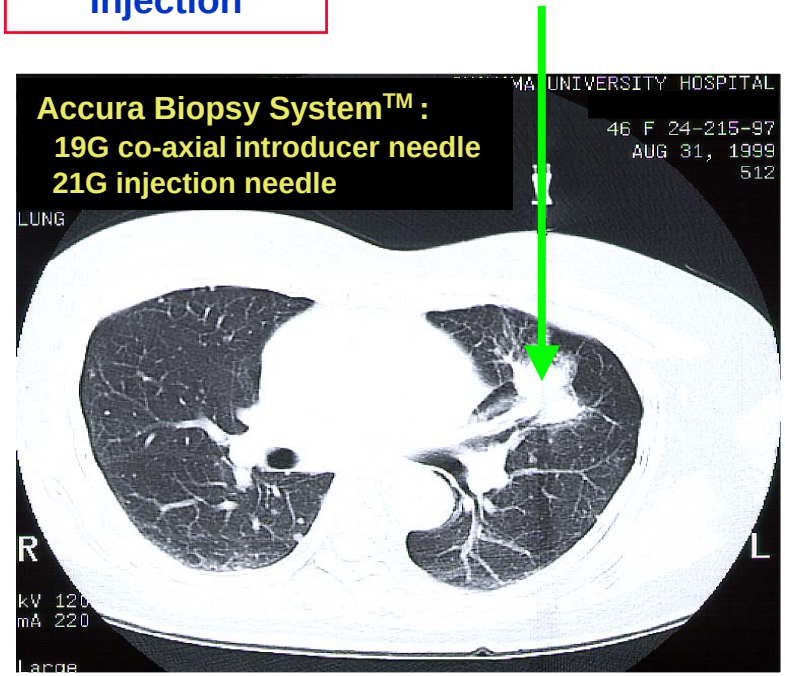
Human wild-type *p53* expression cassette



Bronchoscopic Injection

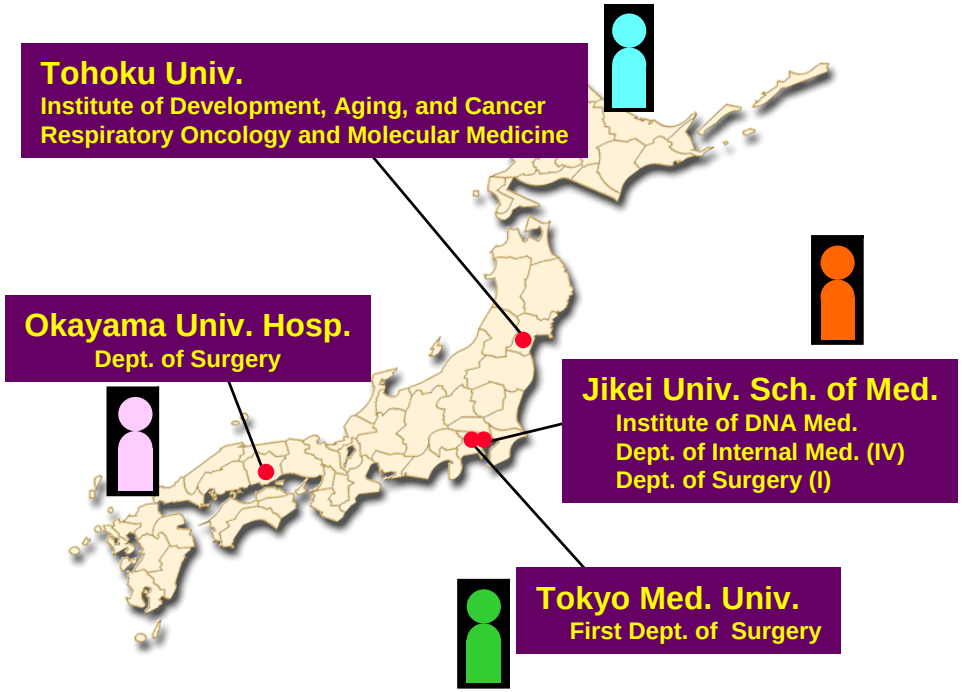


CT-guided Injection

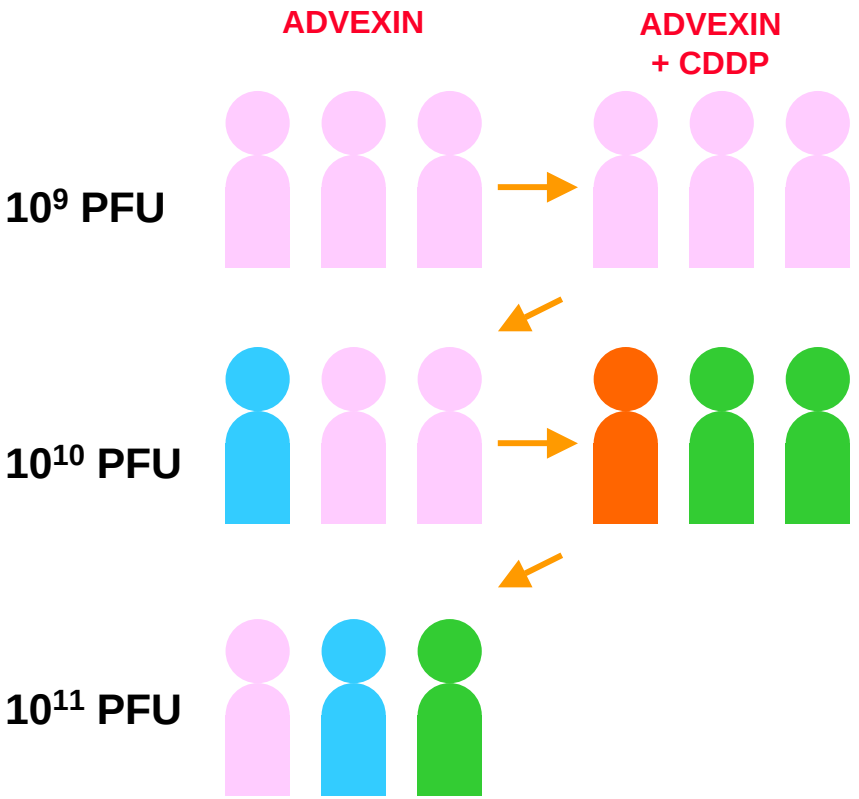


Multicenter Phase I Study

Institutions



Clinical Trial



Total Pt. No.
15 cases

Characteristics of patients that received injections of Advexin®

PT. #	Sex	Age	Histology	Location of tumor	Prior therapy	TNM Staging	Method of injection	Viral dose +/- CDDP	Number of courses
01	Male	58	squamous	carina	chemotherapy radiotherapy	cT4N0M0 St IIIB	BF	10^9 PFU	14 (485)
02	Male	58	squamous	left lower lobe	chemotherapy radiotherapy	cT4N2M0 St IIIB	BF/CT	10^9 PFU	9 (340)
03	Male	66	squamous	right mainstem bronchus	surgery, laser chemotherapy	cT2N0M0 St IB (pre-operative)	BF	10^9 PFU	4 (109)
04	Female	46	adenocar-cinoma	left upper lobe	chemotherapy	cT2N3M1 St IV	CT	10^9 PFU + CDDP	10 (619)
05	Male	56	squamous	right lower lobe	chemotherapy radiotherapy	cT4N1M0 St IIIB	BF/CT	10^9 PFU + CDDP	3 (165)
06 (Tohoku Univ.)	Male	54	squamous	left upper lobe	chemotherapy radiotherapy	cT3N2M0 St IIIB	CT	10^9 PFU + CDDP	2 (207)
07 (Okayama Univ.)	Male	71	squamous	left upper lobe	chemotherapy radiotherapy	cT4N1M0 St IIIB	BF	10^{10}PFU	2 (178)
08 (Okayama Univ.)	Male	52	squamous	right lower lobe	chemotherapy radiotherapy	cT2N2M0 St IIB	BF	10^{10} PFU	1 (115)
09 (Tokyo Jikei Univ.)	Male	66	squamous	left upper lobe	surgery, laser chemotherapy	cT2N0M0 St IB (pre-operative)	CT	10^{10} PFU	4 (590)
10 (Tokyo Med. Univ.)	Male	51	adenocar-cinoma	right lower lobe	chemotherapy	cT2N2M1 St IV	CT	10^{10} PFU + CDDP	1 (150)
11 (Tokyo Med. Univ.)	Male	55	adenocar-cinoma	right upper lobe	chemotherapy	cT4N3M0 St IIIB	BF/CT	10^{10} PFU + CDDP	2 (3y9m)
12 (Okayama Univ.)	Male	61	squamous	right upper lobe	chemotherapy radiotherapy	cT4N2M0 St IIIB	CT	10^{10} PFU + CDDP	2 (390)
13 (Tohoku Univ.)	Male	62	squamous	left mainstem bronchus	chemotherapy radiotherapy	cT4N3M0 St IIIB	BF	10^{11}PFU	1 (88)
14 (Tokyo Med. Univ.)	Male	52	squamous	left upper lobe	chemotherapy	cT2N2M0 St IIIA	BF	10^{11} PFU	4 (2y4m)
15	Male	62	squamous	right upper lobe	chemotherapy radiotherapy	cT4N2M0 St IIIB	BF	10^{11} PFU	4 (94)

Adverse events associated with Advexin® gene therapy in patients with non-small cell lung cancer

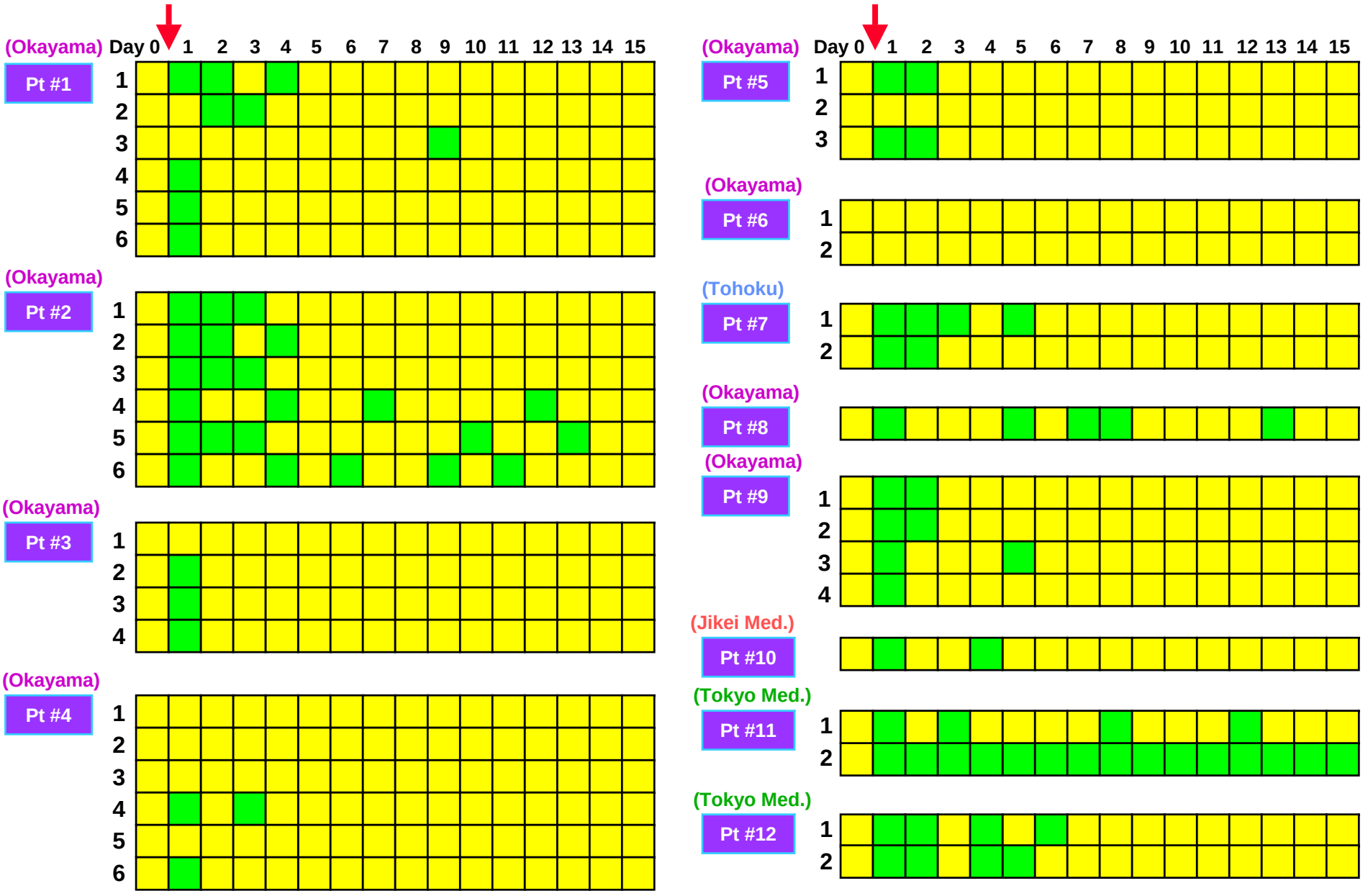
adverse events	grades			Total (100%) n = 12
	mild	moderate	severe	
fever	1	8	2	11 (91.7%)
hemoptysis	7	1	0	8 (66.7%)
dyspnea	6	0	0	6 (50.0%)
nausea	2	3	0	5 (41.7%)
vomiting	3	2	0	5 (41.7%)
cough	4	0	0	4 (33.3%)
constipation	3	0	0	3 (25.0%)
back pain	2	1	0	3 (25.0%)
obst. pneumo.	0	2	1	3 (25.0%)
chest pain	3	0	0	3 (25.0%)
diarrhea	3	0	0	3 (25.0%)
stomatitis	2	0	0	2 (16.7%)
shoulder pain	2	0	0	2 (16.7%)
infection	1	1	0	2 (16.7%)
anemia	0	0	2	2 (16.6%)
pneumonia	0	1	0	1 (8.3%)
atelectasis	0	0	1	1 (8.3%)
abd. fullness	1	0	0	1 (8.3%)
dysesthesia	1	0	0	1 (8.3%)
peumothorax	1	0	0	1 (8.3%)
hematoma	1	0	0	1 (8.3%)
fatigue	1	0	0	1 (8.3%)

adverse events	grades			Total (100%) n = 12
	mild	moderate	severe	
pituita	1	0	0	1 (8.3%)
arm pain	0	1	0	1 (8.3%)
pleu. effusion	1	0	0	1 (8.3%)
anal bleeding	1	0	0	1 (8.3%)
dullness	1	0	0	1 (8.3%)
tooth pain	1	0	0	1 (8.3%)
lumbsgo	1	0	0	1 (8.3%)
bronchitis	0	1	0	1 (8.3%)
dysthymia	1	0	0	1 (8.3%)
paracusis	0	2	0	1 (8.3%)
inj. site pain	1	0	0	1 (8.3%)
finger abscess	1	0	0	1 (8.3%)
weight loss	0	0	1	1 (8.3%)

Okayama University Hospital Center for GENE&CELL Therapy

Biodistribution Data of Advexin®: gargle
(DNA-PCR analysis)

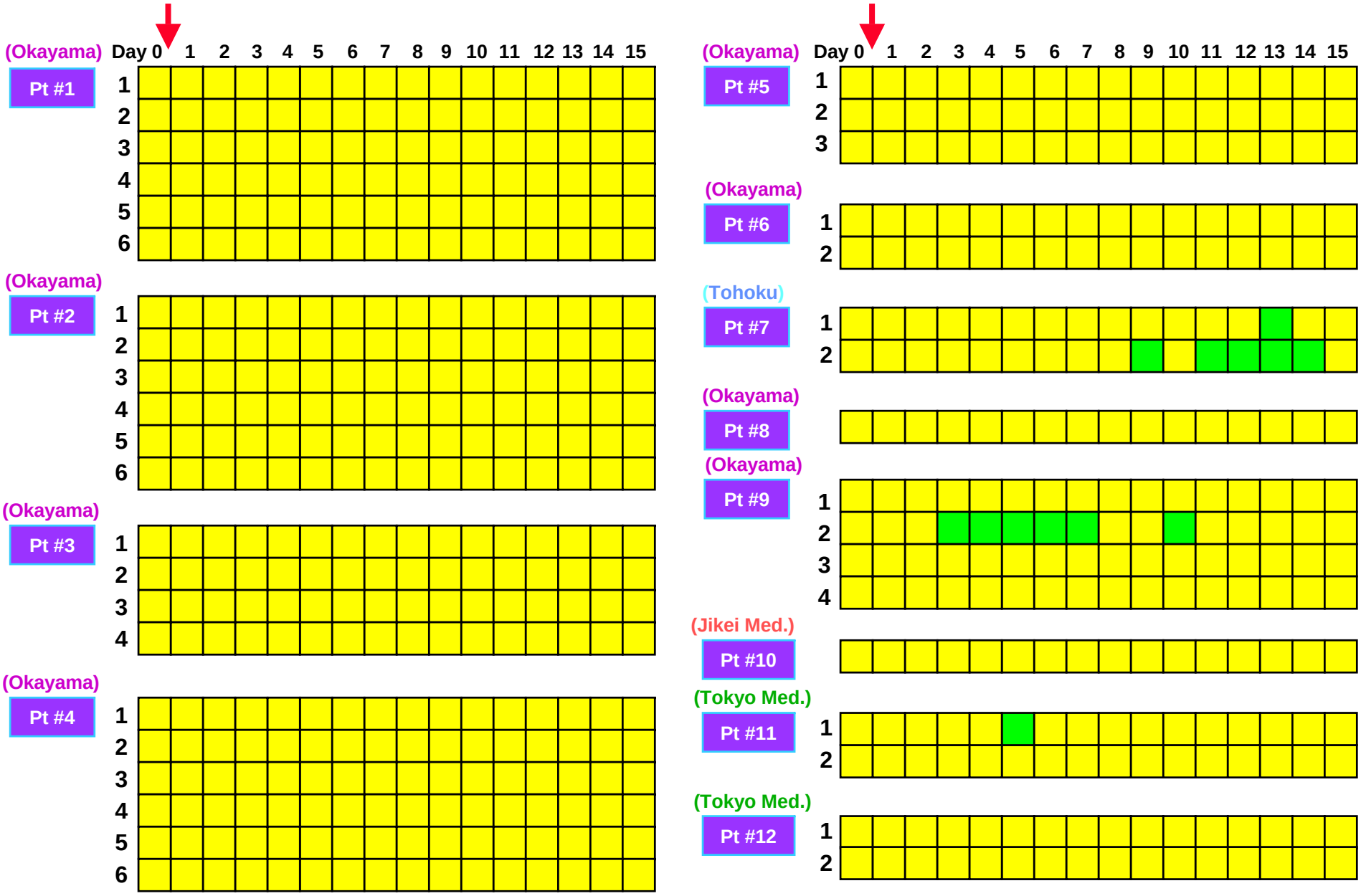
positive
negative



Okayama University Hospital
Center for GENE&CELL Therapy

Biodistribution Data of Advexin®: urine
(DNA-PCR analysis)

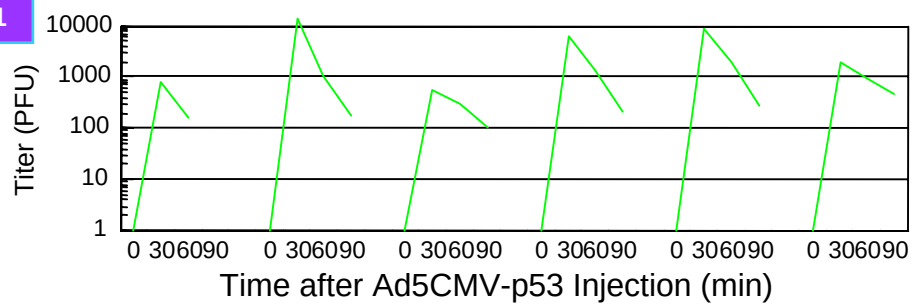
positive
negative



Biodistribution Data of Advexin®: plasma

(DNA-PCR analysis & CPE assay)

Pt #1



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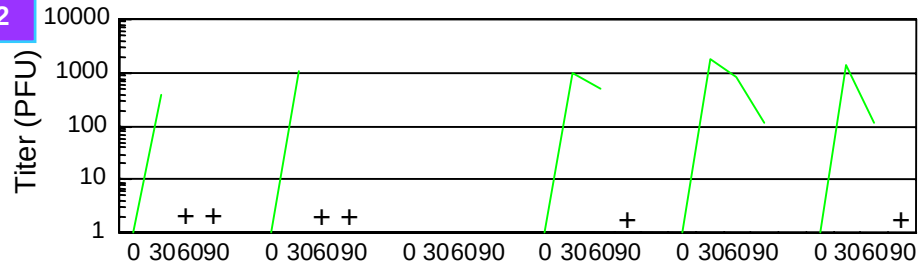

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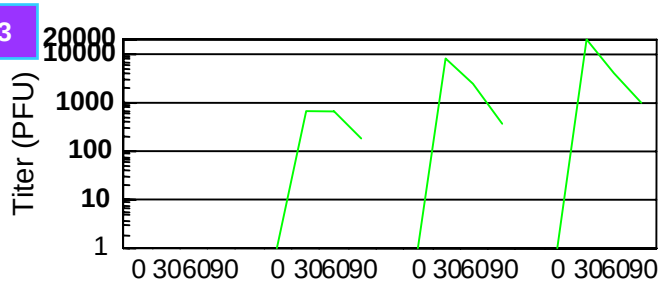
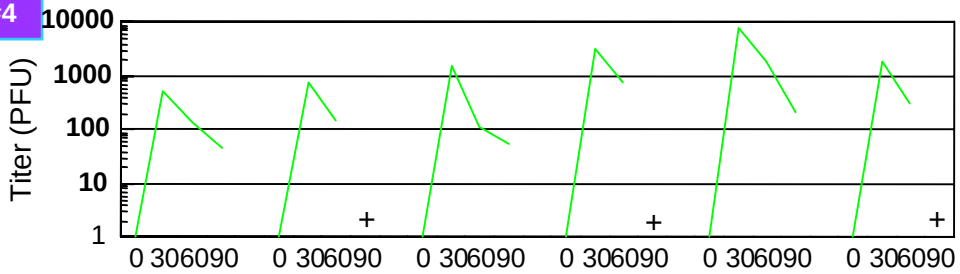


Pt #4



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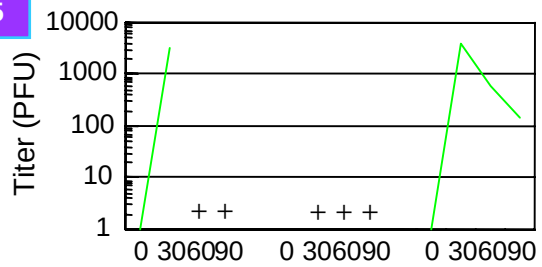

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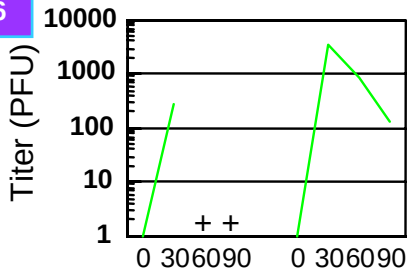

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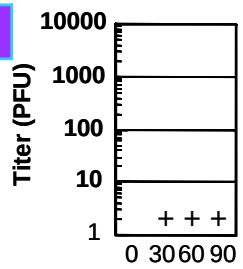

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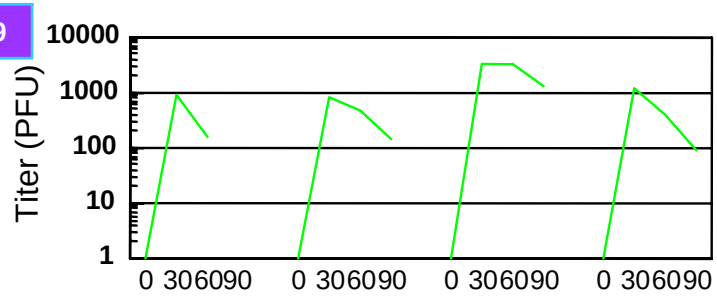
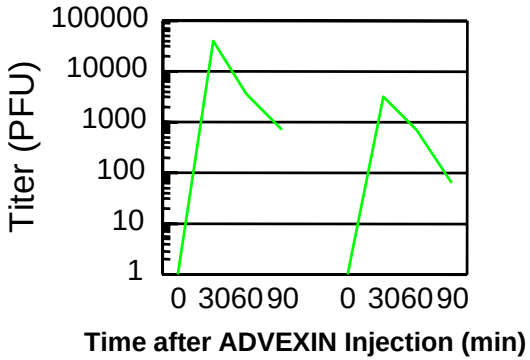


Biodistribution Data of Advexin® : plasma
(DNA-PCR analysis & CPE assay)

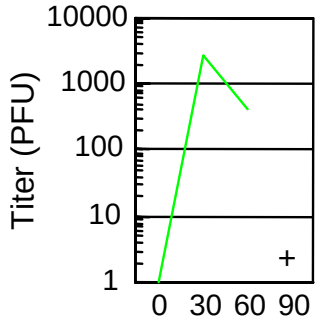
(Tohoku)

Pt #7



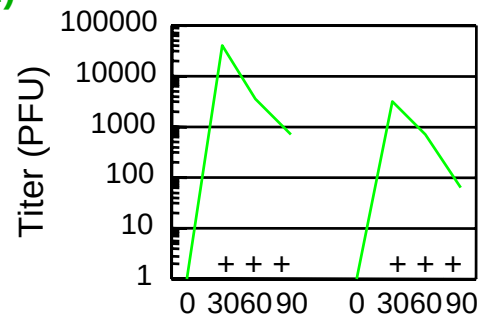
(Jikei Med.)

Pt #10

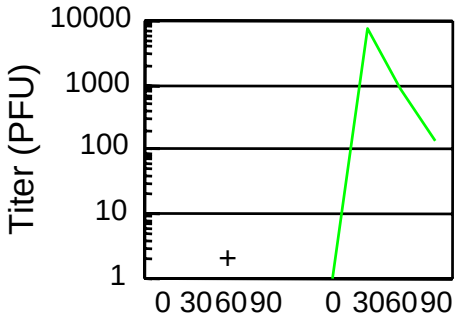


(Tokyo Med.)

Pt #11



Pt #12



Biodistribution Data of Advexin®: autopsy samples
(DNA-PCR analysis)

Patient #3 (Okayama)

Autopsy samples (9/25/99) : 25 days after the 4th Advexin injection (8/31/99)

• Tumor	(+)	• Liver	(-)
• Distant LN	(-)	• Kidney	(-)
• Testis	(-)		

Patient #7 (Tohoku)

Autopsy samples (12/9/00) : 151 days after the 2nd Advexin injection (7/12/00)

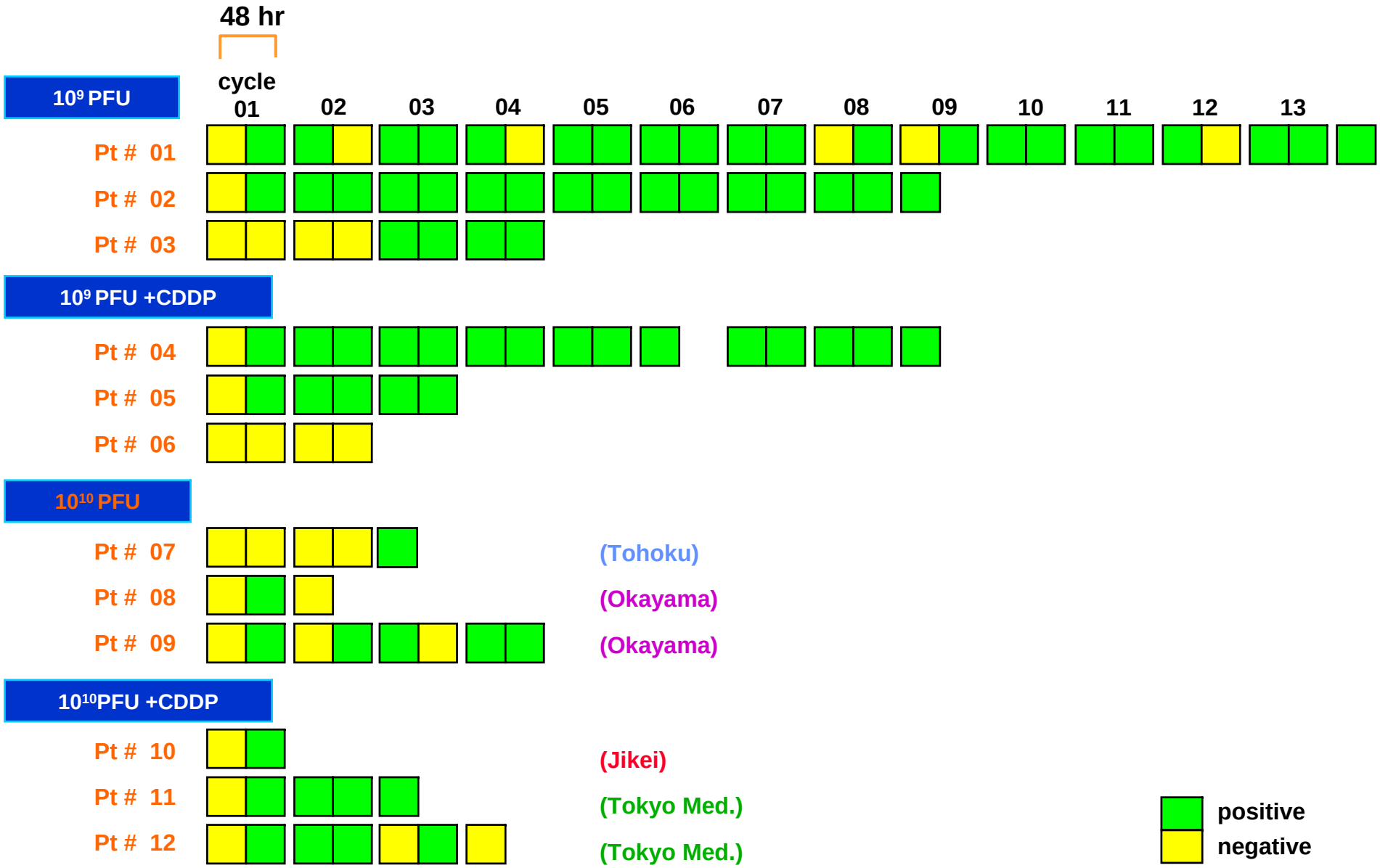
• Tumor	(+)	• Liver	(-)
• Proximal LN	(+)	• Kidney	(-)
• Testis	(-)		

Antibodies in the Serum after Advexin® Administration

Pt No. / Initial	Cycle	Enzyme Immuno Assay: Anti-p53	Enzyme Immuno Assay: Anti-Ad (U/ml) x 1000	Ad5 Neutralisation Assay
Pt #1 / F-A	pre	(-)	17.71	20
	1	(-)	26.46	1280
	2	(-)	29.65	1280
	3	(-)	35.24	2560
	4	(-)	70.63	1280
	5	(-)	51.35	1280
	6	(-)	56.46	2560
	7	(-)		2560
	final	(-)	62.47	5120
Pt #2 / T-M	pre	(-)	12.16	< 20
	1	(-)	31.03	320
	2	(-)	43.53	320
	3	(-)	65.83	640
	4	(-)	56.82	320
	5	(-)	76.47	160
	6	(+)	55.03	160
	final	(-)		340
Pt #3 / S-I	pre	(-)	10.36	< 20
	1	(-)	22.82	20
	2	(-)	23.86	40
	3	(-)	24.63	20
Pt #4 / N-M	pre	(-)	13.14	320
	1	(+)	48.68	160
	2	(+)	14.98	1280
	3	(+)	18.10	1280
	4	(-)		1280
	5	(+)		1280
	6	(+)	30.11	5120
	final	(+)	48.94	2560

Pt No. / Initial	Cycle	Enzyme Immuno Assay: Anti-p53	Enzyme Immuno Assay: Anti-Ad (U/ml) x 1000	Ad5 Neutralisation Assay
Pt #5 / Y-T	pre			40
	1			> 20480
	2	(-)	118.37	2560
Pt #6 / M-H	pre	(-)	8.49	< 20
	1	(-)	89.815	2560
(Tohoku) Pt #7 / I-S	pre	(+)	5.19	20
	1	(+)	55.85	20480
(Okayama) Pt #8 / Y-K	pre	(-)	3.149	20
	1	(-)	114.31	5120
Pt #9 / I-H	pre	(-)	14.378	320
	1	(-)	27.770	5120
	2	(-)	142.68	10240
	3	(-)	177.93	10240
(Jikei Med.) Pt #10 / T-Y	pre	(-)	4.784	40
	1	(-)	68.02	>20480
(Tokyo Med.) Pt #11 / K-K	pre	(+)	8.839	<20
	1	(+)	370.69	80
	2	(+)	391.76	5120
Pt #12 / K-K	pre	(-)	0.885	<20
	1	(-)	9.495	160
	2	(-)	66.84	2560
	3	(-)	81.59	1280

p53 mRNA Expression in Tumor Tissues Following Advexin® Administration (RT-PCR Analysis)



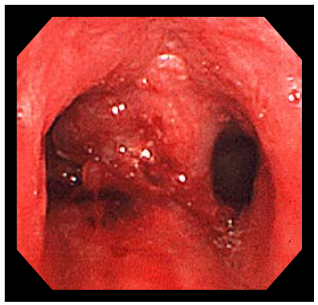
Best Response of Indicated Lesion Assessment of Advexin® for Non-Small Cell Lung Cancer

Treatments		10^9 PFU	10^9 PFU+CDDP	10^{10} PFU	10^{10} PFU+CDDP	10^{11} PFU	Total
Evaluable Patients		3	3	3*	3	3*	15
Response	PR	1					1
	SD	1	3	1	3	2	10
	PD	1		1			2

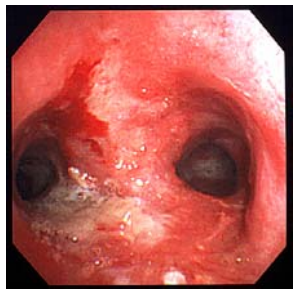
*One patient was not evaluable because their tumor sizes could not be measured due to obstructive pneumonia.

Case #01

Pretreatment

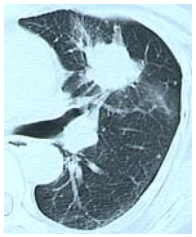


5 months After 4 cycles

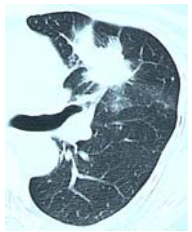


Case #04

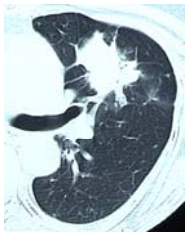
Pretreatment



5 months After 4 cycles



12 months After 11 cycles



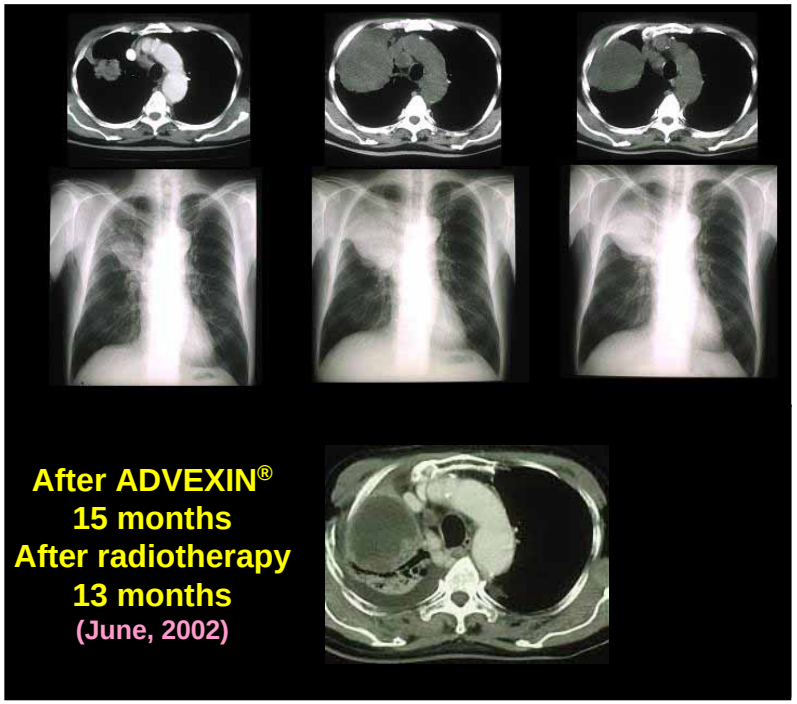
Case #10

(Tokyo Med. Sch.)

Before chemotherapy

Before ADVEXIN®

After ADVEXIN® (March , 2001)



After ADVEXIN®
15 months
After radiotherapy
13 months
(June, 2002)

Lessons Learned from Phase I Study of Advexin® Gene Therapy

Safety

- Vector-related adverse events were minimal. Repeated intratumoral injections of Advexin up to a dose of 10^{11} PFU could be well tolerated.

Biodistribution

- Results of plasma and aerodigestive secretion analysis confirmed transient systemic spread of the vector; however, no vector dissemination-related toxicity was observed. Transgene expression was detected in the presence of neutralizing anti-adenovirus antibody.

Clinical Benefit

- Evidence of clinical antitumor activity, including tumor regression, tumor growth stabilization, and decrease of tumor markers, was observed in some patients. Note the Advexin-mediated radiosensitization, suggesting the clinical feasibility of Advexin gene therapy.

千葉大学大学院医学研究院先端応用外科学
-食道胃腸外科・乳腺甲状腺外科・移植外科-
Department of frontier surgery
Graduate school of medicine, Chiba University

Human Gene Therapy Protocol

Phase I/II Adenoviral *p53* Gene Therapy for Chemoradiation Resistant Advanced Esophageal Squamous Cell Carcinoma

Chiba University Graduate School of Medicine

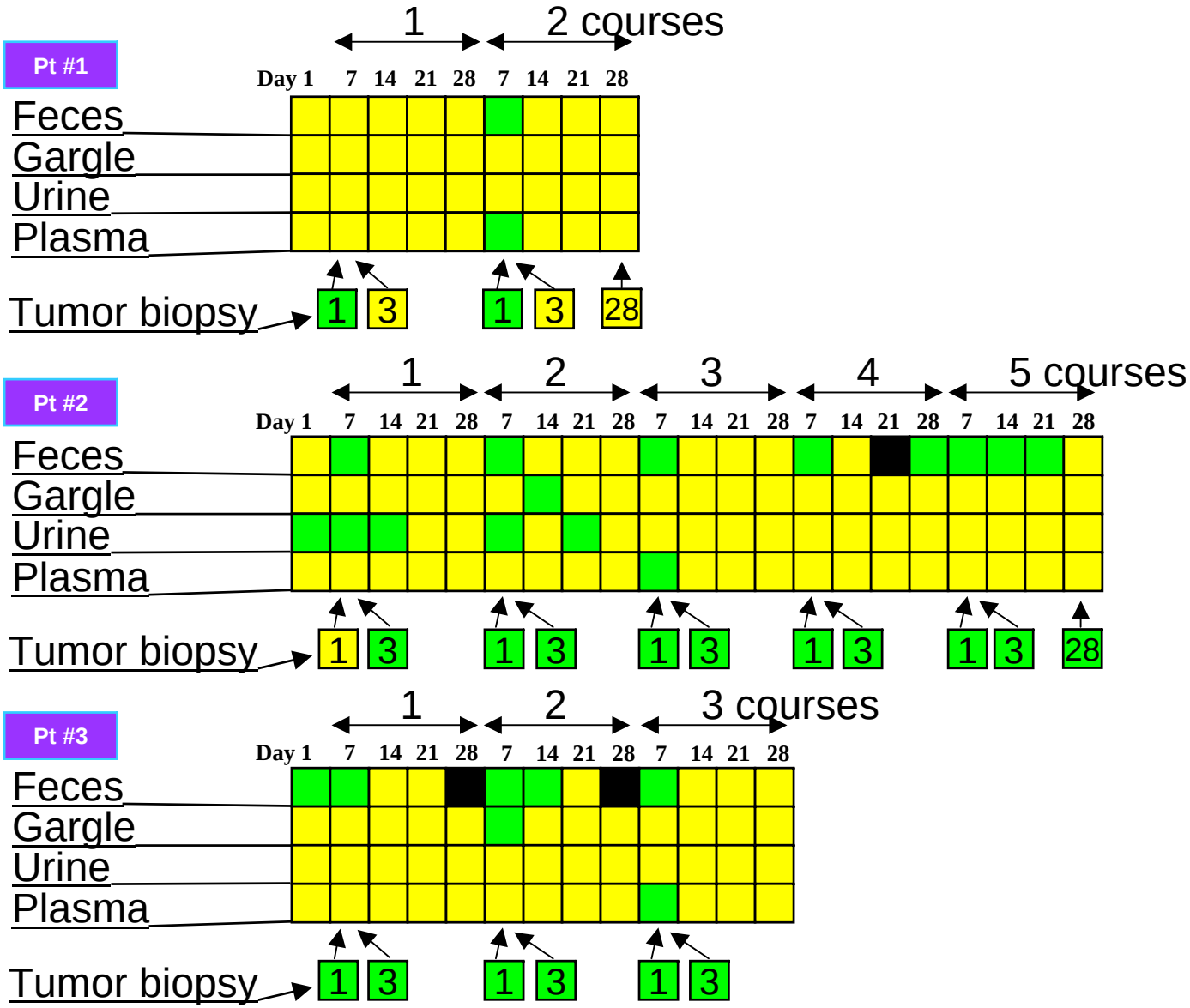
Shimada H, Matsubara H, Shiratori T, Shimizu T,
Myazaki S, Okazumi S, Nabeya Y, Shuto K, Hayashi H,
Tanizawa T, Nakatani Y, Nakase H, Kitada M, Ochiai T

Characteristics of Patients with Esophageal SCC with Advexin®

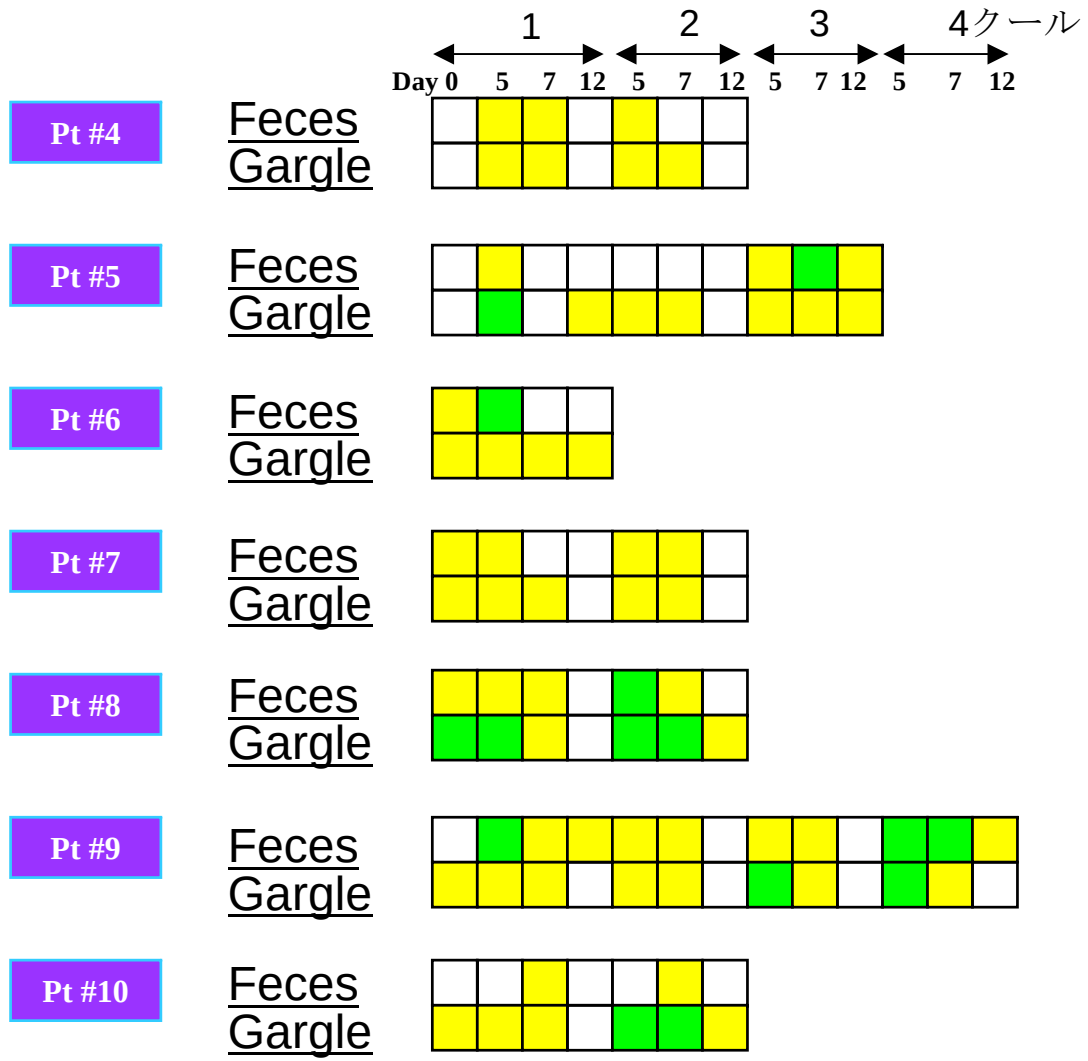
Patient No.	Age Gender	Local Response to CRT	UICC Stage	Injection Area	Exon Site 0f *P53* mutation	Viral particles	Treatment cycles
1	64 M	CR	IIB	E	5	15×10^{11}	2
2	71 M	PR	III	E	7	10×10^{11}	5
3	62 M	SD	IVA	E	negative	15×10^{11}	3
4	78 M	CR	IVA	E	8	10×10^{11}	2
5	66 M	CR	IIA	E	7	10×10^{11}	3
6	60 M	SD	IVB	E	7	20×10^{11}	1
7	67 F	SD	IVA	E	5	10×10^{11}	2
8	58 M	SD	IIA	E	6	10×10^{11}	2
9	48 M	PR	IVA	E	7	25×10^{11}	4
10	77 M	NA	IVA	N	negative	25×10^{11}	2

CR; complete response, PR; partial response, SD; stable disease, NA; not assessed
E; Esophagus, N; lymph nodes

Biodistribution Data of Advexin®



Biodistribution Data of Advexin®



Lessons Learned from Phase I/II Study of Advexin® Gene Therapy for Esophageal Cancer

-Transient shedding of the virus into feces and gargle could be detected in some of the treated patients; however, urine rarely contained the virus.

-Systemic spread of the virus could not be detected in plasma 24 hours after virus injection, although the lung study demonstrated a transient dissemination of the virus just after virus injection.

-Most of biodistribution data in the esophageal study are consistent with those in the lung study.

Acknowledgements

Okayama University

Toshiyoshi Fujiwara, M. D., Ph. D.

Noriaki Tanaka, M. D., Ph. D.

Shunsuke Kagawa, M. D., Ph. D.
Futoshi Uno, M. D., Ph. D.

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Kanazawa University

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Masaki Inoue, M. D., Ph. D.

National Institute of Biomedical Innovation

Hiroyuki Mizuguchi, Ph. D.

Oncolys BioPharma, Inc.

Yasuo Urata
Katsuyuki Nagai
Hitoshi Kawamura, Ph. D.
Daiju Ichimaru

The University of Texas M. D. Anderson Cancer Center

Jack A. Roth, M. D.
Stephen G. Swisher, M. D.

Bingliang Fang, M. D., Ph. D.

Ryo Sakai, M. D.

Elizabeth A. Grimm, Ph. D.

Introgen Therapeutics, Inc.

Sunil Chada, Ph. D.
Peter M. Clarke, Ph. D.
David G. Nance