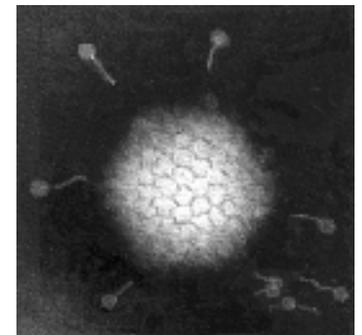


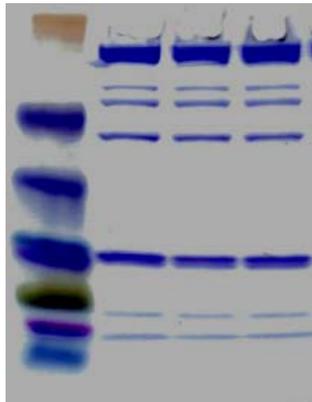
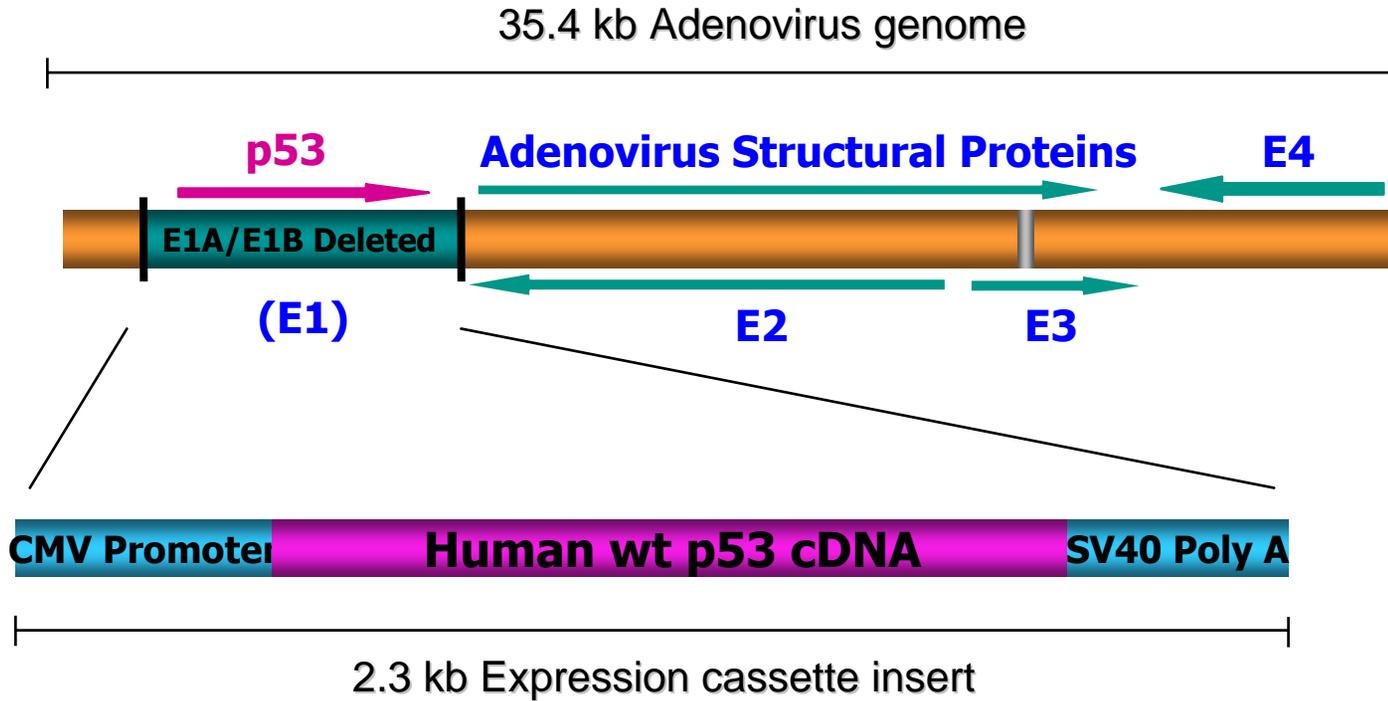
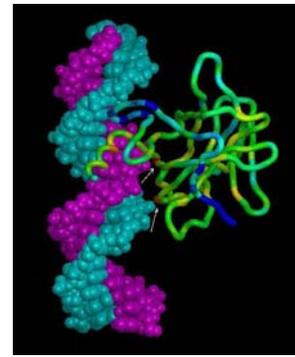
Development of Adenovirus vectors – from preclinical to Phase III

iSBTc Oncology Biologics
Development Primer

Sunil Chada, Ph.D.
s.chada@introgen.com



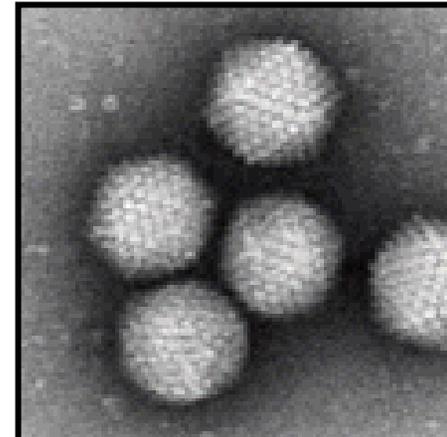
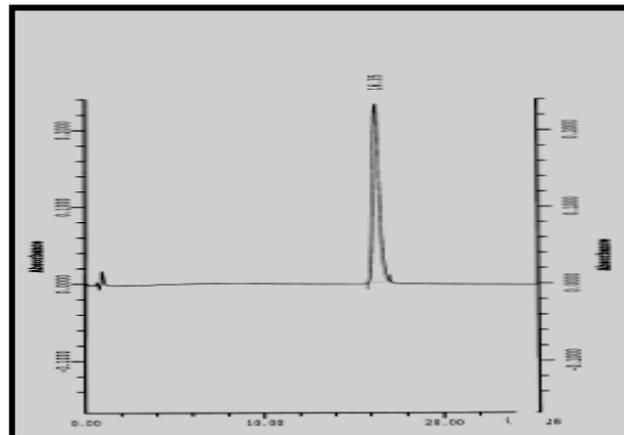
ADVEXIN[®] Construct

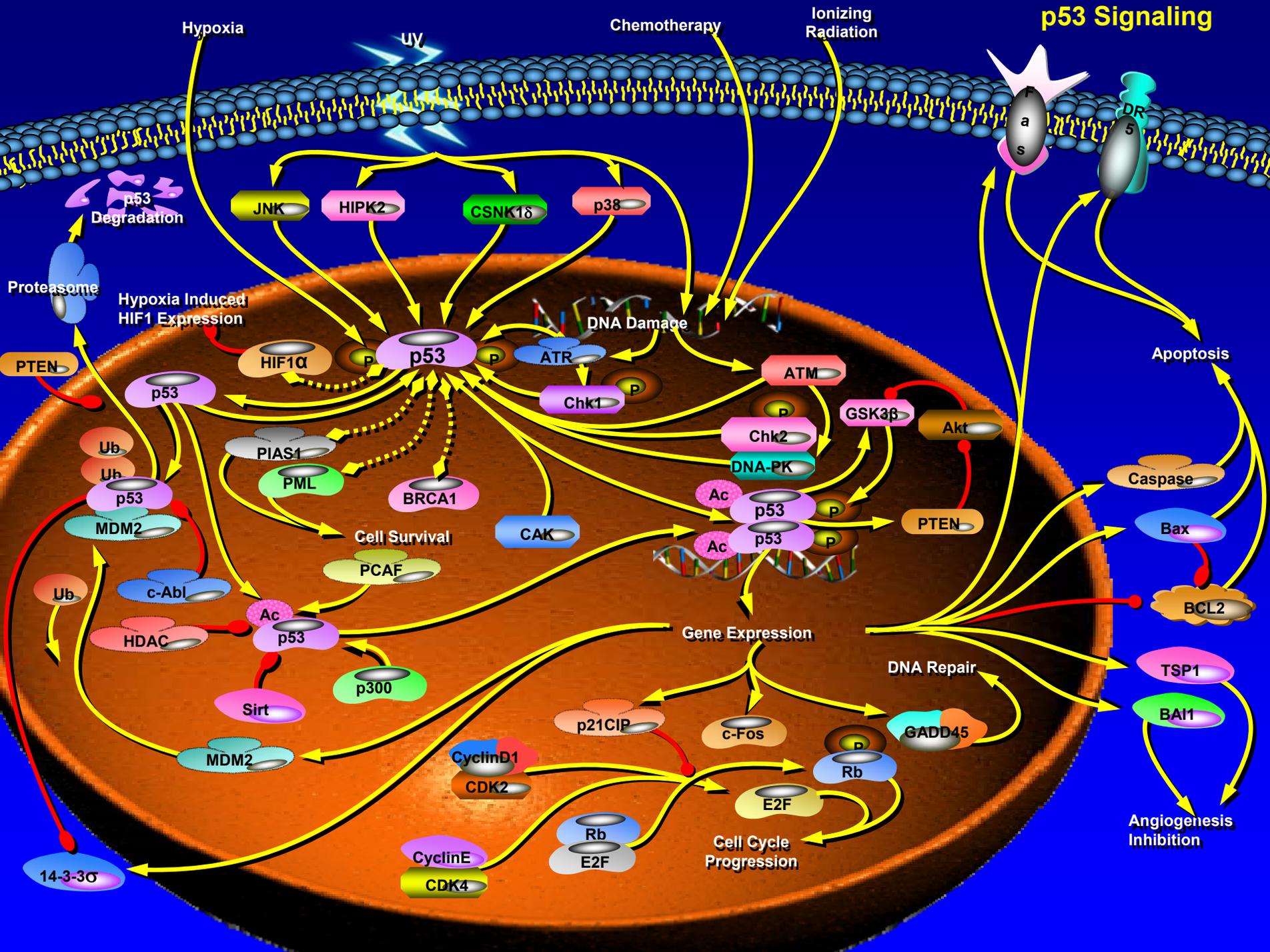


Hexon
Penton
Fiber
Core

Core

Hexon associated



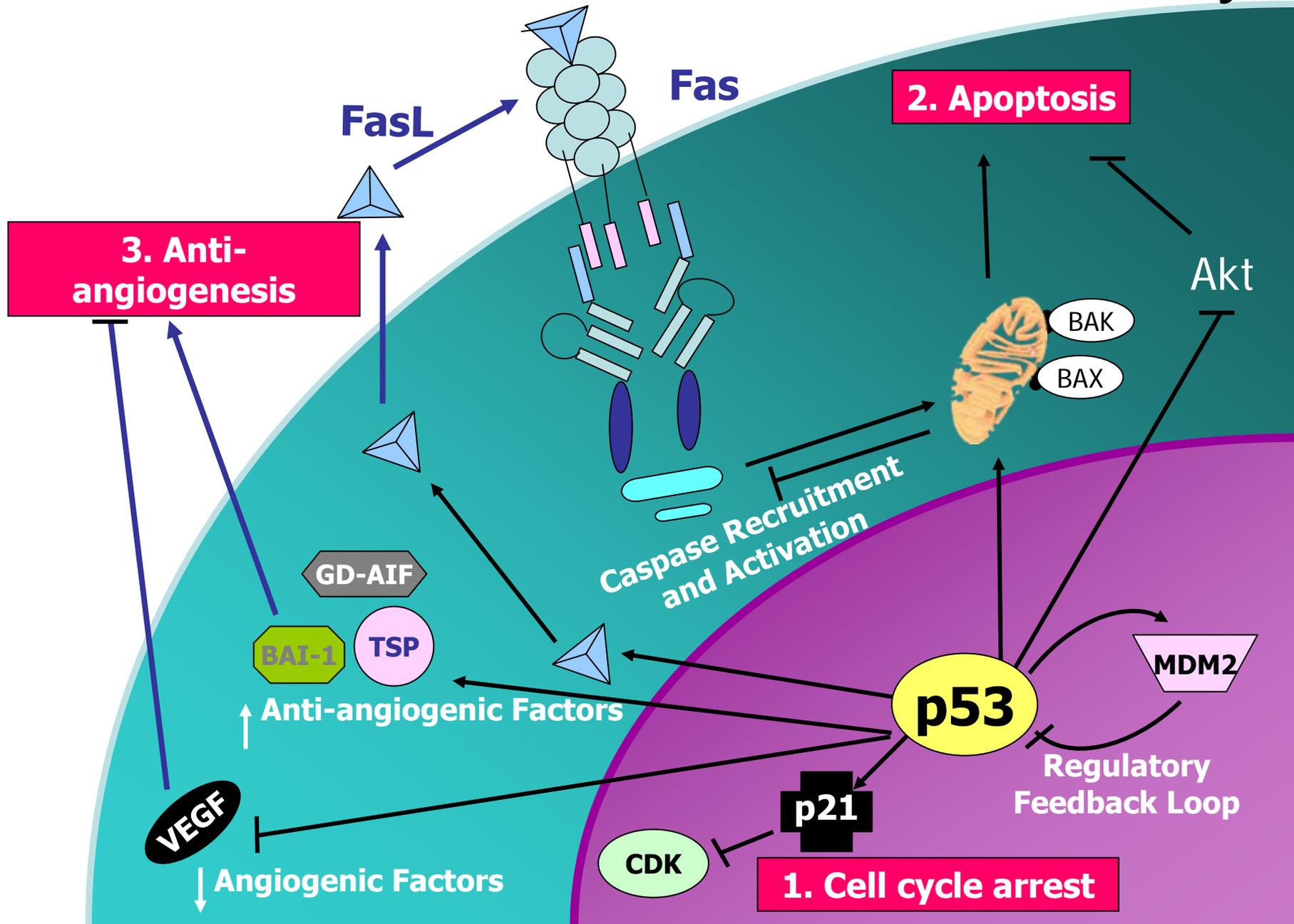


ADVEXIN[®]

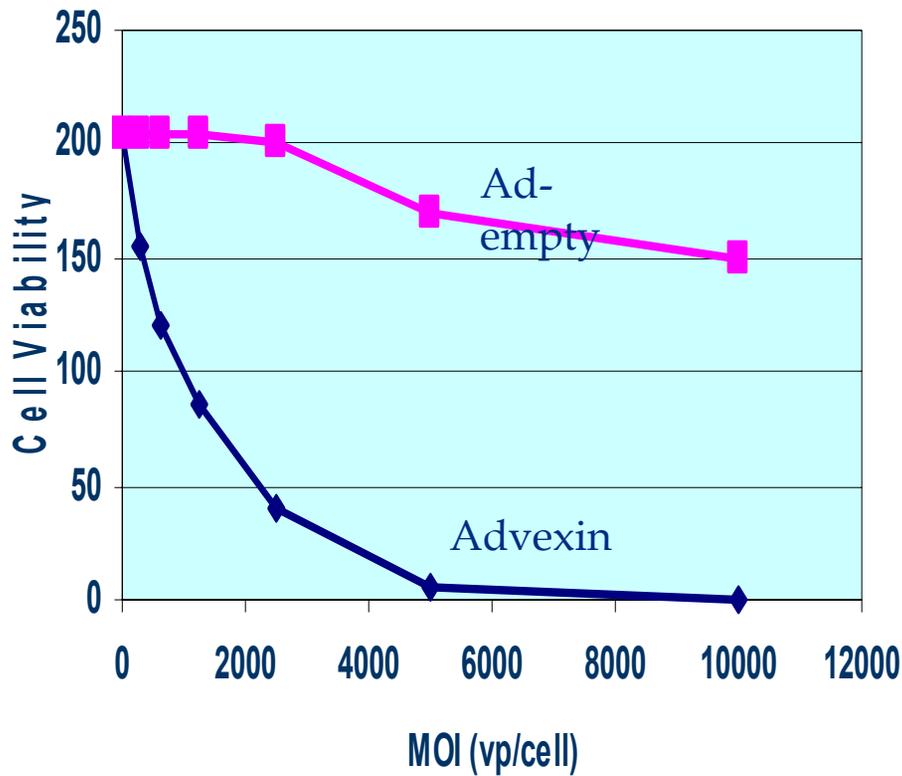
p53 Tumor Suppressor Therapy

- **Selectively kills cancer cells, safe to normal cells**
- **Pharmacologic intervention with p53 protein - targets fundamental molecular defect in cancer**
- **Non-replicating adenovirus; well tolerated >600 patients; >30 trials**
- **Excellent safety profile**
- **Useful alone and in combination with local and systemic modalities — radiotherapy, surgery, chemotherapy, biotherapy**

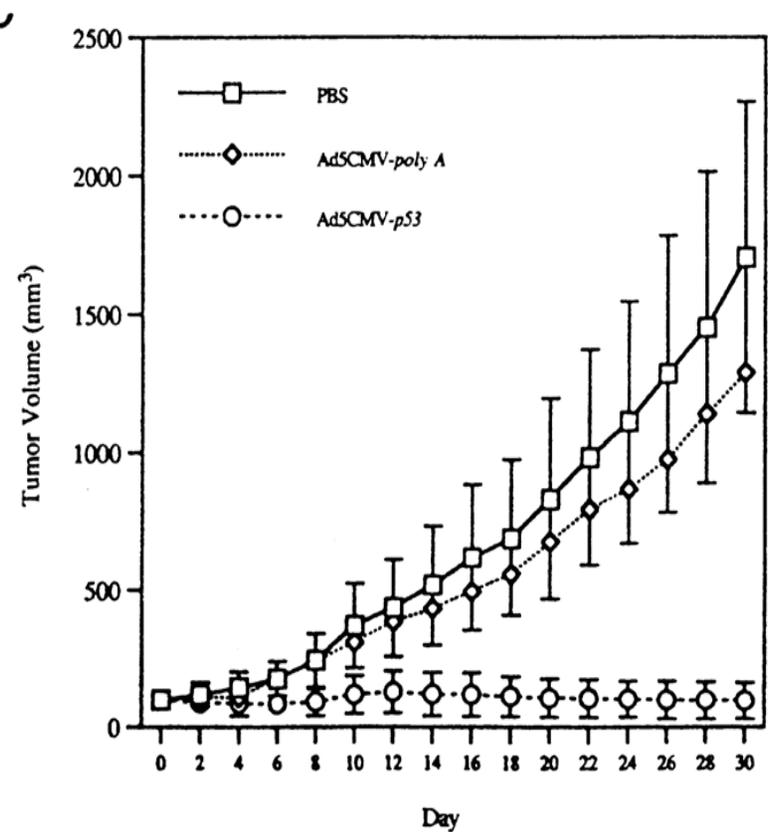
Mechanisms of ADVEXIN[®] Activity



Preclinical studies



HeLa cells
Trypan blue assay



SiHa tumors
1E11 vp IT; 6 injections
Hamada et al, Cancer Res 56: 3047, 1996

Advexin exhibits tumor-selectivity

Tumor type	Killing <i>in vitro</i>	Killing <i>in vivo</i>
SCCHN	√	√
NSCLC	√	√
Breast	√	√
Prostate	√	√
Colorectal	√	√
Ovarian	√	√
HCC	√	√
Glioma	√	√
Pancreatic	√	√
Melanoma	√	n.d.
Cervical	√	√
Bladder	√	√
Sarcoma	√	√
Myeloma	√	n.d.
Normal cells	X	X

ADVEXIN® Inhibits Tumor Growth in Combination with Other Cancer Therapies

Additive or synergistic effects

Tumor types

- SCCHN
- NSCLC
- Breast
- Prostate
- Colorectal
- HCC
- Glioma

Therapies

- XRT
- CDDP
- 5FU
- Taxanes
- CPT-11
- Doxorubicin
- etc

Additional preclinical studies

- 8 GLP toxicology studies: mice, rats, cotton rats
 - Advexin well tolerated: sq; oral; iv; ip; ia
 - The liver is affected at very high doses with iv route, but see no liver effects in the clinic
- Biodistribution (PK) studies
 - $t_{1/2} = 10$ minutes; no gonadal persistence
- Other safety studies
 - Little/ no effect on normal cells; lack of replication or integration

Therapeutic index > 3 logs

Clinical Studies

Advexin® Clinical Program

- > 600 patients treated with > 3,000 doses
- First trial conducted in 1995; published results in 1996
- > 30 active or completed trials
- Most patients treated with intratumoral injection
- Four additional routes of administration: IV, IP, BAL, intravesicle
- Randomized controlled Phase III multinational studies ongoing

Advexin® Clinical Program

- Phase I Trials – **US**; EU; Japan
 - Head & Neck, Lung, Breast, Prostate, Colorectal, Bladder, Ovarian, Brain, Lung + Chemotherapy, Solid Tumors (IV), Oral Premalignancy
- Phase II Trials
 - Head & Neck, Lung + Radiation, Breast + Chemotherapy, Esophageal
- Phase III Trials
 - Head & Neck ± Chemotherapy

ADVEXIN[®] Well Tolerated Safety Data in >600 Treated Patients

Body System	EVENT	All Serious Adverse Events Occurring in > 1% of Patients		SAE - Investigator Related	
		n	%	n	%
Body as a Whole	Fever	13	2.0	8	1.3
	Pain	10	1.6	1	0.2
	Asthenia	7	1.1	0	0
	Infection local	16	2.6	3	0.5
	Tumor hemorrhage	28	4.5	4	0.6
	Procedure (Inpatient scheduled)	8	1.3	0	0
Digestive	Vomit	11	1.8	1	0.2
	Dysphagia	8	1.3	1	0.2
Respiratory System					
	Pneumonia	38	6.1	5	0.8
	Dyspnea	24	3.9	4	0.6
	Apnea	11	1.8	0	0
Cardiovascular System	Hypotension	11	1.8	1	0.2
	Heart arrest	9	1.5	0	0
Metabolic and Nutritional Systems	Dehydration	26	4.2	4	0.6
	Kidney Failure	7	1.1	2	0.3

ADVEXIN[®] Monotherapy Results in Long Term Survival (> 8 years) in SCCHN

Age=48



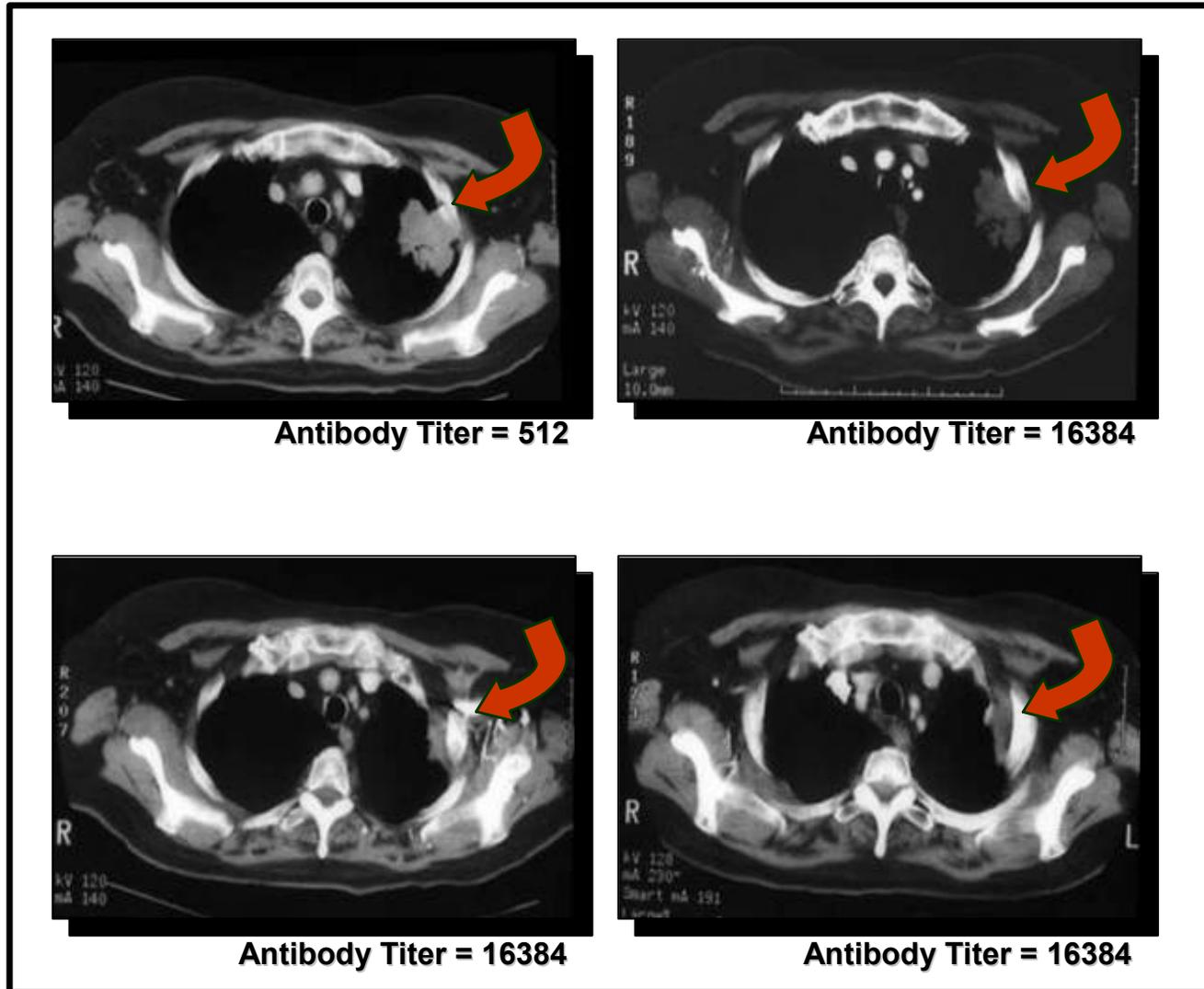
Cycle 13: 21 June 1999
39 injections
≈ 6X10¹³ vp



8 June 2006
276 injections
≈ 4X10¹⁴ vp

Baseline: 27 May 1998

Objective response after Advexin injection in NSCLC

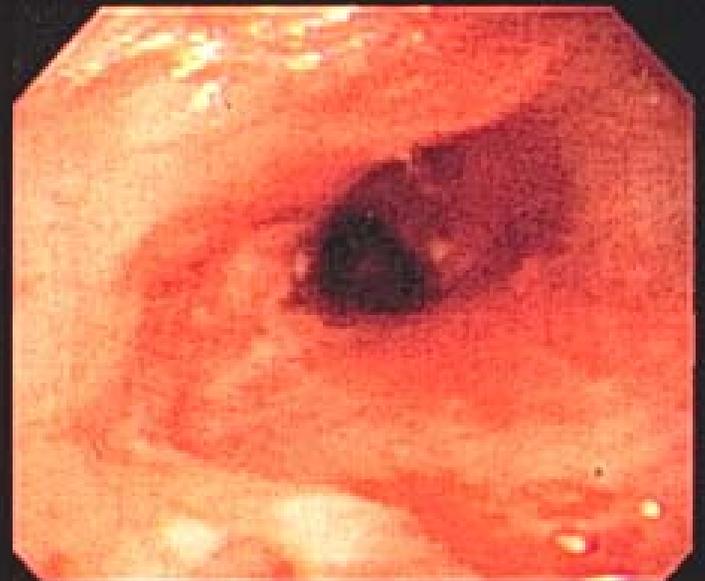


Swisher *et al.*, J Natl Cancer Inst **91**:763-771, 1999. Failed RT and Chemo. Six months of Ad-p53 treatment. Tumor free biopsies after 2 months. Stable off therapy for >6 years

1



2



3



4



Molecular pharmacology and biomarker development

Positive

DNA
Damage

Oncogenes

p14^{ARF}

Negative

HDM2

p53

Upstream
Regulators

Downstream
Effectors

CELL CYCLE

ANGIOGENESIS

APOPTOSIS

TUMOR IMMUNITY

p21

Cdk

G1

PCNA

G1/S

BAI-1

TSP-1

VEGF

Bax

Bak

Bcl2

CD95L/FasL

Genomic integrity
Growth control

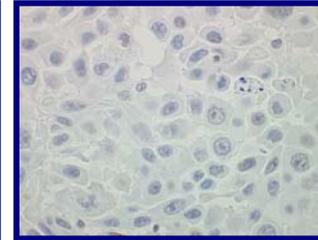
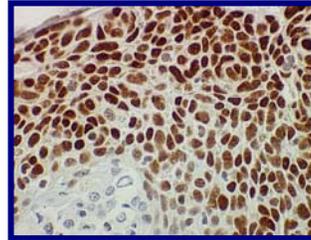
Staining result:

+

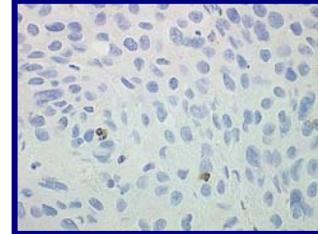
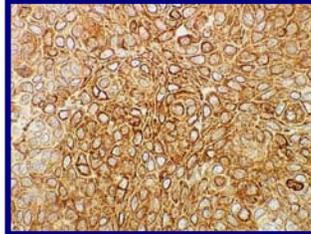
-

Antibody
specificity:

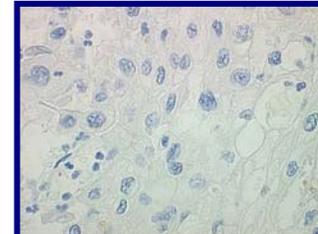
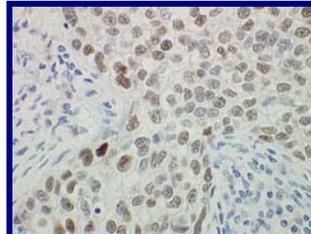
p53



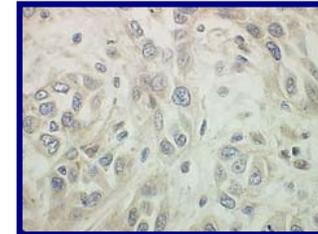
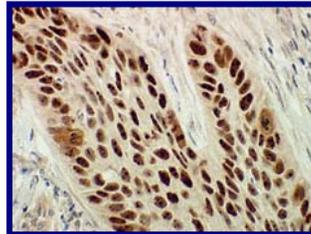
bcl-2



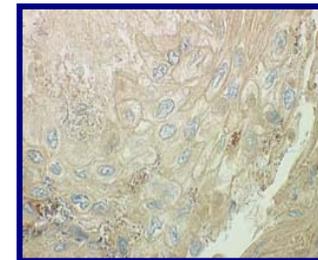
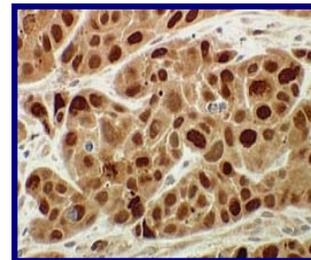
MDM2



p14



survivin



Representative images from Advexin Phase II SCCHN patient tumors depicting examples of positive and negative immunostaining with each antibody

Interrogation of p53 pathway markers for tumor response and survival

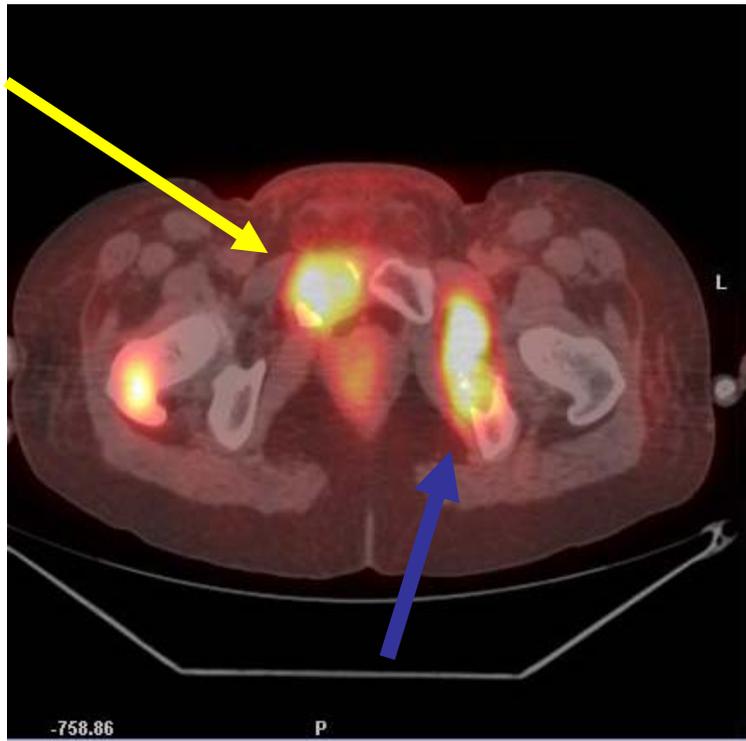
Tumor Response			Overall Survival (Months)				
<i>Univariate Logistic Regression</i>			<i>Log-Rank Test</i>				
Tumor Marker*	N	P-value	Tumor Marker*	N	Median	95% CI	P-value
p53	18	0.03	positive	11	11	(6.5, 16.0)	< 0.01
			negative	7	3	(1.5, 3.5)	
p53-Ser15	18	0.07	positive	8	12.2	(6.5, 16.0)	0.07
			negative	9	3	(2.0, 5.5)	
p14^{ARF}	18	0.28	positive	4	7.8	(1.0, 15.0)	0.65
			negative	14	6	(3.5, 11.0)	
HDM2	17	0.10	positive	8	10.8	(5.5, 16.0)	0.17
			negative	9	3.5	(2.0, 7.0)	
Bcl-2	17	0.17	positive	5	7	(3.5, ---)	0.32
			negative	12	4.8	(3.0, 11.0)	
survivin	13	0.62	positive	10	7.5	(3.5, 15.0)	0.88
			negative	3	3	(1.5, ---)	

* $\geq 20\%$ is positive for p53, p14^{ARF}, HDM2, Bcl2, and survivin; $\geq 5\%$ is positive for p53-Ser15
 --- could not be calculated reliably variability in the data

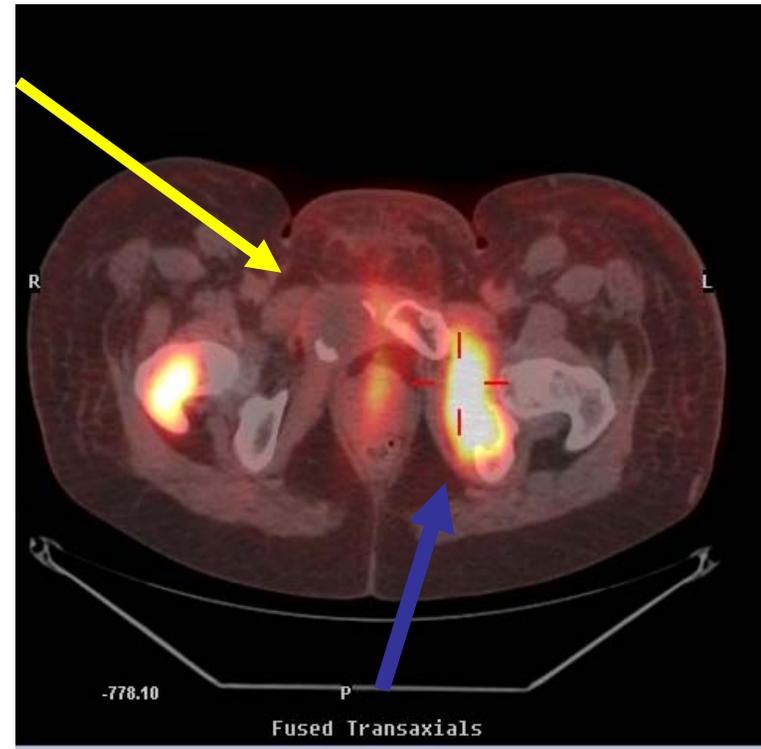
Imaging technology: PET-CT response in LFS patient

Pre-Treatment

Post-Treatment



10-31-2005



12-28-2005

SUV: 80% decrease in injected lesion. Non-injected lesion; 130% increase



Case Studies: Lessons and Issues

- **Key Strategic Decisions**

- Early decision on r & Development company

- Rely upon academic collaborations for “r”, animal data, etc.

- Focus resources on generation of clinical data

- Important to control supply of clinical-grade materials

- Decision made early to create manufacturing infrastructure

- Develop parallel regulatory development paths with FDA and EMEA

- Take industrialized approach to clinical design and biostatistics – avoid repeating studies

- **Impact of Regulatory Interactions**

- Provided valuable guidance

- Early interactions important to avoid surprises

- Regulators don't have all the answers

- Be collaborative, not combative

Case Studies: Lessons and Issues

- **Financial Considerations: Projected Costs vs. Reality**
 - Everything is more expensive and takes longer.....
 - Heavy price for first-in-class development
 - No regulatory precedents
 - Investor reluctance (no comparables)
 - Pharma partner caution
 - Avoid temptation to cut corners on required GLP studies (more expensive to do it twice!!)
 - Outsourcing/ consultants
 - need careful oversight
 - do not assume they are “experts” in your area
 - monitor timelines
- **People matter**
 - Flexible , non-silo people key in the early days
 - Research mindset needs to evolve to industrial/ business approach

Case Studies: Lessons and Issues

- **Impact of long development timelines**
 - Early studies may not meet current standards (e.g., PCR sensitivity; RCA levels)
 - Evolution of clinical standard of care
 - New drug approvals

Lessons learned

1. Early deployment of Clinical Development Plan

- Synchronize research and clinical studies
- Enhances iterative translation-based development program
- Challenges of using CROs:
 - Databases
 - Monitoring
 - Need oversight
- Tough to modify protocols/ CRFs during study
- Stick to the plan!! Avoid tempting, incremental research

Lessons learned

2. Biomarker development

- Goal is to identify responding and non-responding patient populations
- Limited by patient #/ samples/ informed consent/ etc
- Preparation is critical – work closely with PI's on informed consent, CRF's, sample logistics

Words to the Wise

- Have the courage to kill a project
- Impact of having pharma partner early
 - Differences in cultures and risk assessment
 - Keep your eye on the clinical development plan
 - Control your company's/project's destiny
- What is your backup plan ???
 - Financially
 - Balance need for product pipeline with “all eyes on the prize”

Questions?