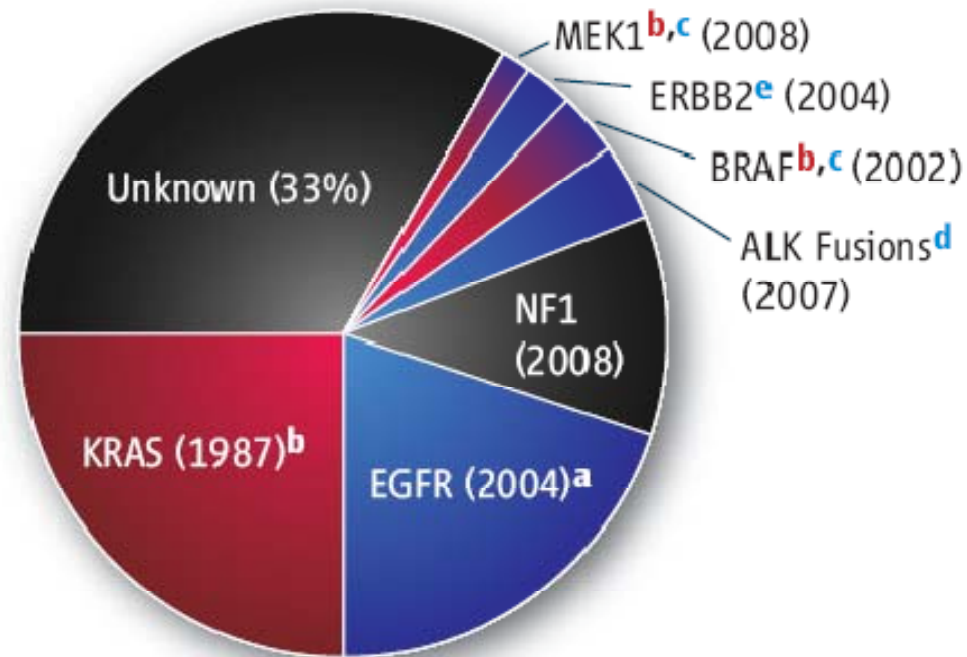


Abstract ID MO15.08

Phase II study of reovirus with paclitaxel (P) and carboplatin (C) in patients with metastatic non-small cell lung cancer (NSCLC) who have Kras or EGFR-activated tumors

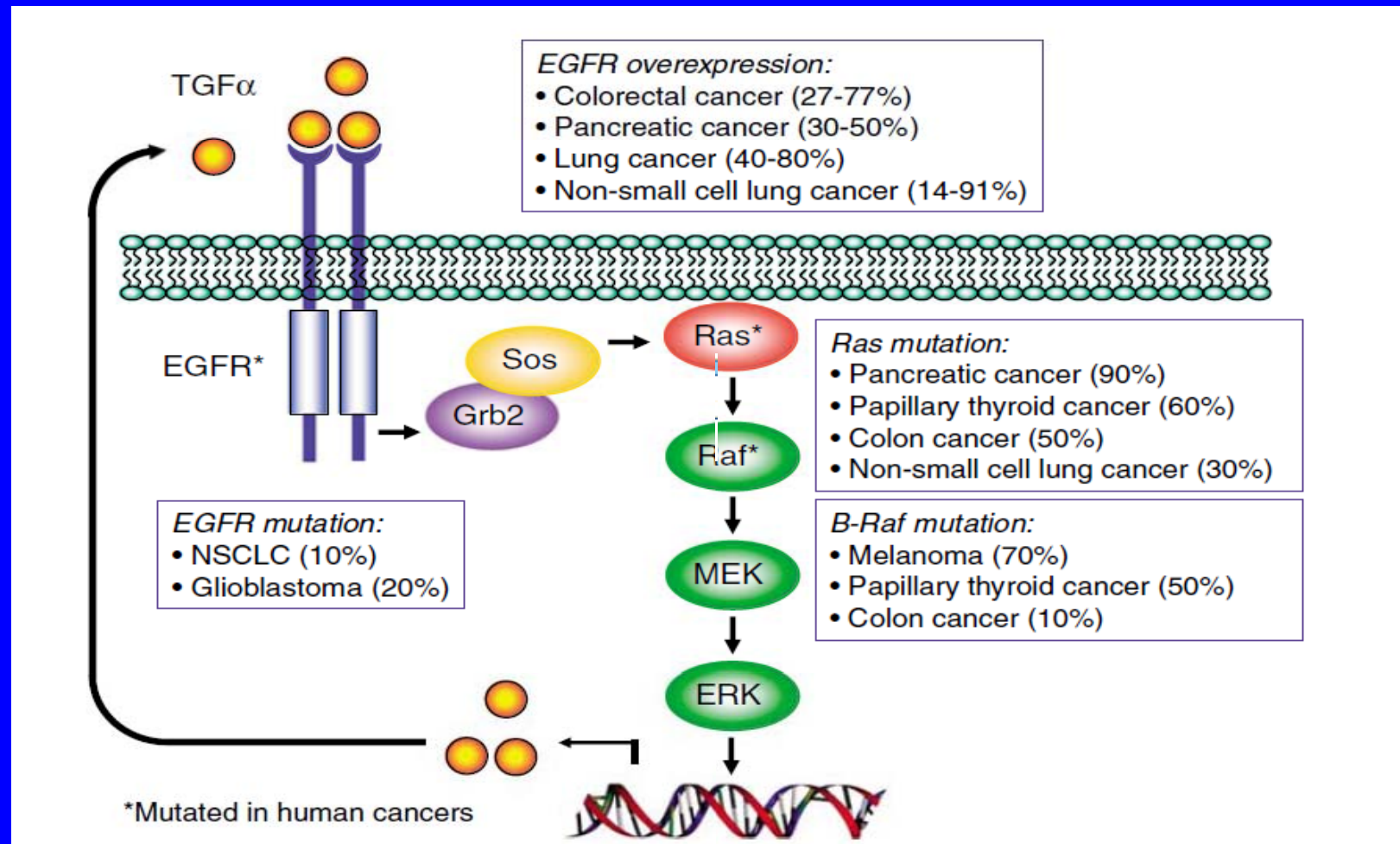
M.A. Villalona-Calero, E. Lam, G. Otterson, W. Zhao, K. Donthireddy, J. Thurmond, D. Subramanian, E. Hade, J. Pennington, M. Knopp, K. Mettinger, M. Coffey

KEY MUTATIONS IN LUNG CANCER



- a** Sensitive to EGFR inhibitors
- b** Resistant to EGFR inhibitors
- c** Sensitive to MEK1 inhibitors
- d** Sensitive to ALK inhibitors
- e** Sensitive to HER2 inhibitors

Ras and the ERK MAPK cascade



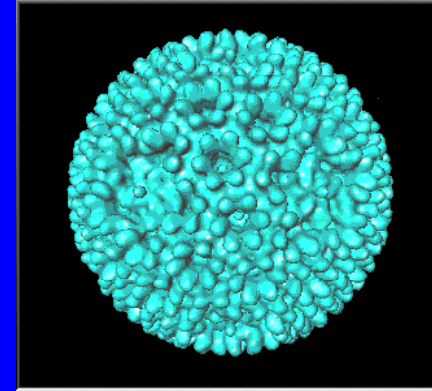
Roberts and Der. *Oncogene* 2007;26:3291-3310

Interferon-induced antiviral state

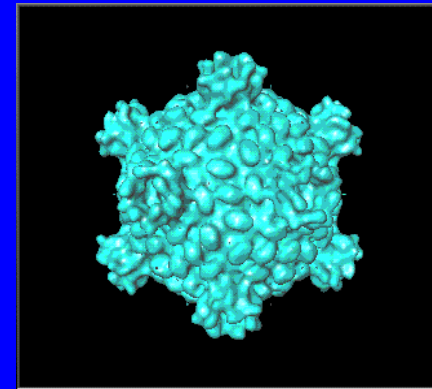
- IFN released by virus-infected cells establishes an anti-viral state in the infected and neighboring cells
- Cells with activated Ras/Raf/MEK pathway allow propagation of virus in the presence of IFN via a MEK-2 induced suppression of IFN response.

The Mammalian Reovirus

- Naturally occurring, unmodified, unattenuated oncolytic virus
- Non-enveloped human reovirus with genome of 10 dsRNA segments
- Two capsids (inner and outer)
- Following internalization, outer capsid is proteolytically degraded



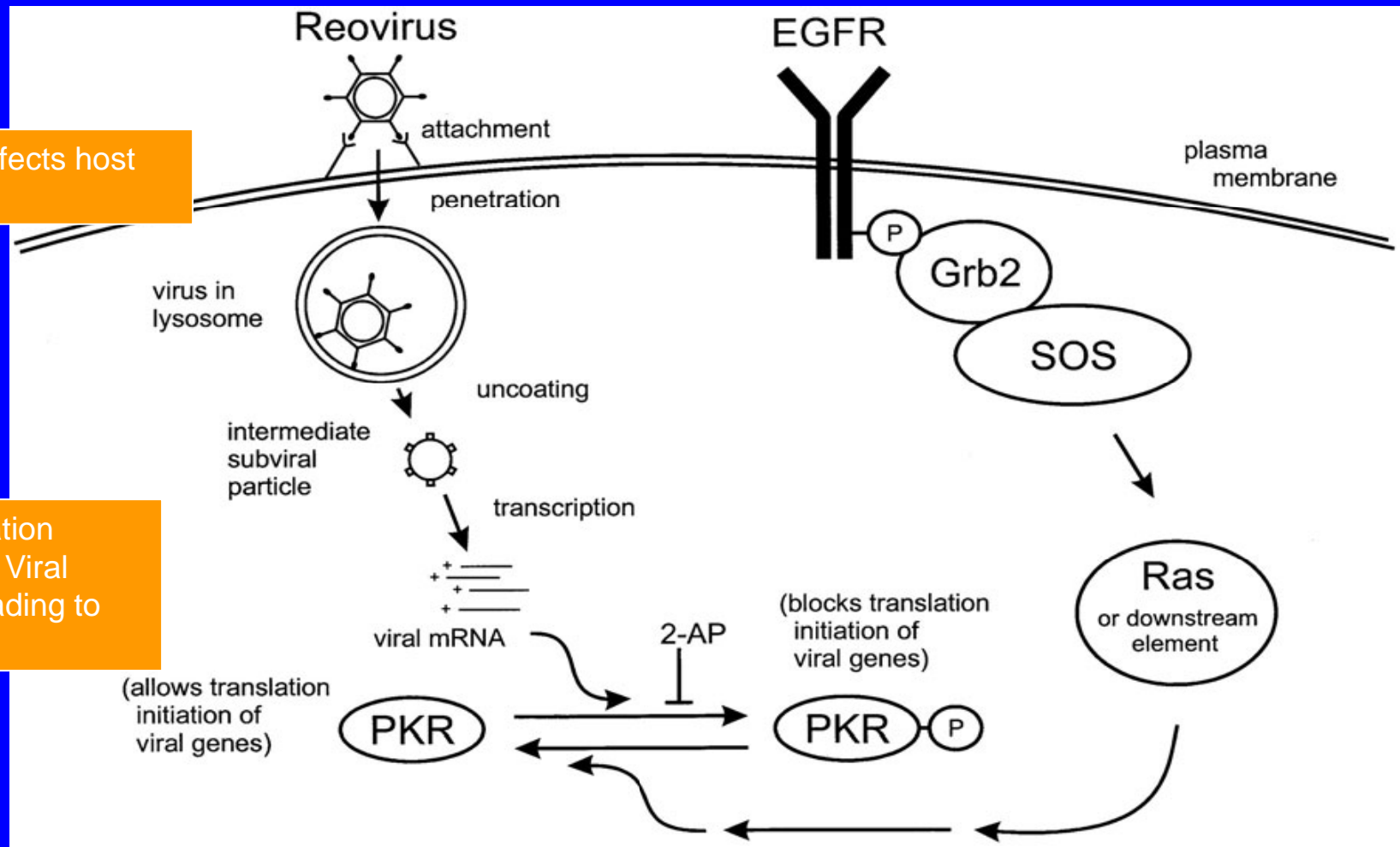
Reovirus Virion



Reovirus Core

Reovirus infects host cell

PKR deactivation enables virus replication leading to cell lysis



With activated Ras pathway, PKR is dephosphorylated (deactivated) downstream

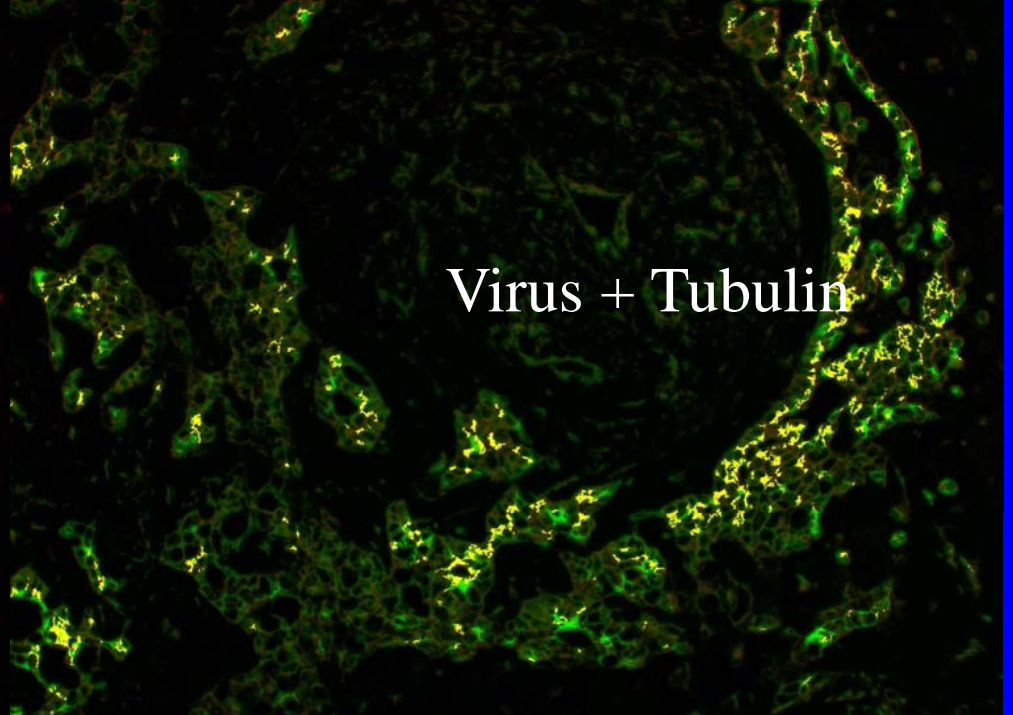
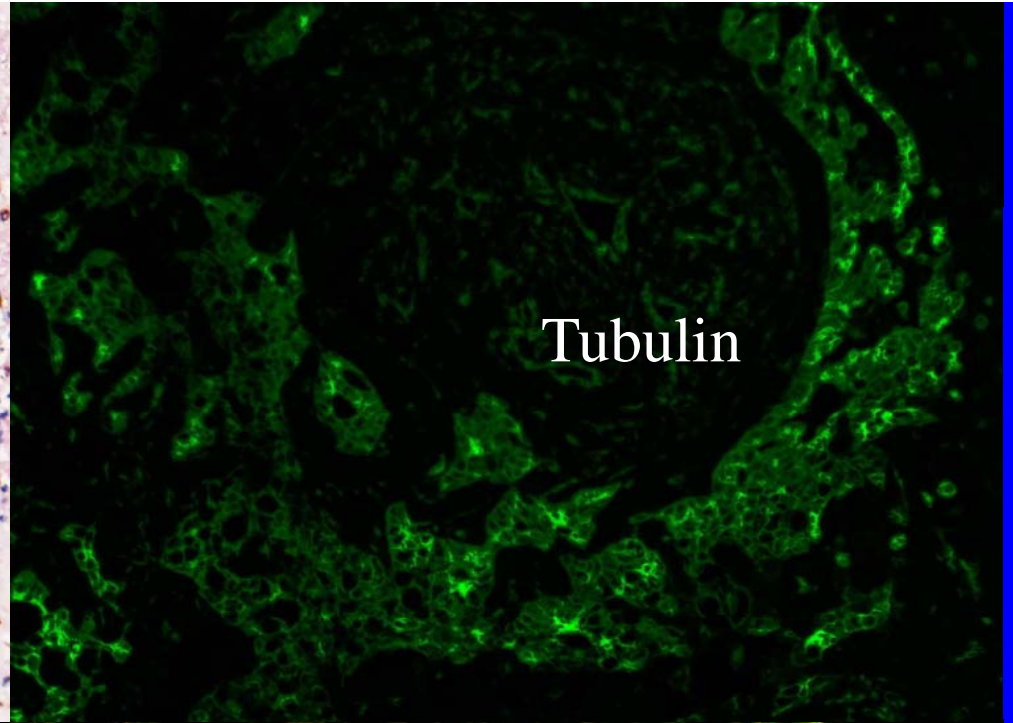
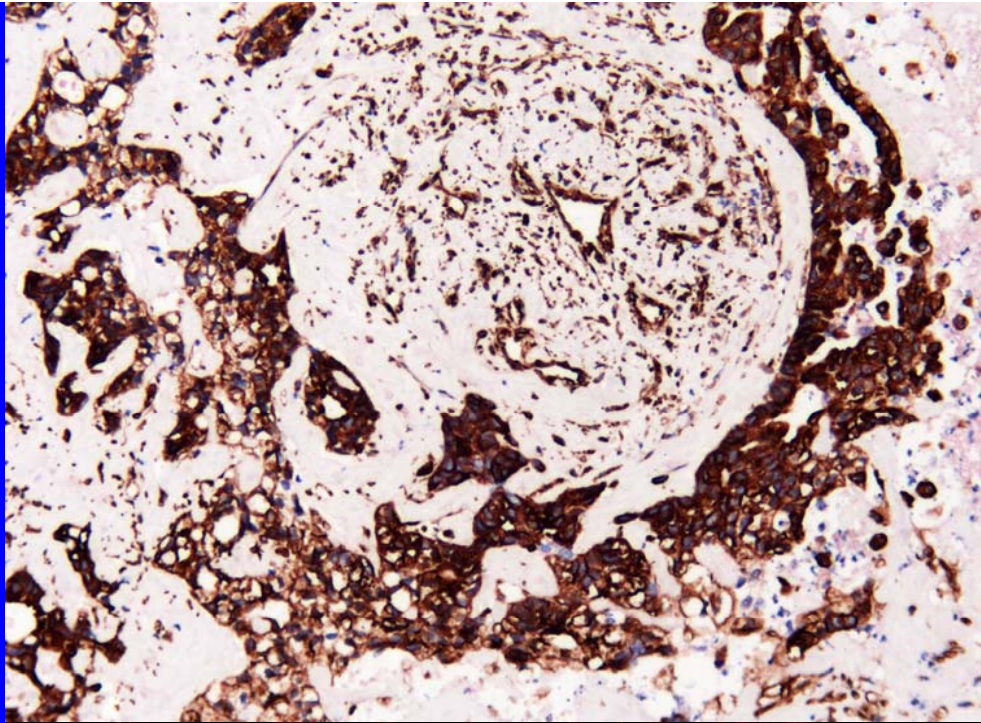
Preclinical Experience

- Reovirus is cytopathic to many human cancer cells *in vitro*, in SQ implanted murine models, and in surgical (GBM) specimens grown in short-term culture.
- Reovirus by intratumoral or intravenous administration caused tumor regression in various *in vivo* mouse models.

Strong et al, 1996. Coffey et al, 1998, Hirasawa et al, 2003. Norman et al, 2002, Alain et al, 2002.

Etoh et al, 2003. Thirukkumaran et al, 2003. Kilani et al, 2003. Yang et al, 2003. Wilcox et al, 2001.

Anticancer Drug and Cell Line	Drug Sensitivity (as a single agent) IC50 (µM)	Combination Ratio* I (drug : ReoT3D MOI)	Combination Index Values				Combination Ratio* II (drug : ReoT3D MOI)	Combination Index Values			
			ED50	ED75	ED90	Mean		ED50	ED75	ED90	Mean
<u>Paclitaxel</u>											
NCI-H460	0.003 ± 0.001	1 : 53	0.903	0.221	0.057	0.394	1 : 533	0.193	0.042	0.013	0.083
HOP-92	38.50 ± 16.01	1 : 20	0.144	0.295	0.613	0.351	1 : 200	0.178	0.420	0.994	0.530
NCI-H23	0.08 ± 0.04	1 : 20	0.577	0.303	0.159	0.347	1 : 200	0.329	0.061	0.011	0.134
EKVX	56.06 ± 15.87	1 : 20	0.294	0.440	0.660	0.464	1 : 200	0.124	0.216	0.374	0.238
NCI-H226	77.79 ± 25.08	1 : 20	0.543	0.344	0.218	0.368	1 : 200	0.004	1.30E-04	4.73E-06	0.001
NCI-H322M	> 100	1 : 20	0.069	0.092	0.133	0.098	1 : 200	0.078	0.081	0.084	0.081
<u>Cisplatin</u>											
NCI-H460	5.31 ± 1.21	1 : 53	0.875	0.313	0.112	0.433	1 : 533	0.661	0.292	0.129	0.361
HOP-92	6.82 ± 3.22	1 : 20	0.352	0.325	0.500	0.392	1 : 200	0.053	0.080	0.195	0.109
NCI-H23	3.51 ± 1.15	1 : 20	0.579	0.487	0.530	0.532	1 : 200	0.546	0.248	0.342	0.379
EKVX	29.34 ± 6.62	1 : 20	0.955	0.559	0.328	0.614	1 : 200	0.933	0.754	0.610	0.766
NCI-H226	14.71 ± 6.60	1 : 20	0.615	0.297	0.145	0.352	1 : 200	0.558	0.081	0.013	0.217
NCI-H322M	> 100	1 : 20	> 10	> 10	> 10	> 10	1 : 200	> 10	> 10	> 10	> 10



Inclusion Criteria

Metastatic or recurrent NSCLC (IIIB/IV by revised IASLC staging)

EGFR mutation, EGFR amplification, or KRAS mutation verified in a CLIA-certified lab

EGFR kinase mutations at exons 18 to 21 and Kras mutation at codons 12, 13, and 61 will be performed using PCR DNA sequencing assays.

EGFR amplification will be detected by FISH using a commercially available

Vysis® dual-color dual-probe kit to determine the ratio of EGFR gene and CEP7.

No prior chemotherapy (except TKI) in the metastatic or recurrent setting

Patients with treated brain metastasis allowed

Normal hepatic, renal and hematologic function

ECOG Performance Status 0-2

Study Design I

Recurrent or metastatic NSCLC
EGFR mutation or amplification, Ras mutation
No previous therapy

Reolysin (D1-5) plus
Carboplatin & Paclitaxel (D1) every 21 days x 4-6 cycles.
Thereafter, Reolysin single agent D1-5 q21day intervals.

First 6 patients will be treated with
Carboplatin AUC 6 and Paclitaxel 200mg/m²

Reolysin dose
(3×10^{10} TCID₅₀)

Dose reduction to C AUC 5 and P 175 mg/m² if intolerable

RECIST measurements at baseline & after every 2 cycles

Study Design II

Objectives:

Primary

1. Determine the objective response rate (Complete Response (CR) + Partial Response (PR)) of REOLYSIN in combination with paclitaxel and carboplatin in patients with metastatic or recurrent non-small cell lung cancer with KRAS- or EGFR-activated tumors.

Secondary

1. Determine the proportion of patients receiving the above treatment who are alive and free of disease progression at 6 months.
2. Determine the median duration of progression-free survival of patients receiving the above treatment.
3. Determine the median to 1-year survival of patients receiving the above treatment.
4. Evaluate the safety and tolerability of REOLYSIN in combination with paclitaxel and carboplatin in this patient population.

Statistical Considerations

To determine the proportion of patients demonstrating objective anti-tumor response, we will use a Fleming/A'Hern stage, phase II trial design. A PR requires a RECIST CT response or at least 40% reduction of uptake in PET avid lesions. We will consider the Reovirus plus chemotherapy regimen ineffective if the true response rate is 20% or less; while on the other hand, worthy of further study if the response rate is 40% or greater. This is based on an assumed 20% objective response rate for paclitaxel/carboplatin. Using a Fleming/A'Hern single stage a maximum of 36 patients will be recruited. An improvement in response rate from 20% to 40%, with the addition of REOLYSIN, would be excluded with 90% power and an alpha of 0.1, if fewer than 10/36 responses were observed.

Analysis of Secondary Endpoints

Secondary endpoints will include progression free and overall survival, 6 month progression free survival and description of safety and tolerability. Survival endpoints will be described by the Kaplan-Meier method and confidence intervals for median survival time will be calculated by the Brookmeyer-Crowley method. Summary statistics will be used to characterize secondary endpoints by patient characteristics and response. Confidence intervals and hypothesis tests will provide information in planning future studies. P-values may be adjusted for multiple comparisons by the Holms procedure.

Patients Characteristics I

Patients Treated/Evaluable	22/21
Sex (male/female)	7/15
Median age, years (range)	66 (49-82)
IASLC Stage	
IV A	9
IV B	13
Histology	
Adenocarcinoma	15
PD 3 MD 2 WD 2 NS 8	
BAC	1
Squamous	3
NOS	3
Previous Radiotherapy	6
Previous Chemotherapy (adj)	4
Previous Molecular Targeted Tx	3

Patients Characteristics II

Study ID	EGFR FISH	EGFR-M	KRAS-m	KRAS-M	KRAS-M%
016-102-01	neg	neg	c.34G>C	G12R	F=33% , R=24%
016-102-02	neg	neg	c.34G>T	G12C	F=17%, R=17%
016-102-03	amp >2	neg	neg	neg	neg
016-102-04	pos	neg	c.34G>T	G12C	F=41.6%, R=40.4%
016-102-05	amp >2	ex19 del	neg	neg	neg
016-102-06	pos	neg	c.35G>A	G12D	F=34%, R= 28.5%
016-102-07	pos	neg	c.36T>G, c.37G>C	silent, G13R,	F=50%, R=40.5%; F=31.5%, R= 30%
016-102-08	amp > 2	neg	neg	neg	neg
016-102-09	pos	neg	neg	neg	neg
016-102-10	pos	neg	neg	neg	neg
016-102-11	pos	ex19 del	neg	neg	neg
016-102-12	pos	neg	neg	neg	neg
016-102-13	neg	neg	c.34G>T	G12C	F=11.6%, R=13.5%
016-102-14	neg	neg	c.37G>T	G13C	F=6% , R=7%)
016-102-15	pos	neg	neg	neg	neg
016-102-16	pos	neg	neg	neg	neg
016-102-17	neg	neg	c.34G>T, c.35G>T	G12F, G12V	F=14.%/R=17% F=17.%/R=23
016-109-18	neg	ex19 del	neg	neg	neg
016-102-19	pos	neg	neg	neg	neg
016-102-20	pos	neg	c.34G>T	G12C	F=27%, R=27%
016-109-21	pos	ND	ND	ND	ND
016-102-22	pos	neg	neg	neg	neg
	16 pts	3 pts	G>T 8	9 pts	
	3 amp > 2		G>C 2	2 double mut	
			G>A 1		

HEMATOLOGIC TOXICITIES

Dose Level

P (mg/sqm)

C (AUC)

Number of patients with

Reo (3×10^{10} TCID₅₀)

Neutropenia

Anemia

Platelets

pts

G2

G3

G4

Febrile

G3

G4

G2

G3

G4

200/6

6

1

1

2

2

2

0

1

0

0

175/5

16

2

2

2

1

2

0

0

1

0

MOST FREQUENT NON-HEMATOLOGIC TOXICITIES*

Dose Level P/C		Number of patients with									
		Hypotension		Diarrhea		N/V		Fatigue		Confusion	
	# pts	G2	G3	G2	G3	G2	G3	G2	G3	G2	G3
200/6	6	0	0	1	2	0	1	0	3	0	0
175/5	16	0	3	0	0	0	1	0	2	0	1

* Revised NCI Common Toxicity Criteria 3

**NCI Toxicity Grade

1 pt PEA, likely related to carboplatin sensitivity

Study ID	EGFR FISH	EGFR-M	KRAS-m	KRAS-M	KRAS-M%	Number of Cycles	Best response	Actual TTP (days)	Reason off Study
016-102-01	neg	neg	c.34G>C	G12R	F=33%, R=24%	3	PR	169	Off due to AE
016-102-02	neg	neg	c.34G>T	G12C	F=17%, R=17%	4	SD	205	Off due to AE
016-102-03	amp >2	neg	neg	neg	neg	3	PR	88	Disease Progression
016-102-04	pos	neg	c.34G>T	G12C	F=41.6%, R=40.4%	6	SD	120	Disease Progression
016-102-05	amp >2	ex19 del	neg	neg	neg	6	PR	129	Disease Progression
016-102-06	pos	neg	c.35G>A	G12D	F=34%, R= 28.5%	6	SD	119	Disease Progression
016-102-07	pos	neg	c.36T>G, c.37G>C	silent, G13R,	F=50%, R=40.5%; F=31.5%, R= 30%	1	withdrew consent		Consent withdrawal, went to hospice care
016-102-08	amp > 2	neg	neg	neg	neg	8	SD	170	Disease Progression
016-102-09	pos	neg	neg	neg	neg	6	SD	472	Switched maintenance pemetrexed after 6 cycles
016-102-10	pos	neg	neg	neg	neg	2	PD	36	Disease Progression
016-102-11	pos	ex19 del	neg	neg	neg	1	PD	4	Disease Progression brain met on head ct done shortly after study initiation
016-102-12	pos	neg	neg	neg	neg	4	PR		Referred for microwave ablation of liver lesion
016-102-13	neg	neg	c.34G>T	G12C	F=11.6%, R=13.5%	4	SD	80	Disease Progression
016-102-14	neg	neg	c.37G>T	G13C	F=6% , R=7%)	4	SD	78	Disease Progression
016-102-15	pos	neg	neg	neg	neg	4	SD	78	Disease Progression
016-102-16	pos	neg	neg	neg	neg	8	PR	168	Disease Progression
016-102-17	neg	neg	c.34G>T, c.35G>T	G12F, G12V	F=14.%/R=17% F=17.%/R=23	4	SD		Off due to SAE, placed on Alimta maintenance
016-109-18	neg	ex19 del	neg	neg	neg	4	SD		
016-102-19	pos	neg	neg	neg	neg	4	SD		
016-102-20	pos	neg	c.34G>T	G12C	F=27%, R=27%	4	SD		
016-109-21	pos	ND	ND	ND	ND	4	PR		
016-102-22	pos	neg	neg	neg	neg	3	SD		
							6 PR, 13 SD,		
							2PD, 1 NE		
16 pts		3 pts	G>T 8	9 pts					
3 amp > 2			G>C 2	2 double mut					
			G>A 1						

8/18/2009

6/25/2009



8/18/2009

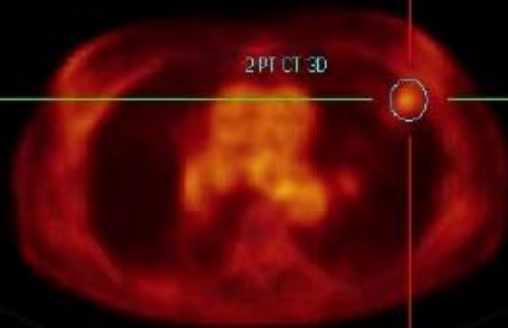
6/25/2009

016-102-0004
11/21/01/1537
8/18/2009
9:35:22 AM

FUSED MPR
Follow-up 1

AR
2
Min: 0.46 SUV bw, -1009 HU
Avg: 1.20 SUV bw, -364 HU
Max: 2.73 SUV bw, 570 HU
Max Diameter: 2.6 cm
Max Size: X: 2.5 cm, Y: 2.0 cm, Z: 2.5 cm
Volume: 8.98 cm3
SP (Max): -523.0 mm

Biograph64
VG10A



R

F

SP F820.3
SL: 4.000

CTPT: 4/ 96
W: 300 T: 5.07 SUV bw
C: 40 B: 0.00 SUV bw

1
2



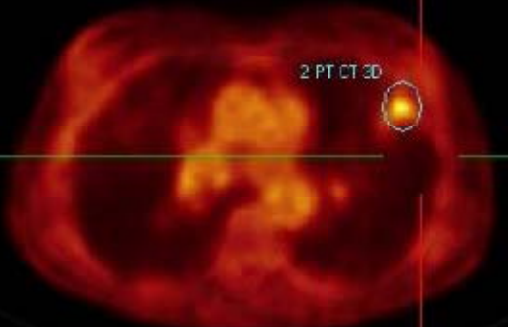
T: 20%
B: 19%

016-102-0004
11/21/01/1537
6/25/2009
12:25:00 PM

FUSED MPR
Baseline

A
2
Min: 0.54 SUV bw, -1024 HU
Avg: 1.04 SUV bw, -373 HU
Max: 5.27 SUV bw, 547 HU
Max Diameter: 3.2 cm
Max Size: X: 2.5 cm, Y: 3.2 cm, Z: 2.5 cm
Volume: 10.49 cm3
SP (Max): 17.6 mm

Biograph64
VG10A



R

F

1
2



TrueD: MIP Control [X]

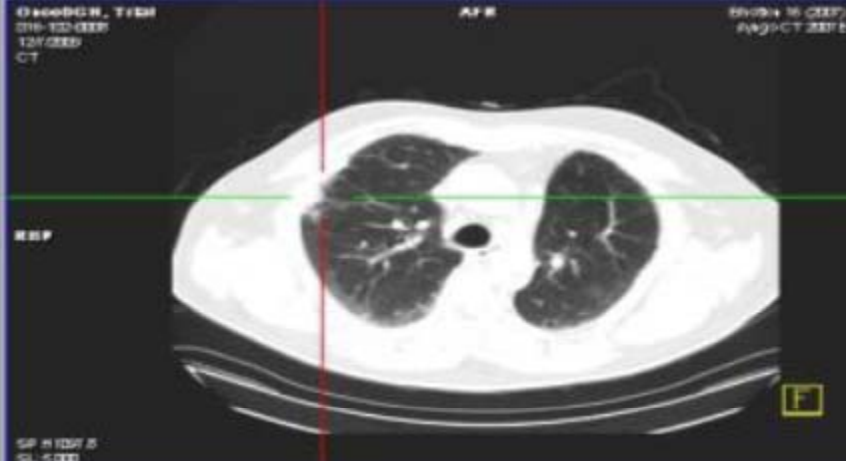
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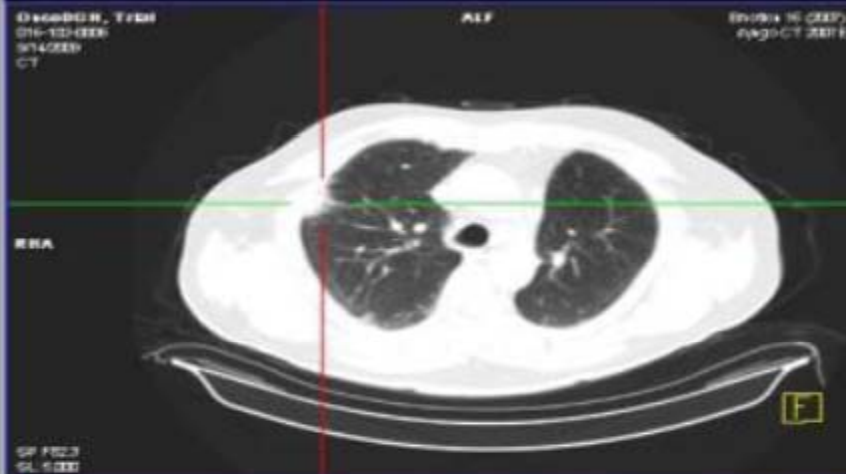
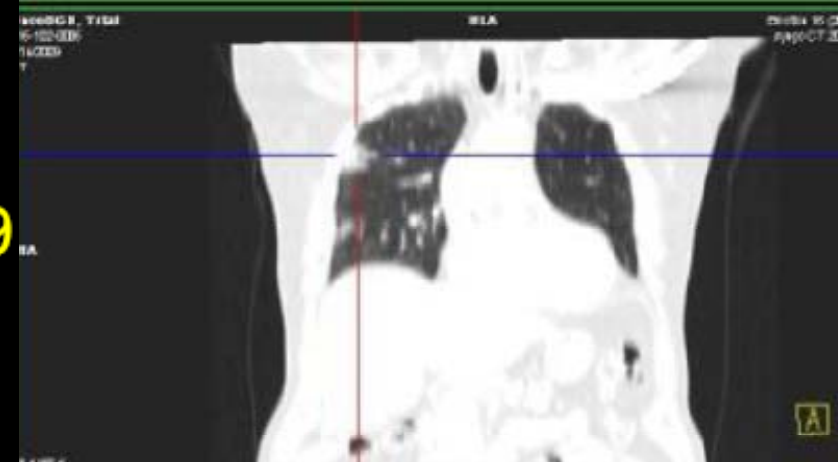
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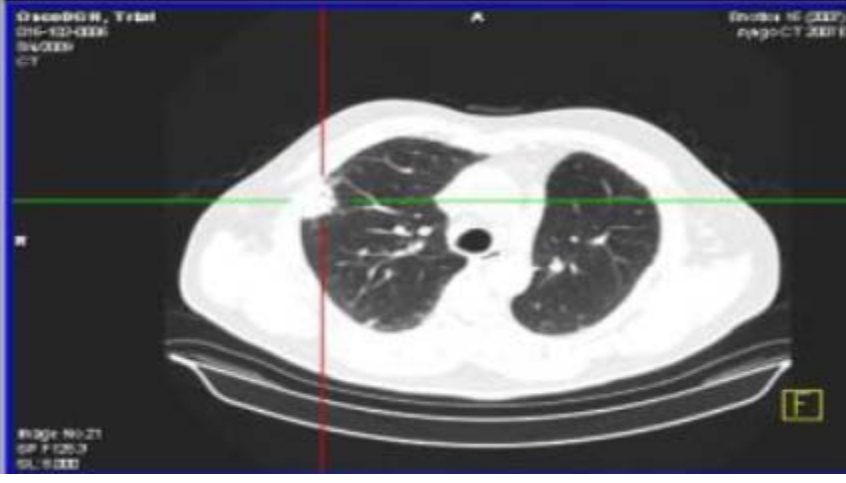
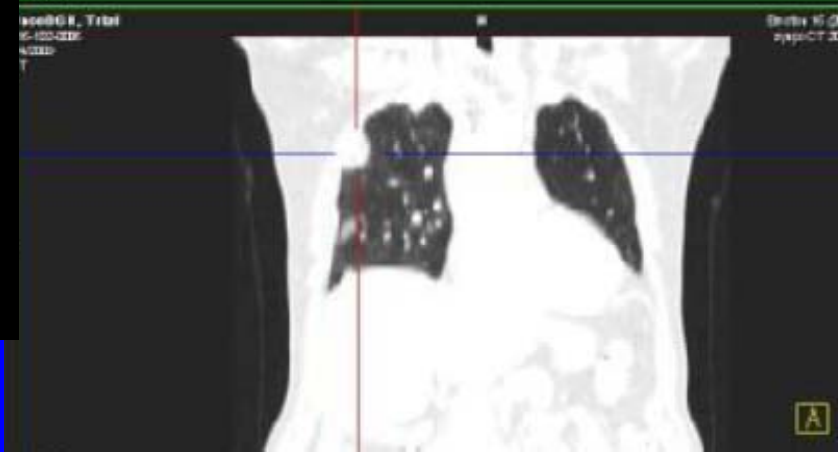
12/7/2009



09/14/2009



8/4/2009



Conclusions

- Patient selection based on molecular profile for first line therapy in NSCLC is feasible.
- Reovirus can be administered safely in combination with C/P
- Clinical benefit observed so far is encouraging
- Historical controls are not informative on the effect of chemotherapy in Kras activated NSCLC
- A randomized trial to differentiate the contribution of the addition of the reovirus to chemotherapy appears warranted.