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Oncolytic Reovirus in Combination With Chemotherapy in Metastatic or Recurrent Non–Small Cell Lung Cancer Patients With KRAS-Activated Tumors

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Abstract

BACKGROUND—The type 3 Dearing reovirus (Reolysin) is a naturally occurring virus that preferentially infects and causes oncolysis in tumor cells with a *Ras*-activated pathway. It induces host immunity and cell cycle arrest and acts synergistically with cytotoxic agents.

METHODS—This study evaluated Reolysin combined with paclitaxel and carboplatin in patients with metastatic/recurrent KRAS-mutated or epidermal growth factor receptor (EGFR)–mutated/amplified non–small cell lung cancer.

RESULTS—Thirty-seven patients were treated. Molecular alterations included 20 KRAS mutations, 10 EGFR amplifications, 3 EGFR mutations, and 4 BRAF-V600E mutations. In total, 242 cycles (median, 4; range, 1-47) were completed. The initial doses were area under the curve (AUC) 6 mg/mL/min for carboplatin, 200 mg/m^2 for paclitaxel on day 1, and 3×10^{10} 50% tissue

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CONFLICT OF INTEREST DISCLOSURES

Gregory Otterson reports grants from Pfizer, Genentech, Boeh-ringer Ingelheim, Clovis, NewLink Genetics, Celgene, and Bristol-Myers Squibb outside the submitted work. George M. Gill is employed by Oncolytics Biotech, Incorporated, as chief safety officer. Matthew Coffey is a cofounder of Oncolytics Biotech, Incorporated (US Patent 6,110,461). Giovanni Selvaggi is Vice President of Clinical Development for Oncolytics Biotech, Incorporated. Bo Chao is currently an employee of Eli Lilly and Company.

culture infective dose for Reolysin on days 1 to 5 of each 21-day cycle. Because of diarrhea and febrile neutropenia (in the first 2 patients), subsequent doses were reduced to 175 mg/m^2 for paclitaxel and AUC 5 mg/mL/min for carboplatin. Toxicities included fatigue, diarrhea, nausea/vomiting, neutropenia, arthralgia/myalgia, anorexia, and electrolyte abnormalities. Response Evaluation Criteria in Solid Tumors 1.0 responses included the following: partial response for 11 patients, stable disease (SD) for 20 patients, progressive disease for 4 patients, and not evaluable for 2 patients (objective response rate, 31%; 90% 1-sided lower confidence interval, 21%). Four SD patients had >40% positron emission tomography standardized uptake value reductions. The median progression-free survival, median overall survival, and 12-month overall survival rate were 4 months, 13.1 months, and 57%, respectively. Seven patients were alive after a median follow-up of 34.2 months; they included 2 patients without disease progression at 37 and 50 months.

CONCLUSIONS—Reolysin in combination with paclitaxel and carboplatin was well tolerated. The observed response rate suggests a benefit of the reovirus for chemotherapy. A follow-up randomized study is recommended. The proportion of patients surviving longer than 2 years (30%) suggests a second/third-line treatment effect or possibly the triggering of an immune response after tumor reovirus infiltration.

Keywords

BRAF; epidermal growth factor receptor (EGFR); lung cancer; KRAS; oncolytic virus

INTRODUCTION

Epidermal growth factor receptor (EGFR) dysregulation and KRAS mutations occur commonly in non–small cell lung cancer (NSCLC), and both lead to downstream activation of *Ras*-dependent pathways. Patients with non–EGFR-mutated/EGFR-amplified tumors derive little benefit from EGFR tyrosine kinase inhibitors (TKIs), whereas no effective KRAS-targeted therapy is currently available. Targeting *Ras*-dependent pathways is, therefore, a major area of unmet therapeutic need in NSCLC.

The type 3 Dearing strain reovirus (Reolysin) is a naturally occurring, ubiquitous, nonenveloped human reovirus with a genome that consists of 10 segments of double-stranded RNA. In preclinical studies, it has been shown to induce host immunity and cell cycle arrest and to act synergistically with chemotherapy.[1] A reovirus infection begins with the internalization of the virus via the attachment of the reovirus sigma 1 protein to the cell surface sialic acid residues.[2] Enhanced infection efficiency has been observed with either functional EGFR or the *v-erb B* oncogene.[3,4] In unsusceptible cells, a reovirus infection results in the autophosphorylation of double-stranded RNA–activated protein kinase R (PKR). The phosphorylation event activates PKR, which in turn phosphorylates the α subunit of eukaryotic initiation factor 2 and subsequently inhibits viral protein synthesis.[5,6] In reovirus-susceptible cells, the active *Ras*-signaling pathway inhibits the autophosphorylation of PKR and thereby permits the synthesis of viral proteins and results in the lysis of the host cell (Fig. 1). In EGFR-, Sos-, or *ras*-transformed cells, PKR is held in a nonphosphorylated state, and the replication of the reovirus proceeds uninhibited.[6] The dependence of reovirus activity on activation or mutation of the Ras pathway may be indication-specific.[7,8]

A reovirus administered with paclitaxel was synergistic in all NSCLC cell lines examined, including those with high-level resistance to paclitaxel or the reovirus.[9] Phase 1 clinical trials involving Reolysin dispensed as a monotherapy or in combination with gemcitabine, cyclophosphamide, docetaxel, or paclitaxel and carboplatin demonstrated its good tolerability as monotherapy (mild to moderate flulike symptoms and gastrointestinal symptoms were the major side effects) and a lack of exacerbation of the toxicities of the chemotherapeutic agents when it was given in combination.[10-13]

Because of the preferential activity for Reolysin observed in *Ras*-activated cells, we set out to screen NSCLC patients to select those with a KRAS-activated pathway through KRAS mutations or EGFR mutations or amplification and to establish the safety and efficacy of Reolysin in combination with paclitaxel and carboplatin in those patients.

MATERIALS AND METHODS

Patients

The institutional review boards of Ohio State University and Georgetown University approved this study (NCT 00861627 at ClinicalTrials.gov). Patients who were 18 years old or older and had recurrent or metastatic NSCLC with EGFR activation (EGFR-activating mutations in exons 18-21 or EGFR fluorescence in situ hybridization [FISH] amplification) or KRAS mutations (exon 2, codons 12, 13, and 61) and no previous cytotoxic chemotherapy for metastatic disease (except previous therapy for localized disease or TKIs for EGFR-mutant patients) were eligible. Other eligibility requirements included the following: the signing of written informed consent, a lapse of at least 6 months from prior adjuvant chemotherapy or chemoradiation therapy, an Eastern Cooperative Oncology Group performance status≤2, and normal organ and marrow function (absolute neutrophil count≥1.5×10^9, platelets≥100×10^9, hemoglobin ≥9g/dL, serum creatinine and bilirubin ≤1.5 times the upper limit of normal, and aspartate aminotransferase/alanine aminotransferase ≤2.5 times the upper limit of normal). TKI treatment and palliative radiation must have been discontinued for at least 4 weeks, and the toxicities must have been reduced to grade 1 or lower. Exclusion criteria included pregnancy, brain metastases (resected oligometastases were allowed), grade 2 or higher peripheral neuropathy, uncontrolled concurrent illness (including a known cardiac ejection fraction < 40%), known human immunodeficiency virus infection, and active hepatitis B or C.

Molecular Characterization

All molecular studies were performed centrally at the Molecular Pathology Core Facility at Ohio State University (OSU-PCF). FISH for EGFR amplification was performed with a commercially available Vysis dual-color, dual-probe kit to determine the ratio of the EGFR gene (orange) to CEP7 (green) in the tumor cells under a fluorescence microscope, and it was interpreted by a board-certified pathologist. Positive amplification was scored if any of the following were observed: 1) EGFR/CEP7 ratio≥2, 2)≥15 copies of EGFR per cell in 10% or more of analyzed cells, and 3) EGFR copy numbers per nucleus≥4 in 20% or more of analyzed cells.

For the identification of EGFR and KRAS mutations, genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue with the Qiagen DNA preparation kit, and the target sequences containing the targeted mutations were amplified by polymerase chain reaction with specific primers for the genes of interest. After purification, the polymerase chain reaction products were sequenced with an ABI 3130xl DNA analyzer and were interpreted by a pathologist at OSU-PCF.

Study Design and Treatment Plan

The study was a single-arm, Fleming-A'Hern, single-stage, open-label, phase 2 study[14,15] with a 6-patient run in a mini–phase 1 portion to ensure the appropriateness of chemotherapy doses for combination with Reolysin. Paclitaxel was administered as a 3-hour intravenous infusion, and carboplatin was administered as a 30-minute intravenous infusion. The chemotherapy was followed by Reolysin, which was administered as a 60-minute intravenous infusion at a dose of 3×10^{10} 50% tissue culture infective dose ($TCID_{50}$) on day 1. On days 2 through 5, Reolysin alone was administered at the same dose and with the same method used on day 1.

The study was designed to reject the combination therapy with a 90% chance (10% 1-sided type I error) if the objective response rate (ORR) was 20% and to consider the combination worthy of further study with a high chance (90%; 10% type II error) if the true ORR was 40% or more. Thirty-six response-evaluable patients were planned for recruitment, and an improvement was determined if more than 10 of the 36 patients were observed to have objective response. A partial response (PR) required a Response Evaluation Criteria in Solid Tumors (RECIST) 1.0 computed tomography (CT) response or at least a 40% uptake reduction in positron emission tomography (PET)–avid lesions.

For the phase 1 portion, dose-limiting toxicity was defined as grade 4 neutropenia for > 7 days or with sepsis or fever, grade 4 thrombocytopenia, or grade 3/4 nonhematologic toxicity. Toxicities were graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (version 3.0). The starting doses were area under the curve (AUC) 6 mg/mL/min (Calvert formula) for carboplatin and 200 mg/m^2 for paclitaxel with a planned dose reduction for subsequent patients in the phase 2 portion to AUC 5 mg/mL/min for carboplatin and 175 mg/m^2 for paclitaxel if the first dose was intolerable in at least 2 of 6 patients. Cycles were repeated every 3 weeks.

Dose Modifications

For subsequent treatment courses in patients with toxicities meeting the aforementioned criteria for dose-limiting toxicity, dose reductions (25% of the dose of paclitaxel and decreases of 1 mg/mL/min in the AUC dose of carboplatin) were planned as long as a clinical benefit could be documented. Paclitaxel was discontinued for grade 3 or worse peripheral neuropathy. Dose reductions of Reolysin were to be undertaken only for moderate to severe symptoms not attributable to chemotherapy, such as flulike symptoms, rhinorrhea, and diarrhea. For these events, the Reolysin dose was decreased to 1×10^{10} $TCID_{50}$. In the absence of intolerable toxicity or tumor progression, the combination regimen was given for

4 to 6 cycles (according to physician preference), and this was followed by Reolysin as single-agent maintenance with the same schedule of 5 days every 3 weeks.

Study Endpoints

The primary endpoint was the ORR (complete response + PR) as per a RECIST 1.0 CT response[16] or at least a 40% reduction of uptake in PET-avid lesions.[17] CT was performed at the baseline and after every 2 cycles for all patients, whereas PET was introduced in this fashion, after an amendment, for the last 26 patients. Secondary endpoints included progression-free survival (PFS), overall survival (OS), safety, and tolerability.

Statistical Methods

Exact binomial confidence intervals (CIs) for the ORR were calculated to summarize the primary endpoint for all response-evaluable patients and by subgroup.[18] Survival endpoints were summarized with the methods of Kaplan and Meier,[19] with Brookmeyer-Crowley CIs used for the median survival time and with Greenwood's method used for 6- and 12-month survival estimates. PFS was defined from the date of study entry to the date of disease progression. Patients who withdrew from the study, were lost to follow-up, died without progressive disease, or began alternative treatments before progression was noted were censored on the date of withdrawal, last follow-up, or new treatment initiation. OS was determined as the time from study entry to the date of death or was censored on the date on which a patient was last known to be alive. The final review was completed on March 15, 2015. All analyses were unadjusted for multiple comparisons and were performed with STATA (version 13.1; StataCorp, College Station, Texas).

RESULTS

Screening

Patients with metastatic or recurrent NSCLC who were found with a routine EGFR amplification/KRAS mutation assessment performed at OSU-PCF were referred for participation in the study. From March 2009 to February 2013, 37 patients were enrolled at the Ohio State University Medical Center (n = 33) and Georgetown University Medical Center (n = 4). The characteristics of these patients are depicted in Table 1. The majority of tumors had an adenocarcinoma histology (n = 27), which reflected the association of testing for KRAS and EGFR mutations with adenocarcinomas; 2 patients with a mixed adenosquamous histology, 1 patient with squamous carcinomas, and 1 patient with a bronchioloalveolar histology were also enrolled. Six patients had NSCLC that was not otherwise specified. KRAS mutations were present in 20 patients, whereas EGFR amplification alone was present in 10 patients. Three patients' tumors exhibited EGFR-activating mutations, and 4 patients' tumors (all EGFR-amplified) also had concurrent BRAF-V600E mutations.

Dose Evaluation and Toxicities

Two patients were treated at the starting doses of AUC 6 for carboplatin, 200 mg/m^2 for paclitaxel, and 3×10^{10} $TCID_{50}$ for Reolysin, and both experienced unacceptable toxicities (febrile neutropenia in one and grade 3 diarrhea in the other). These patients were dose-

reduced for subsequent cycles to AUC 5 for carboplatin and 175 mg/m^2 for paclitaxel; Reolysin was kept at 3×10^{10} $TCID_{50}$ as previously planned, and these starting doses were used for all subsequently enrolled patients. Overall, 242 cycles (including 105 for Reolysin-only maintenance) were administered to the 37 patients (median, 4; range, 1-47). Chemotherapy was further reduced because of adverse events for only 6 additional patients (17%; 18 of 240 cycles or 8%). Only 1 patient had to reduce the Reolysin dose; more than 95% of planned doses were administered at the full dose over the course of the study. The type, grade, and frequency of the toxicities observed with the combination are depicted in Table 2. The combination at the reduced doses was generally well tolerated. The most frequent moderate to severe toxicities included fatigue (8 patients with grade 3, 1 patient with grade 4), neutropenia (7 patients with grade 3, 1 patient with grade 4), diarrhea (5 patients with grade 3), and nausea/vomiting (3 patients with grade 3). Four patients developed hypotension, and 2 patients developed confusion. Fever was noted only during Reolysin-only maintenance.

Antitumor Activity

Table 3 depicts the best response according to RECIST tumor assessment results, the percentage change in the standardized uptake value (SUV) maximum sum of tumor lesions on PET scans performed after 2 cycles versus the baseline, and PFS and OS according to molecular and histologic characteristics. Two patients were not evaluable for a response because of consent withdrawal shortly after they had received 1 cycle. Eleven of 35 evaluable patients had a RECIST response; all were partial (ORR, 31%; 90% 1-sided lower CI, 21%) as assessed by investigators and were confirmed at a 4-week scan. Twenty patients had stable disease (SD) as their best response, whereas 4 patients experienced disease progression on their first postbaseline assessment. The percentage of patients who had a RECIST PR with a KRAS tumor genotype (5 PRs among 18 evaluable patients) and the percentage of patients who had a PR with an EGFR amplification–alone tumor genotype (3 PRs among 10 evaluable patients) were similar (odds ratio of EGFR amplification alone vs KRAS, 1.1; 95% CI, 0.20-6.11; $P = .90$). Two PRs occurred among 4 patients with BRAF-mutated tumors (50%; 95% CI, 7%-93%). Twenty-four patients had PET scans performed simultaneously with the CT assessments, before treatment, and after every 2 cycles. Ten of these patients (6 with PRs and 4 with SD according to RECIST) had a > 40% decrease in the SUV sum of the lesions after 2 cycles. No significant increases in SUV were observed after 2 cycles in any of the patients treated. Thus, the RECIST + PET ORR was 43% (90% 1-sided lower CI, 31%).

The median PFS, OS, and 12-month OS rate were 4 months (95% CI, 2.9-6.1 months), 13.1 months (95% CI, 9.2-21.6 months), and 57% (95% CI, 39%-72%), respectively (Table 3). Ten patients went on to receive Reolysin-only maintenance after 4 to 6 cycles of the combination, whereas 3 patients opted to switch to pemetrexed maintenance at the completion of paclitaxel, carboplatin, and Reolysin without progression or significant toxicity, and 1 patient (carrying an EGFR-activating mutation) was switched to erlotinib after 6 cycles. These patients were censored at the time of the switch to the alternative therapy for the PFS calculations. Kaplan-Meier PFS and OS plots according to the molecular phenotype are depicted in Figures 2 and 3. Seven patients were alive after a

median follow-up of 34.2 months (range, 26.9-71.5 months); they included 2 patients (with an EGFR mutation and a KRAS G12 mutation) with no evidence of disease progression to date (37 and 50 months, respectively; Table 3).

DISCUSSION

A systematic evaluation of tumor genetics in NSCLC has led to the identification of tumor drivers and, in some cases, to effective targeted therapies. Notable examples are EGFR mutations and anaplastic lymphoma kinase trans-locations, for which several targeted agents are available for clinical use.[20,21] KRAS constitutive activation has been more challenging; this has led some to conclude that it is not a druggable target. The preclinical data for the Reolysin virus showing selectivity for *Ras* have, therefore, high appeal for an evaluation in *Ras*-activated NSCLC patients. The *Ras* pathway can be activated through an upstream event, such as EGFR mutations and amplification, or through a downstream event, such as BRAF mutations. This study included a group of patients with diverse molecular aberrations that had the common denominator of *Ras* activation, and it showed good feasibility for the population-enriched approach undertaken as well as a good safety profile for the combination of Reolysin with chemotherapy in this population.

In this study, the RECIST response rate for paclitaxel, carboplatin, and Reolysin according to the investi gators' assessment was significantly increased (31%; 90% 1-sided lower CI, 21%) in comparison with the assumed historical response rate for paclitaxel and carboplatin alone (20%). However, the firmness of historical data is limited, and the comparison being used here is hypothesis-generating because the prognostic or predictive value of KRAS mutations is still a matter of debate. Studies in early-stage disease offer contradictory information.[22-24] The First-Line Erbitux in Lung Cancer (FLEX) study, performed with patients with metastatic NSCLC, randomized EGFR-expressing patients by immunohisto-chemistry to receive either cisplatin and vinorelbine or this chemotherapy in combination with cetuximab.[25] OS, but not PFS, was better for the cetuximab-containing arm. In a retrospective molecular characterization study that used tumors from patients in the FLEX trial,[26] the response rate for patients with KRAS mutations was 36.8% for patients receiving cetuximab and chemotherapy and 21.6% for those receiving chemotherapy alone. PFS was 5.5 months (95% CI, 3.1-6.9 months) for cetuximab and chemotherapy and 2.9 months (95% CI, 1.8-5.8 months) for patients with KRAS mutations receiving chemotherapy alone. However, OS was only 8.9 months for the combination versus 11.1 months for chemotherapy alone. In the group of patients enrolled with evidence of EGFR amplification by FISH, the response rates were 36.7% and 26.4% for the cetuximab/chemotherapy and chemotherapy arms, respectively, whereas PFS was 4.2 and 4.4 months and OS was 11.6 and 9.9 months for the cetuximab/chemotherapy and chemotherapy arms, respectively.

Although the response rate (31% according to RECIST) and the median survival rate (13.1 months) for our trial compare favorably with those for the chemotherapy-alone arm in the FLEX trial, the lack of a randomized control group in our trial does not allow us to make firm interpretations of superior antitumor efficacy for the approach tested in this study. Furthermore, we did not observe significant differences in clinical outcomes for patients enrolled according to the genotype leading to *Ras* activation (Figs. 2 and 3), and because of

the lack of a non–*Ras*-activated control cohort, we could not confirm or reject the utility of *Ras* activation as a predictor of Reolysin/chemotherapy activity.

Significant increases in neutralizing antireoviral antibodies (median, 250-fold) have been demonstrated in humans with advanced cancers after exposure to Reolysin,[27] and coadministration of cyclophosphamide was shown not to be able to attenuate the host antiviral response to a reovirus.[28] However, despite the presence of neutralizing antibodies, it has been demonstrated that a reovirus can evade neutralization by associating with peripheral blood mononuclear cells for up to 10 days after treatment in patient samples.[28,29] Further demonstration of the ability of a reovirus to escape neutralization can be found in the persistence of an infectious, replicating virus in patient tumor samples at various periods up to weeks after reovirus administration despite the presence of neutralization antibodies. Infectious reoviruses have been isolated from various solid tumor types after systemic administration, including ovarian cancer, melanoma, pleural mesothelioma, breast cancer, pancreatic cancer, colon cancer, and head and neck cancer,[10,12,29-33] and from hematopoietic malignancies such as multiple myeloma.[34]

Thus, it is very tempting to speculate on the potential for Reolysin to create immunogenicity against the tumor cells infiltrated by the virus. This response could theoretically be capable of bypassing operating mechanisms of immune tolerance and result in longer survival. The current study was designed for patients to receive maintenance reovirus alone after initial induction with Reolysin and chemotherapy; this was done except for the 4 patients who switched to pemetrexed or erlotinib for maintenance. An intriguing and potentially better approach could be the use of a checkpoint inhibitor in that setting, which could benefit from the immunogenicity created by the virus during the induction phase. In support of this hypothesis, $CD8^+$-enriched splenocytes have been shown to secrete augmented levels of interferon-γ when they are cocultured with Reolysin ver sus an ultraviolet-inactivated reovirus; this indicates reovirus recognition by $CD8^+$ cells and proinflammatory response stimulation through interferon-γ secretion.[35] Furthermore, therapy combining a reovirus with programmed death 1 blockade has been shown to produce a significant survival benefit by augmenting tumor-specific natural killer responses and specifically attenuating tumor-specific immunosuppression.[36]

In summary, Reolysin in combination with paclitaxel and carboplatin was well tolerated, and the observed response rate met study specifications. A randomized trial that could elucidate both the contribution of Reolysin to the effect of chemotherapy and the utility of a predictive biomarker is planned. It would be interesting to explore serum immune response markers as well as tumor biopsy before and after Reolysin therapy to evaluate the extent of tumor infiltration with markers of an immune response such as $Fox\text{-}P3^+$ regulatory T cells. Because of the benefits shown with immune checkpoint inhibitor therapy in lung cancer and the intriguing data of immune stimulation after viral exposure, a study combining the 2 approaches would be an exciting follow-up study.

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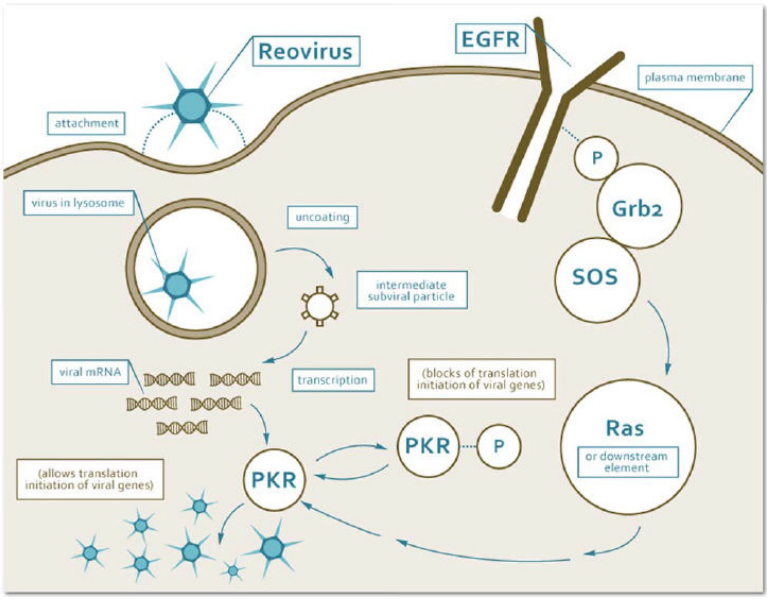

Figure 1.

Stepwise and schematic representation of the reovirus type 3 Dearing mechanism of action in the cytoplasm of cancer cells with an activated *ras* signaling pathway. The phosphorylated proteins (EGFR, and PKR) are tagged with *P*. EGFR indicates epidermal growth factor receptor; mRNA, messenger RNA; PKR, protein kinase R.

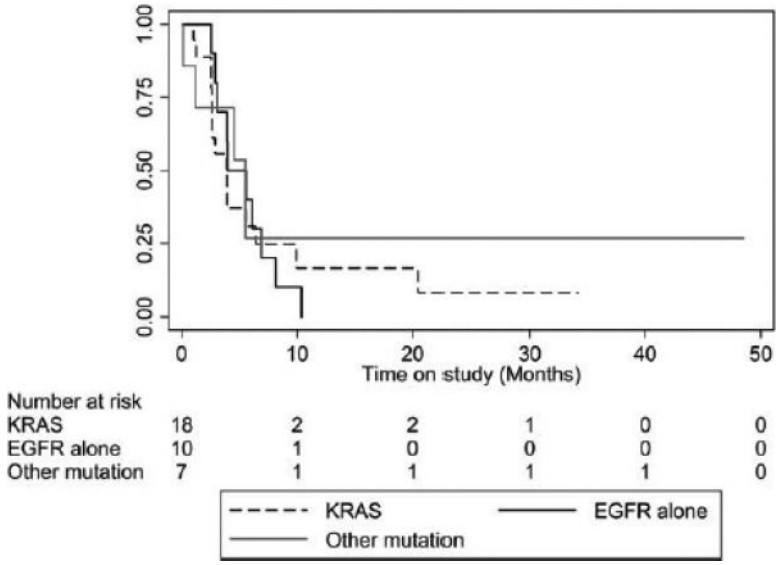

Figure 2.

Progression-free survival rates versus time for patients receiving Reolysin in combination with paclitaxel and carboplatin according to molecular abnormalities. The data are presented as months. EGFR indicates epidermal growth factor receptor.

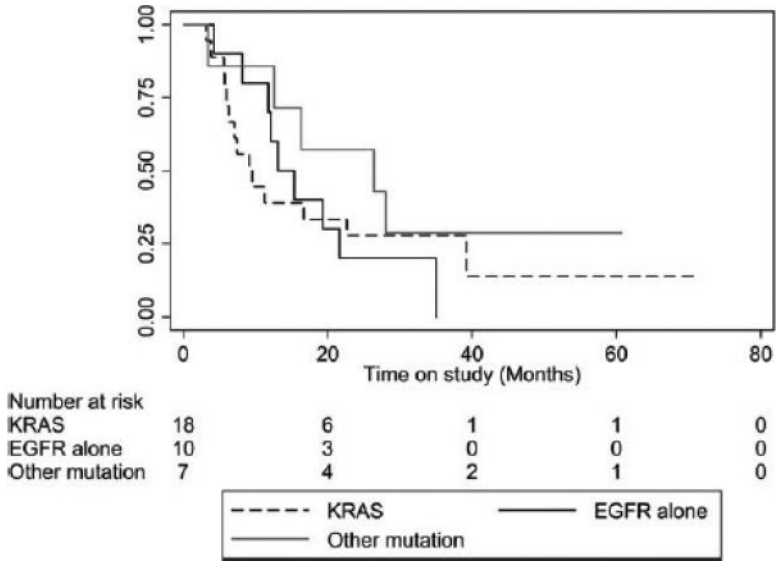

Figure 3.

Overall survival rates versus time for patients receiving Reolysin in combination with paclitaxel and carboplatin according to molecular abnormalities. The data are presented as months. EGFR indicates epidermal growth factor receptor.

TABLE 1

Patient Characteristics

Enrolled patients, No.	**37**
Age, y	
Median	65
Range	47-82
Sex	
Men	15
Women	22
Race/ethnicity	
White	32
African American	5
Hispanic/Latino	0
ECOG performance status	
0	23
1	13
2	1
Stage	
IVA	14
IVB	23
Recurrent	8
Histology	
Adenocarcinoma	26
Adenosquamous	2
Squamous	2
Bronchioloalveolar	1
NSC NOS	6
Molecular Abnormality	
KRAS mutation only	14
EGFR-amplified only	10
EGFR mutant only	1
KRAS mutation + EGFR amplification	6
EGFR mutation + EGFR amplification	2
BRAF mutation + EGFR amplification	4
Prior anticancer therapies	
Targeted agents	3
Chemotherapy (adjuvant)	4
Surgery	13
Thoracic surgery for NSCLC	9
Resection of brain metastasis	4
Radiation	15
Adjuvant (mediastinum)	5

Enrolled patients, No.	**37**
Palliative	12

Abbreviations: ECOG, Eastern Cooperative Oncology Group; NOS, not otherwise specified; NSC, non–small cell; NSCLC, non–small cell lung cancer.

TABLE 2

Toxicities for Reolysin in Combination With Paclitaxel and Carboplatin

	Reolysin Plus Paclitaxel With Carboplatin at 200mg/m^2/AUC 6 (n = 2), %				Reolysin Plus Paclitaxel With Carboplatin at 175 mg/m^2/AUC 5 (n = 36), %			
Toxicity	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Hematologic								
Neutropenia	0	0	0	50	0	14	19	0
Anemia	0	0	50	0	6	14	3	0
Thrombocytopenia	0	50	0	0	14	3	3	0
Lymphocytopenia	0	0	0	0	8	11	6	0
Febrile neutropenia	0	0	50	0	0	0	0	0
Non hematologic								
Nausea	50	50	0	0	64	8	3	0
Vomiting	0	0	0	0	22	8	8	0
Anorexia	0	50	0	0	17	11	0	0
Diarrhea	0	0	100	0	72	17	8	0
Confusion	0	0	0	0	6	3	3	0
Headaches	0	0	0	0	3	0	0	0
Transaminitis	0	0	0	0	14	6	3	0
Fatigue/asthenia	0	50	50	0	83	33	19	3
Arthralgia	0	0	0	0	11	17	0	0
Myalgia	0	0	0	0	17	28	0	0
Peripheral neuropathy	50	0	0	0	14	3	0	0
Hypotension	0	0	0	0	0	0	0	3
Peripheral edema	0	0	50	0	3	0	0	0
Fever	0	0	0	0	36	8	3	0

Abbreviation: AUC, area under the curve.

TABLE 3

Clinical Benefit Assessment of Paclitaxel, Carboplatin, and Reolysin in Non-Small Cell Lung Cancer

Patient No.	Abnormality	Amino Acid	Nucleotide	Histology	Cycle No.	Best Response	PET SUV Change, %	Progression-Free Survival, d	Overall Survival, d
0023	KRAS, EGFR Amp	G12C	c.34G>T	Adenocarcinoma	2	PD	IC	31	193
0004	KRAS, EGFR Amp	G12C	c.34G>T	NSC NOS	6	SD	−49.8	120	1195
0006	KRAS, EGFR Amp	G12D	c.34G>A	Adenocarcinoma	6	SD	ND	119	690
0020	KRAS, EGFR Amp	G12C	c.34G>T	Adenocarcinoma	4	SD	1.4	78	343
0026	KRAS, EGFR Amp	G12C	c.34G>T	NSC NOS	8	SD	−51.8	196	279
0027	KRAS, EGFR Amp	G12V	c.34G>T	Adenocarcinoma	4	SD	−22.3	80	508
0001	KRAS	G12R	c.34G>C	Adenocarcinoma	3	PR	−80.6	170	227
0002	KRAS	G12C	c.34G>T	Adenocarcinoma	4	PR	ND	624	>2164
0007	KRAS	G13R	c.37G>C	Adenocarcinoma	1	NE	ND	N/A	Unknown
0013	KRAS	G12C	c.34G>T	Adenocarcinoma	4	SD	ND	80	96
0014	KRAS	G13C	c.37G>T	Adenocarcinoma	4	SD	ND	78	182
0017	KRAS	G12C; G12V	c.34G>T; c.35G>T	Adenocarcinoma	C4D1	SD	−64.0	235	290
0025	KRAS	G12C	c.34G>T	Adenocarcinoma	1	NE	ND	90	228
0028	KRAS	G12S	c.34G>A	NSC NOS	4	SD	0.0	88	172
0032	KRAS	G12V	c.35G>T	Adenocarcinoma	14	PR	−48.5	302	>999
0034	KRAS	G12V	c.35G>T	Adenocarcinoma	4	SD	−36.3	80	175
0035	KRAS	G12A	c.35G>C	Adenosquamous	6	SD	−34.5	120	>828
0037	KRAS	G12C	c.34G>T	Adenocarcinoma	6	PR	−0.1	Died without PD	216
0031	KRAS	G12A	c.35G>C	Adenocarcinoma	47	PR	−100.0	>1105	>1105
0038	KRAS	G12C	c.34G>T	Adenocarcinoma	2	PD	ND	38	118
0005	EGFRm, EGFR Amp	p.E746_T51 del	c.2237_2251del15	Bronchioloalveolar	6	PR	ND	139	498
0011	EGFRm, EGFR Amp	p.E746_T51 del	c.2237_2251del15	NSC NOS	1	PD	ND	4	105
0018	EGFRm	p.E746_T51 del	c.2237_2251del15	Adenocarcinoma	C6D1	SD	−13.9	>1486	>1486
0003	EGFR Amp			Adenosquamous	3	SD	−37.6	88	359
0008	EGFR Amp			NSC NOS	8	SD	ND	170	589
0015	EGFR Amp			Adenocarcinoma	4	SD	ND	78	129
0019	EGFR Amp			Adenocarcinoma	10	PR	−34.3	210	399

Patient No.	Abnormality	Amino Acid	Nucleotide	Histology	Cycle No.	Best Response	PET SUV Change, %	Progression-Free Survival, d	Overall Survival, d
0022	EGFR Amp			Adenocarcinoma	6	SD	0.0	120	249
0024	EGFR Amp			Adenocarcinoma	6	SD	−50.2	121	1067
0029	EGFR Amp			Adenocarcinoma	4	PR	−63.1	93	369
0030	EGFR Amp			Adenocarcinoma	14	SD	−18.9	316	658
0033	EGFR Amp			Adenocarcinoma	14	SD	−30.7	248	>837
0021	EGFR Amp			Squamous	8	PR	−80.8	185	468
0009	BRAF, EGFR Amp	V600E	c.1799T>A	Adenocarcinoma	6	SD	−34.0	575	854
0010	BRAF, EGFR Amp	V600E	c.1799T>A	Adenocarcinoma	2	PD	0.0	36	382
0012	BRAF, EGFR Amp	V600E	c.1799T>A	Adenocarcinoma	4	PR	ND	1460	>1850
0016	BRAF, EGFR Amp	V600E	c.1799T>A	NSC NOS	8	PR	−91.3	168	803

Abbreviations: Amp, amplified; C4D1, cycle 4 day 1; C6D1, cycle 6 day 1; EGFR, epidermal growth factor receptor; EGFRm, EGFR mutated; IC, incomplete; N/A, not available; ND, not determined; NE, not evaluable; NOS, not otherwise specified; NSC, non-small cell; PD, progressive disease; PET, positron emission tomography; PR, partial response; SD, stable disease; SUV, standardized uptake value.