Int J Clin Exp Med 2016;9(12):23560-23567
www.ijcem.com /ISSN:1940-5901/IJCEM0021101

*Original Article*

# Clinical trial of the rAd-p53 in combination with lenvatinib for treatment of renal cell carcinoma

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Received December 3, 2015; Accepted June 8, 2016; Epub December 15, 2016; Published December 30, 2016

**Abstract:** The objectives of this clinical study were to assess the effects of rAd-p53 in combination with Lenvatinib in patients with renal cell carcinoma (RCC). In order to determine the maximum tolerated dose (MTD) and preliminary evidence of its anti-tumor efficacy or rAd-p53 and Lenvatinib, Lenvatinib was administered orally once-daily in the indicated dose and rAd-p53 was injected intravenously twice-daily continuous schedule in 14-day treatment cycles for RCC patients. The anti-tumor efficacy for RCC patients was assessed every two day. Our clinic results showed that dose-limiting toxicities of Lenvatinib were grade 3 proteinuria at 30 mg, and the MTD was defined as 26 mg. The dose-limiting toxicities of rAd-p53 were $10^{12}$ pfu, and the MTD was defined as $10^{10}$ pfu. The most common treatment-related treatment-emergent adverse events were pain (85%) and hemorrhagic spot in injection site of rAd-p53 (50%), fatigue 32%, mucosal inflammation 43%, proteinuria, diarrhea, vomiting, hypertension, and nausea, each 46%. Lenvatinib and rAd-p53 demonstrated dose-linear kinetics without drug accumulation after 14-day treatment administration. Taken together, Lenvatinib and rAd-p53 are well tolerated at the indicated dose. Gene and target therapy for RCC could be further enhanced by synergistic effects of Lenvatinib and rAd-p53.

**Keywords:** rAd-p53, Lenvatinib, RCC, gene therapy, target therapy

## Introduction

Renal cell carcinoma (RCC) is one of the most common diseases of human cancer in the world [1, 2]. And the majority of cancer patients finally develop metastatic renal cell carcinoma [3-5]. RCC is generally resistant to chemotherapy, radiotherapy, and hormonotherapy [6, 7]. Though organ transplantation is beneficial for patients with RCC, immunological rejection, organ failure and recurrences are frequent and the survival rate of patients remains properly poor after postoperation [8-10]. Therefore, new clinical treatments for clinicians are urgently needed in order to improve the fewer efficacies of patients with RCC in clinic.

Gene and target therapies offer new therapeutic options for patients with RCC and are considered to be effective drugs for other human diseases [11-16]. Gene therapy drug of rAd-p53 is the first generation gene drug and been approved for human cancer therapy. Previous clinical trials have shown that the side-effects of rAd-p53 are acceptable in the majority of the cases [17, 18]. Target therapy drug of Lenvatinib is an oral drug and targeted vascular endothelial growth factor receptor 1-3 (VEGFR1-3), fibroblast growth factor receptor 1-4 (FGFR1-4), platelet-derived growth factor receptor-β (PDGFR-β), RET, and kinase insert domain receptor (KIT) [19, 20]. Lenvatinib has shown clinical benefit in patients with RCC [21]. And Wayne Kuznar et al reported that that Lenvatinib administrated with everolimus significantly prolonged the survival of patients with RCC compared with everolimus or Lenvatinib alone [22]. Therefore, combination gene therapy with target therapy may be efficient clinical treatments for patients with RCC or other cancers.

The purpose of this study was to assess the safety and tolerability of Lenvatinib and rAd-p53 in patients with advanced RCC. Furthermore, we intended to determine the maximum tolerated dose (MTD) and pharmacokinetic (PK) profile Lenvatinib and rAd-p53. At last, we explored and provided the preliminary evidences of anti-tumor efficacy of our clinical treatments. Our clinic results indicate that

**Table 1.** Patient's characteristics

| | Number of Patients | % |
|---|---|---|
| Total patients with RCC | 382 | 100 |
| Gender | | |
| Female | 176 | 46.1 |
| Male | 206 | 53.9 |
| Performance status (Karnofsky) | | |
| 100 | 153 | 40.1 |
| 90 | 86 | 22.5 |
| 80 | 143 | 37.5 |
| Prior treatment | | |
| Other anti-cancer medication | 38 | 9.9 |
| Surgery | 142 | 37.2 |
| Radiotherapy | 108 | 28.3 |
| Chemotherapy | 94 | 24.6 |
| Drugs treatment | | |
| rAd-p53 | 114 | 29.8 |
| Lenvatinib | 116 | 30.4 |
| rAd-p53 plus Lenvatinib | 152 | 39.8 |

Lenvatinib administrated with rAd-p53 extended overall survival significantly compared with Lenvatinib or rAd-p53 alone in patients with RCC. This study also suggests that patients with RCC were improved progression-free survival (PFS) after treated by Lenvatinib and rAd-p53 compared with Lenvatinib or rAd-p53 alone.

## Patients and methods

### *Standard protocol approvals, registrations, and patient consents*

The phase-I study (CH21010110) was administrated in Guide of Chinese clinical experiments from January 2009 to January 2015. All study was conducted in accordance with European Medicines Agency requirements. All patients provided written informed consent before any study-related procedures were performed.

### *Patient eligibility*

Eligibility criteria included age ≥18 years, with a Karnofsky performance status ≥80%; adequate hematological (platelet count of ≥100 × $10^9$/L; absolute neutrophil count of ≥1.5 × $10^9$/L; and hemoglobin ≥8.5 g/dL), hepatic (serum alanine aminotransferase; bilirubin ≤25 μmol/L and aspartate transaminase ≤3 × the upper limit of normal) and renal function (a creatinine clearance ≥60 mL/min or serum creatinine ≤1.5 × the upper limit of normal by Cockcroft-Gault formula). Previous treatment (including surgery and radiotherapy) should be completed at least 8 weeks before study entry.

### *Dose-confirmation design*

Dose confirmation was conducted with an accelerated design. The next dose level was open for patient accrual only after the first patient in the previous cohort had completed with no grade ≥1 drug-related toxicity. Dose increases in subsequent cohorts were by 100% increments until any patient at a given dose level experienced grade ≥2 toxicity, following which dose escalation increments were ≤50% and dose escalation only occurred when all three patients at a given dose level had completed one treatment cycle. Doses confirm continued until the maximum tolerated doses were determined.

### *Disease evaluation and objective response assessments*

Pre-treatment evaluation included a complete history and clinical examination, vital signs, assessment of performance status, full blood count, biochemical profile, 12-lead electrocardiogram, urinalysis, pregnancy test (if appropriate) and tumor assessments, all of which were performed within 14 days of study treatment. Tumors size was evaluated by computed tomography or magnetic resonance imaging, chest X-ray, and by physical examination in patients prior to starting study therapy. Assessments were repeated after every 2 cycles of treatment. Responses to treatment were defined according to Response Evaluation Criteria in RCC. The clinical benefit ratio was calculated as the sum of all patients experiencing a complete response, partial response or stable disease, divided by the total number of patients who were evaluable for response. Progression-free survival was defined as the elapsed time between treatment initiation and tumor progression or death from any cause. Time to tumor progression was defined as the elapsed time between treatment initiation and tumor progression.

### *Treatment administration*

Lenvatinib (once-daily) and rAd-p53 (twice-daily) were administered orally and intratumor

**Table 2.** Treatment-related hypertension and proteinuria by Common Toxicity Criteria grade

| | Total (n = 21) | Lenvatinib 12.5 mg (n = 7) | Lenvatinib 16 mg (n = 7) | Lenvatinib 26 mg (n = 7) |
|---|---|---|---|---|
| Adverse event | | | | |
| Hypertension | 10 | 2 | 2 | 6 |
| Grade 1 | 2 | 0 | 0 | 2 |
| Grade 2 | 3 | 1 | 1 | 1 |
| Grade 3 | 5 | 1 | 1 | 3 |
| Proteinuria | 11 | 2 | 3 | 6 |
| Grade 1 | 2 | 0 | 1 | 1 |
| Grade 2 | 5 | 2 | 1 | 2 |
| Grade 3 | 4 | 0 | 1 | 3 |

**Table 3.** Treatment-related adverse event of rAd-p53 with an overall incidence ≥10%

| | Total (n = 48) | $10^4$-$10^6$ (n = 16) | $10^8$-$10^{10}$ (n = 20) | $10^{12}$-$10^{14}$ (n = 12) |
|---|---|---|---|---|
| Adverse event | | | | |
| Pain | 8 | 2 | 3 | 3 |
| Hypertension | 11 | 1 | 3 | 7 |
| Diarrhea | 7 | 1 | 2 | 4 |
| Proteinuria | 8 | 1 | 2 | 5 |
| Nausea | 3 | 1 | 1 | 1 |
| Vomiting | 9 | 1 | 3 | 5 |
| Lethargy | 2 | 0 | 1 | 1 |
| Rash | 7 | 1 | 2 | 4 |
| Fatigue | 10 | 3 | 3 | 4 |
| Constipation | 4 | 0 | 2 | 2 |
| Weight decreased | 4 | 1 | 1 | 2 |
| Decreased appetite | 14 | 4 | 5 | 5 |
| Epistaxis | 2 | 0 | 0 | 2 |
| Hypertriglyceridemia | 2 | 0 | 1 | 1 |
| Edema peripheral | 2 | 1 | 0 | 1 |

injection respectively, on a continuous 14-day administration schedule. Treatment did not terminate until unacceptable toxicity, progressive disease or death. Doses confirm were performed with experiment hypertension and proteinuria and continued until the MTD was determined. In addition, no food was allowed for 4 h following administration of Lenvatinib (once-daily) and rAd-p53 (twice-daily).

*Evaluation of toxicity*

Toxicity was graded using the National Cancer Institute Common Toxicity Criteria (version 3.0). Physical examination, full blood count, biochemical profile measurement of blood pressure and urinalysis were performed every two days during combined therapy. Electrocardiograms and biochemical detection were performed every three days. A DLT was defined as any of the following drug-related toxicities in previous study [23].

*Statistical methods*

All data were reported as means and SEM. Statistical significance of differences between mean values was assessed by Student's t test for unpaired data. Comparisons of data between multiple groups were performed with analysis of variance (ANOVA). Patients' survival probability was analyzed by Kaplan-Meier plots. Continuous variables were reported as mean and 95% confidence interval (CI). Treatment effect is presented as median reduction in seizure frequency over the treatment period. Robust nonparametric Hodges-Lehmann estimates of median drugs treatment effects and 95% confidence interval are provided. Responder rates and treatment adverse events were analyzed by $x^2$ test. $P<0.05$ was considered statistically significant.

## Results

*Patient characteristics*

Patients with RCC were conducted clinical practice from January 2009 to January 2015 at Tianjin Cancer Institute, Tianjin, China. Patients' age was ranged from thirty-two to sixty-eight years (average = forty-four years old). In this study, the numbers of men and women were roughly equal in the statistics. Most of these patients with RCC had undergone surgery or/and chemotherapy (**Table 1**). Notably, almost 86% of patients had received at least one prior systemic therapy regimen. And about 27% of patients had received two systemic therapy regimens. In addition, about 15% of patients had received three systemic therapy regimens and 8% had received with four or more systemic therapy regimens.

**Table 4.** Treatment-related adverse event of Lenvatinib with an overall incidence ≥10%

| | Total (n = 63) | 0.2-3.2 mg (n = 14) | 12.5-16 mg (n = 18) | 26 mg (n = 20) | 32 mg (n = 11) |
|---|---|---|---|---|---|
| Adverse event | | | | | |
| Hypertension | 15 | 2 | 4 | 4 | 5 |
| Diarrhea | 5 | 1 | 1 | 1 | 2 |
| Proteinuria | 14 | 3 | 3 | 3 | 5 |
| Nausea | 8 | 1 | 2 | 2 | 3 |
| Vomiting | 12 | 2 | 4 | 3 | 3 |
| Lethargy | 4 | 0 | 1 | 1 | 2 |
| Rash | 5 | 1 | 1 | 2 | 1 |
| Fatigue | 17 | 5 | 3 | 5 | 4 |
| Constipation | 2 | 0 | 0 | 1 | 1 |
| Weight decreased | 6 | 1 | 2 | 2 | 1 |
| Decreased appetite | 18 | 3 | 5 | 4 | 6 |
| Hypertriglyceridemia | 6 | 0 | 2 | 1 | 3 |
| Edema peripheral | 1 | 0 | 0 | 0 | 1 |

*Maximum tolerated dose*

Patients (n = 48) with RCC received rAd-p53 in rAd-p53 group in dose cohorts: $10^4$, $10^6$, $10^8$, $10^{10}$, $10^{12}$, $10^{14}$ pfu. Patients (n = 63) with RCC received Lenvatinib in Lenvatinib group in dose cohorts: 0.2, 0.8, 1.6, 3.2, 12.5, 16, 21, 26 and 32 mg. The MTD dose cohort of rAd-p53 was defined as $10^{10}$ pfu. The MTD dose cohort of Lenvatinib was defined as 26 mg. Partial patients required to reduce drug dose for cumulative toxicity after the dose-limiting toxicity (DLT) assessment period at a higher hypertension and protein uria (**Table 2**). Therefore, most of patients were enrolled at a dose of 16 mg to meet further clinical experiment of the tolerability and anti-tumor effect of Lenvatinib.

*Toxicity of rAd-p53 and Lenvatinib*

Dose-limiting toxicities occurred in patients at doses of $10^6$ pfu rAD-p53 (n = 1, grade 3 Diarrhea), $10^8$ pfu (n =2, grade 3 Rash), $10^{10}$ pfu (n = 1, grade 3 hypertension and grade 3 fatigue) $10^{12}$ pfu (n = 2, grade 3 hypertension grade 3 pain in injected site) and $10^{14}$ pfu (n = 3, grade 3 proteinuria and grade 3 pain in injected site). Dose-limiting toxicities of Lenvatinib occurred in patients at doses of 1.6 mg (n = 1, grade 3 Diarrhea), 12.5 mg (n = 1, grade 4 Rash), 16 mg (n = 1, grade 3 hypertension) and 32 mg (n = 5, both grade 3 proteinuria). Most of patients with RCC treated at the $10^{10}$-pfu of rAd-p53 (82%) or 26-mg dose of Lenvatinib (84%) showed relative few toxicities. In addition, we concluded that dose of 32 mg Lenvatinib and $10^{12}$ pfu rAd-p53 were not tolerable for patients with RCC. Therefore, the MTD of rAd-p53 was identified as $10^{10}$ pfu and the MTD of Lenvatinib was identified as 26 mg.

In treatment-related adverse event experiment, we observed that the most frequent drug-related toxicities of rAd-p53 were pain in injected site (n = 8, 16.67%), constipation (n = 4, 22.92%), fatigue (n = 10, 20.83%), vomiting (n = 9, 18.75%), and decreased appetite (n = 4, 0.5%) in rAd-p53-treated groups. Most of these toxicities were below grade 2. Grade 3 hypertension occurred in eleven patients (22.9%) and grade 3 proteinuria in eight patients (16.7%), with a trend towards an increase in hypertension and proteinuria with increasing doses of rAd-p53 (**Table 3**). And in treatment-related adverse event in Lenvatinib-treated groups, the most frequent drug-related toxicities of Lenvatinib were proteinuria (n = 14, 22.22%), hypertension (n = 15, 23.81%), and gastrointestinal toxicities including stomatitis (n = 26, 32%), nausea (n = 30, 37%), vomiting (n = 8, 12.70%) and diarrhea (n = 12, 19.05%) (**Table 4**). Hematological toxicities were occurred 11.2% patients in rAd-treated group in dose of $10^{14}$ pfu and 13.5% patients in Lenvatinib-treated group in dose of 32 mg.

*Anti-tumor activity*

In order to test our design clinic experiment of Lenvatinib and rAd-p53 for patients with RCC, anti-tumor activities were observed in patients diagnosed with RCC (**Table 5**). Fifty-two patients (53%) improved progressive disease (PD) in a best response manner in three treated groups. Clinical benefit (defined as PD rate plus stable disease (SD rate) occurred in 97 patients (95.1%). In this clinic experiment, 102 patients remain on treatment with Lenvatinib and rAd-

**Table 5.** Treatment duration and response according to response evaluation criteria in RCC

| Dose level (mg or pfu) | No. of patients | Duration in weeks (range) | Best response, n (%) | | | |
|---|---|---|---|---|---|---|
| | | | Partial response | Stable disease | Progressive disease | Not evaluated |
| rAd-p53 ($10^{10}$) | 104 | 0-92 | 10 (9.6) | 57 (54.8) | 36 (34.5) | 1 (1) |
| Lenvatinib (26) | 106 | 0-108 | 14 (13.2) | 42 (39.6) | 48 (45.3) | 2 (1.9) |
| Combined treatment | 102 | 0-168 | 3 (2.9) | 44 (43.1) | 53 (52.0) | 2 (2.0) |
| Total | 312 | 0-168 | 27 (8.7) | 143 (45.8) | 137 (43.9) | 5 (1.6) |

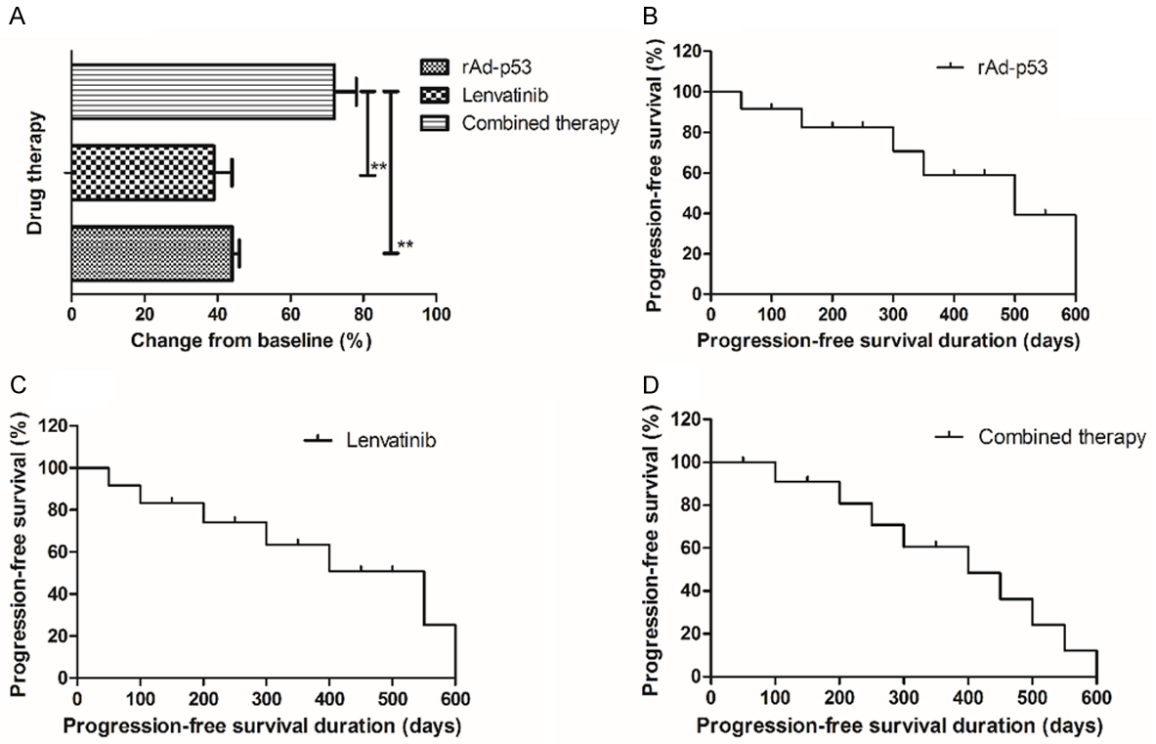


**Figure 1.** Waterfall plots displaying tumor responses to (A) rAd-p53 ($10^{10}$ pfu) (B) Lenvatinib (16 mg) and (C) combined therapy. (D) Kaplan-Meier plots representing progression-free survival for patients with RCC. Measurements were recorded as per the Response Evaluation Criteria in RCC. Abbreviation: CI = confidence interval.

p53. Percent of three patients (2.9%) with renal cancer treated with Lenvatinib and rAd-p53 had partial response. In **Figure 1** showed that the volumes of tumors were decreased in all patients with renal cancer ranging from 34.6 to 72.4%. The change of tumor for all patients with RCC showed a decrease of approximately 85% in the size of tumor diameters. The results showed that all patients with renal cancer treatment with Lenvatinib and rAd-p53 had a median progression-free survival (PFS) of 424 days.

## Discussion

Because rAd-p53 tumor suppressor gene therapy agent has general tumor-inhibit efficacy for human malignancies [17, 24]. Therefore, rAd-p53 has become a leading candidate for clinical cancer studies including renal cell carcinoma, hepatic carcinoma and melanomas [17, 25, 26]. Clinical studies utilizing kinds of human cancer patients have shown more efficient cell cycle arrest, apoptosis, transduction, and enhanced cell death following treatment with rAd-p53 alone or in combination with cytotoxic chemotherapy, radiotherapy and resection [17, 25]. Results from previous studies revealed better clinical efficacy in models of several malignancies, which suggest promise for the strategy of rAd-p53 gene therapy as a novel cancer gene therapeutic approach. Most clinical data to date indicated that rAd-p53 wasdifficult to reach lesions when using the method

of intravenous injection, in contrast to intratumor injection could facilitate drug to reach lesions [24, 27]. Although some clinical cases suggest that the treatment effects of rAd-p53 are sufficient to treat cancer patients, more cancer patients have found that cancer cells are generally resistant to single treatment method.

Therapeutic drugs targeting of VEGF-mediated pathways have become trend treatment for human cancers, including single-target and multi-target potent drugs [22, 28, 29]. Lenvatinib is a multi-targeted tyrosine kinase inhibitor of multi receptors-mediated angiogenesis, which was identified as important factors in the development and metastasis of RCC [19, 20]. Lenvatinib that target the VEGFR1-3, FGFR1-4, PDGFR-β, Ret, and KIT pathway, have also shown clinical benefit in RCC and other human cancers [21]. However, there remain challenges need to be improved as far as be concerned about targeted treatment outcomes for the reason of resistance to single-agent therapy of Lenvatinib in clinic [19]. Therefore, there is a continued need for more effective combinations of targeted agents and gene therapy drugs to improve efficacy treatment for patients with RCC.

In this study, the anti-tumor effects of rAd-p53 in combination with Lenvatinib were assessed in patients with RCC. Lenvatinib (16 mg) or/and rAd-p53 ($10^{10}$ pfu) are well tolerated when administered by a single or co-treatment in clinical schedule. The combined treatment mitigated the disease in patients with RCC. Treatment-related abdominal complaints was reported in 17% of patients, in keeping with the incidence of blood spot reported in 20% of patients treatment with rAd-p53, as well as in studies of other cancers that treated by rAd-p53 [17, 18].

Treatment-related hypertension was observed in 48% of patients with RCC after treatment with Lenvatinib (16 mg) and rAd-p53 (1010 pfu) in this work, which is higher than the incidence of hypertension reported in the other clinical study with Lenvatinib combination with other assisted drugs [30, 31]. The incidence of treatment-related proteinuria was reported in 52% of patients with RCC after treatment with Lenvatinib and rAd-p53, which was much higher than with previous reports [22, 28, 32-35]. However, our clinical protocols were much better compared to other clinical reports as for as therapeutic effects.

In conclusion, our study suggests that Lenvatinib (16 mg) or/and rAd-p53 ($10^{10}$ pfu) are well tolerated when administered to patients with RCC. Encouraging anti-tumor effects were observed in patients with RCC. These results encourage our clinical protocols to use in cancer types, including melanoma, ovarian cancer and hepatic carcinoma.

## Disclosure of conflict of interest

None.

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