# NanoPac® Inhalation Treatment of NSCLC in a Nude Rat Orthotopic Lung Cancer Model

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## Abstract (#8535)

**Background**: NanoPac is patented submicron particle paclitaxel in stable powder form without coating or carrier agents. In a previous PK study, healthy male rats inhaled a single exposure of nebulized NanoPac (0.37 mg/kg or 1.0 mg/kg) or IV Abraxane® (5.0 mg/kg) with a final necropsy time-point at 14 days. $T_{1/2}$ of NanoPac and Abraxane were 56hrs and 20hrs with drug present at 14 days and not after 3 days, respectively. Tissue examined from the last time point were microscopically indistinguishable from non-treated controls.

**Materials and Methods**: 120 nude rats were assigned to 6 groups and intratracheally instilled with Calu-3 lung tumor cells ($2x10^6$). Target doses and treatment schedules; Group 1 (Control: untreated), Group 2 (Abraxane: IV 5 mg/kg q1wx3), Group 3 (NanoPac: Inhaled (IH) 0.5 mg/kg q1wx4), Group 4 (NanoPac: IH 1.0 mg/kg q1wx4), Group 5 (NanoPac: IH 0.5 mg/kg q2wx4), Group 6 (NanoPac: IH 1.0 mg/kg q2wx4). Nebulized NanoPac (2µm Mass Median Aerodynamic Diameter) was delivered via nebulization into a nose-only exposure chamber; necropsy occurred (Group 2: 14 days), (Groups 3-6: 1 - 4 days) post final treatment. Histology was performed on sections of left lung stained with H&E from 10 animals in each group and scored using a 4-point grading scale.

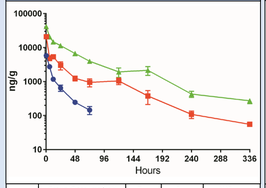
**Results**: Average lung/brain weight ratios for Group 2 (2.3) and Groups 3-6 (2.7) indicated a therapeutic effect vs Group 1 (3.2). Lung tumor burden decreased in combined Groups 3-6 as compared to Groups 1 and 2; characterized by the histological measures of tumor mass, primitive tumor cell population and tumor regression. Average primitive tumor cell population was significantly less ($p<0.05$) for Groups 3-6 (0.3) compared to Group 1 (0.9) or Group 2 (1.0). Combined NanoPac Groups 3-6 (22/40) exhibited a significant ($p<0.05$) incidence of tumor regression compared to Group 1 (0/10) and Group 2 (1/10).

**Conclusion**: NanoPac treatment groups compared to Control and Abraxane groups demonstrated a therapeutic effect as measured by lower lung/brain weight ratio and lower overall lung tumor burden without apparent adverse events. Histological analysis of lung tumor burden treated with NanoPac IH showed a decrease in tumor mass, a decrease in primitive tumor cell population, and an increase in tumor regression.

## Background

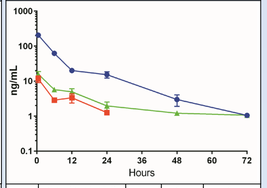
- NanoPac is in clinical development to locally treat ovarian, prostate, and pancreatic cancers as well as pancreatic cysts.
- Prior to use, this powder is suspended in physiological saline containing 0.1% polysorbate 80, without the requirement of Cremophor® EL, or binding/carrying agents.
- Intraperitoneal administration of NanoPac in the clinic has exhibited prolonged local drug residence with de minimus systemic exposure, providing a depot release mechanism direct to the tumor.
- To evaluate the potential for local delivery of NanoPac to the lungs via inhalation, a preclinical pharmacokinetic study was first conducted to confirm prolonged paclitaxel residence in the lung when administered via nebulized inhalation in healthy male Sprague-Dawley rats in one of two inhaled doses (0.38 or 1.18 mg/kg) compared to intravenous nab-paclitaxel (2.9 mg/kg).
- NanoPac was nebulized with 2 parallel Up-Mist (Hospitak, Convatec, McAllen, TX) compressed air jet nebulizers with an average Mass Median Aerodynamic Diameter (MMAD) of 1.8 µm, and 1.9 µm, for the 0.38 mg/kg and 1.18 mg/kg arms respectively. Animals were sacrificed (n =3) at 0.5, 6, 12, 24, 48, 72, 120, 168, 240, and 336 hours post administration. Quantifiable levels of paclitaxel were present in the lung tissue (LLOQ = 50 ng/g) at study completion, two weeks post-inhalation.

Figure 1. Lung Tissue Paclitaxel Concentration Time Curve and PK Data




| | Group [Dose (mg/kg)] | $T_{1/2}$ (hours) | $C_{max}$ (ng/g) | $AUC_{0(last)}$ (h*ng*mg/kg/kg) |
|---|---|---|---|---|
| ▲ | Inhaled NanoPac [1.18] | 56.0 | 41,600 | 997,985 |
| ■ | Inhaled NanoPac [0.38] | 56.3 | 21,000 | 914,095 |
| ● | IV nab-paclitaxel [2.9] | 19.9 | 5,800 | 23,112 |

Figure 2. Plasma Paclitaxel Concentration Time Curve and PK Data




| | Group [Dose (mg/kg)] | $T_{1/2}$ (hours) | $C_{max}$ (ng/mL) | $AUC_{0(last)}$ (h*ng*mg/mL/kg) |
|---|---|---|---|---|
| ● | Inhaled NanoPac [1.18] | 8.3 | 15.9 | 193 |
| ▲ | Inhaled NanoPac [0.38] | 7.9 | 11.6 | 264 |
| ■ | IV nab-paclitaxel [2.9] | 8.7 | 206 | 528 |

## Objective

- Evaluate the efficacy of inhaled NanoPac compared to a clinical reference dose of IV nab-paclitaxel in reducing tumor burden in an orthotopic model of Calu-3 lung cancer in athymic nude rats.

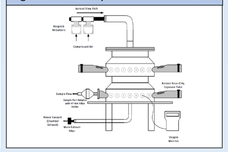
## Materials and Methods

- 120 x-irradiated nude rats were intratracheally instilled with 20 x $10^6$ Calu-3 cancer cells; followed by a 3-week engraftment period, and randomized by weight into one of the following:

Table 1. Treatment Arms (n = 20)

| Group | Treatment | Frequency |
|---|---|---|
| 1 | Control (no treatment) | N/A |
| 2 | IV nab-paclitaxel; 5.0 mg/kg | Q1wx3 |
| 3 | Inhaled NanoPac; 0.5 mg/kg | Q1wx4 |
| 4 | Inhaled NanoPac; 1.0 mg/kg | Q1wx4 |
| 5 | Inhaled NanoPac; 0.5 mg/kg | Q2wx4 |
| 6 | Inhaled NanoPac; 1.0 mg/kg | Q2wx4 |

Figure 3. Nose-only inhalation chamber



- Nab-paclitaxel was administered via tail-vein injection
- NanoPac was delivered via 2 parallel Up-Mist compressed air jet nebulizers into a rodent nose-only exposure chamber. Exposure windows for the 0.5 mg/kg and 1.0 mg/kg doses were 33 minutes and 65 minutes, respectively. Aerosol concentration monitoring was conducted by collecting pre-weighed GF/A 47-mm filters measured every 10-minutes, extracted and analyzed via high performance liquid chromatography.
- Terminal body weights, brain weights, and lung weights were recorded at necropsy; left lungs were paraffin embedded, sectioned at 4 µm, mounted and stained with hematoxylin and eosin (H&E). The lungs from the first 10 animals in each group were evaluated for histopathology and graded for adenocarcinoma, primitive tumor cell, and tumor regression.

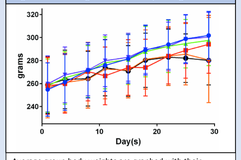
## Results

- All animals survived to scheduled necropsy, exhibited no adverse clinical observations due to treatment, and gained weight at the same rate throughout the study.
- Inhaled suspensions were nebulized with an average MMAD of 2.01 µm; average paclitaxel aerosol concentrations ranged between 244.82 - 270.51 µg/L across treatment groups.

Table 2. Total Paclitaxel Administered (mg/kg)

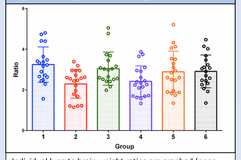
| Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 |
|---|---|---|---|---|---|
| 0.00 | 14.04 | 2.62 | 4.66 | 5.12 | 9.41 |

Figure 4. Average Group Body Weights




Average group body weights are graphed with their respective standard deviation (Blue – Group 1; Red – Group 2; Green – Group 3; Purple – Group 4; Orange – Group 5; Black – Group 6).

Figure 5. Lung to Brain Weight Ratios

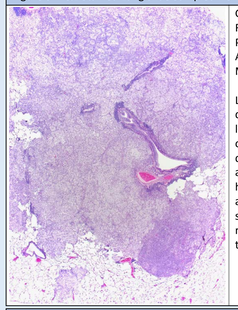



Individual lung to brain weight ratios are graphed (open circles) with the average and standard deviation for each group (bar chart).

Group 2 and 4 were statistically different than Group 1 ($p<0.05$).

## Histopathology

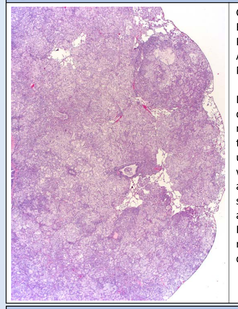
Figure 6. H&E Stained Lung Slide Sampled from **Group 1 (Control Group)**



Group 1 (Control Group)
Regression Grade: 0
Primitive Tumor Cell Grade: 1
Adenocarcinoma Grade: 3
Magnification: 2x

Low-power magnification showing the general distribution of undifferentiated, pleomorphic, large, anaplastic tumor cells within alveolar spaces or lining the alveolar septae. The majority of cells do not have features of adenocarcinoma and appear in sheets of contiguous tumor. Many cells have basophilic staining cytoplasm, while others are large, anaplastic and contain pale amphophilic-staining. Note the presence of a pre-existing resident population of alveolar macrophages and the absence of tumor regression.

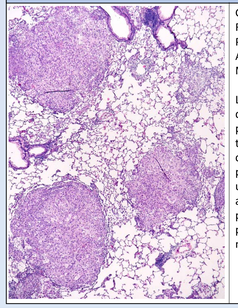
Figure 7. H&E Stained Lung Slide Sampled from **Group 2 (IV nab-paclitaxel; 5.0 mg/kg; q1wx3)**



Group 2 (IV nab-paclitaxel; 5.0 mg/kg; q1wx3)
Regression Grade: 0
Primitive Tumor Cell Grade: 1
Adenocarcinoma Grade: 3
Magnification: 2x

Low-power magnification showing the general distribution of large expansive tumor mass filling most alveolar spaces as well as neoplastic cells in the periphery. Most tumor cells are predominantly undifferentiated, pleomorphic, large, anaplastic with pale amphophilic-staining. The primitive cells are smaller, ovoid, and have more basophilic staining cytoplasm with variable, vesicular nuclei and moderate to marked anisokaryosis. Inflammatory cell infiltration are predominantly neutrophils and macrophages. This image demonstrates an absence of tumor regression.

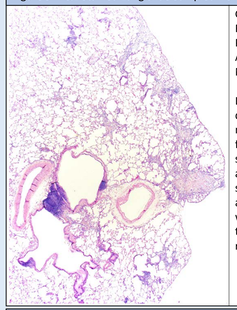
Figure 8. H&E Stained Lung Slide Sampled from **Group 2 (IV nab-paclitaxel; 5.0 mg/kg; q1wx3)**



Group 2 (IV nab-paclitaxel; 5.0 mg/kg; q1wx3)
Regression Grade: 1
Primitive Tumor Cell Grade: 1
Adenocarcinoma Grade: 1
Magnification: 4x

Low-power magnification showing the general distribution of tumor masses predominantly at the periphery as well as multiple smaller expansive tumor masses filling alveolar spaces. The tumor cells are pleomorphic, large, anaplastic and have pale amphophilic-staining, varying from undifferentiated to differentiated patterns of adenocarcinoma. Evidence of tumor regression is present around the periphery of the mass and primarily characterized by the infiltration of macrophages.

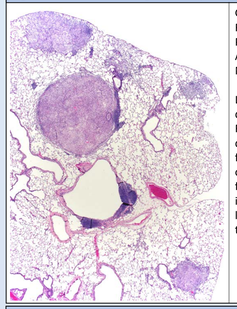
Figure 9. H&E Stained Lung Slide Sampled from **Group 4 (Inhaled NanoPac; 1.0 mg/kg; q1wx4)**



Group 4 (Inhaled NanoPac; 1.0 mg/kg; q1wx4)
Regression Grade: 4
Primitive Tumor Cell Grade: 0
Adenocarcinoma Grade: 0
Magnification: 2x

Low-power magnification showing the general distribution of previously populated tumor masses, the presence of multiple small areas of fibrous connective tissue, central collagenous stroma and fibrocytes are seen at the peripheral alveolar spaces as well as thickened alveolar septae supports evidence of tumor regression. In addition, the alveolar spaces are commonly filled with infiltrate of macrophages and lymphocytes together with additional evidence of tumor regression.

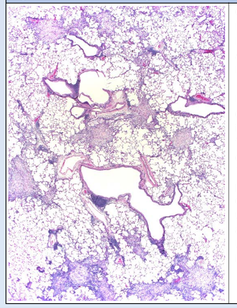
Figure 10. H&E Stained Lung Slide Sampled from **Group 5 (Inhaled NanoPac; 0.5 mg/kg; q2wx4)**



Group 5 (Inhaled NanoPac; 0.5 mg/kg; q2wx4)
Regression Grade: 3
Primitive Tumor Cell Grade: 0
Adenocarcinoma Grade: 1
Magnification: 2x

Low-power magnification showing the general distribution of previously populated tumor masses. Regressing masses are variably small and randomly distributed. Fibrous connective tissue is seen filling/replacing alveolar spaces and suggests foci of regressing adenocarcinoma. Acute necrosis, fibrous connective scaffolding, mixed cell infiltration of macrophages, giant cells and lymphocytes in the epithelium as well as around the stroma are signs of tumor regression.

Figure 11. H&E Stained Lung Slide Sampled from **Group 6 (Inhaled NanoPac; 1.0 mg/kg; q2wx4)**



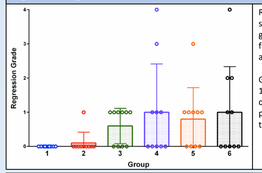
Group 6 (Inhaled NanoPac; 1.0 mg/kg; q2wx4)
Regression Grade: 3
Primitive Tumor Cell Grade: 0
Adenocarcinoma Grade: 1
Magnification: 2x

Low-power magnification showing the general distribution of previously populated tumor masses in multiple small areas of fibrous connective tissue filling/replacing the alveolar spaces suggesting foci of previous infiltrates of adenocarcinoma cells. Tumor regression is evidenced by fibrosis of previously populated tumor masses, central collagenous stromal core and fibrous connective tissue at the periphery filling/replacing the alveolar spaces, thickening of the septae as well as the presence of fibrocytes filling the alveolar space infiltrated by lymphocytes and macrophages.

Table 3. Histopathology Grading Score Definitions

| 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| No evidence of involvement | Minimal evidence (~1-25% of lung section involved) | Mild evidence (~26-50% of lung section involved) | Moderate evidence (~51-75% of lung section involved) | Marked evidence (~76-100% of lung section involved) |

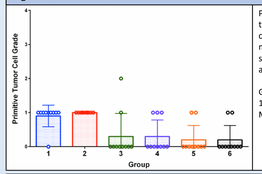
Figure 12. Regression Grades




Regression was characterized by the presence of scalloping of the edges of the individual tumor masses, gradual to complete loss of tumor cells with residual fibrous connective tissue of the lung parenchyma and accompanied by invasion of foamy macrophages.

Groups 3-6 were each significantly different than Group 1 (Control); Group 5 (inhaled NanoPac 0.5 mg/kg, q2wx4) was significantly different than Group 2 (IV nab-paclitaxel 5 mg/kg, q1wx3) ($p<0.05$, Mann-Whitney U test).

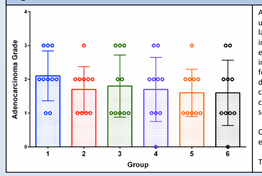
Figure 13. Primitive Tumor Cell Grades




Primitive Tumor Cell grades were characterized by small to moderate amounts of pale basophilic staining cytoplasm, ovoid and variably vascular nuclei, moderate anisokaryosis growing randomly and in sheets, increased numbers of mitotic figures and apoptotic bodies.

Groups 3-6 were each significantly different than Group 1 (Control) and Group 2 (IV nab-paclitaxel) ($p<0.05$, Mann-Whitney U test).

Figure 14. Adenocarcinoma Grades




Adenocarcinoma grades were characterized by undifferentiated adenocarcinoma morphology of pleomorphic, large, anaplastic, pale amphophilic-staining with fine intracytoplasmic vacuoles resembling mucoid vesicles, exhibiting moderate to marked anisokaryosis, observed to be individualized or growing in sheets and lacking clear cut features towards differentiation to adenocarcinoma; and differentiated adenocarcinoma morphology demonstrating clear acinar gland differentiation, amphophilic staining tumor cells arranged in nests or glandular patters bound by alveolar septae.

One animal from Group 4, and another in Group 6 had no evidence of Adenocarcinoma upon necropsy.

There was no statistical difference between groups.

## Summary

- Both inhaled NanoPac and IV nab-paclitaxel were found to be safe throughout the study; all groups gained weight at the same rate; no loss of ambulation; no inflammatory reaction and no granuloma formation was noted on histological evaluation of the lungs.
- Inhaled NanoPac was found to be both safe and effective in reducing (and in some cases eradicating) tumor burden in an orthotopic Calu-3 lung cancer model in T-cell deficient rats.
- H&E stained slides indicate inhaled NanoPac stimulated an immunological response, eliciting lymphocytic infiltration into the lung tumors.
- The immune stimulatory role may be in part due to the prolonged, high concentration of paclitaxel in the lung at quantifiable levels to at least 14-days (Figure 1), as recruitment of the endogenous immune system to infiltrate tumors was not seen to the same extent in Group 1 or Group 2.
- Follow-on studies to identify the lymphocytic infiltration through immunohistochemical staining and flow cytometry will further characterize the kinetics of the NanoPac-induced immunological response.
- IND enabling toxicology studies are underway in preparation for clinical trials.



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**Ongoing Clinical Trials**
NCT03029585: Ovarian Cancer
NCT03077659: Prostate Cancer
NCT03077685: Pancreatic Cancer
NCT03188991: Pancreatic Cysts
NCT03101358: Cutaneous Metastases

**Patents / Expiry**
US 7,744,923 / Oct 11, 2027
US 8,221,779 / Jul 02, 2024
US 9,814,685 / Jun 06, 2036
US 9,918,957 / Jun 06, 2036
US 9,925,512 / Jun 06, 2033

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