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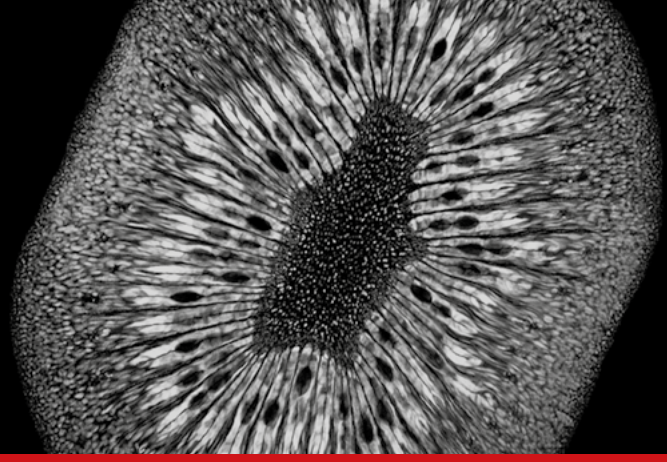
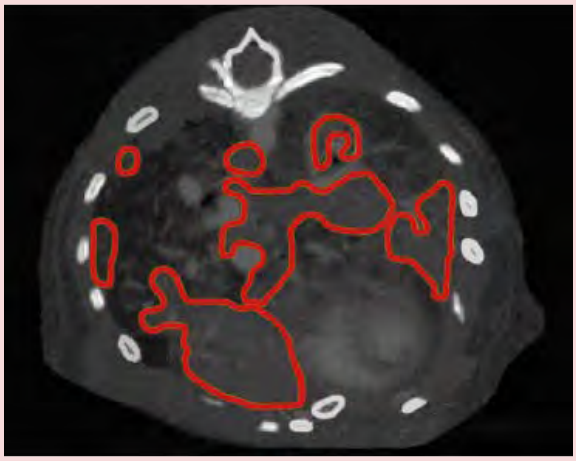


µCT of lung adenocarcinoma



Monitoring of lung adenocarcinoma progression by ExiTron™ nano 12000

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Introduction

The genetically-engineered mouse model, Kras$^{G12D\text{-}LSL}$ represents a most useful preclinical model system to study tumor growth and progression, constituting a valuable tool to investigate the benefit of new therapies[1]. In this study we tested the potency of two drug candidates, PD-0325901 (hereafter PD-901) and PF-04691502 (hereafter PF-502) as single agents or in combination, in suppressing the growth of lung tumors in Kras$^{G12D\text{-}LSL}$ mice. Previous studies have shown that PF-502 exhibits anti-tumor activity in several preclinical models[2] and that PD-901 shows potent tumor growth inhibition in papillary thyroid carcinoma cells[3]. Also, a combination of both compounds significantly inhibited tumor growth and increased survival compared with either monotherapy in a genetically engineered mouse model of ovarian cancer[4]. Since micro-computed tomography (µCT) has proven to be a reliable method to image lung tumors at different stages of tumorigenesis[5], the ability of the two drug candidates to suppress lung adenocarcinoma progression was investigated using µCT in combination with the innovative Viscover™ contrast agent ExiTron™ nano 12000.

Materials and methods

Tumor development

Lung tumors were generated in Kras$^{G12D\text{-}LSL}$ mice (3-4 weeks of age) by intranasally administering an adenovirus vector expressing the Cre recombinase protein (Adeno-Cre)[6]. Standard histological techniques were performed at various timepoints (4, 8, 12, and 16 weeks post-inhalation) to ensure tumor formation and progression.

Therapeutic treatment

Treatment with anti-cancer agents in Kras$^{G12D\text{-}LSL}$ mice was initiated at 14 weeks after inhalation with Adeno-Cre when lung lesions were at an advanced stage of tumorigenesis (adenocarcinoma). Tumor-bearing mice were treated with anti-cancer compounds, PF-502 (7.5 mg/kg/day), PD-901 (1.5 mg/kg/day) and a combination of PF-502 and PD-901 at the respected dose, for a period of 8 weeks.

µCT Imaging

In vivo µCT of tumor-bearing Kras$^{G12D\text{-}LSL}$ mice treated with vehicle (blank), PF-502, PD-901, or a combination of PF-502 and PD-901 was performed 4 weeks after treatment initiation at an advanced stage of tumor progression. Imaging was also performed on non-inhaled (control wild type) mice. All mice were anesthetized using isoflurane inhalation and were imaged at 15 min post injection of 100 µL of the CT contrast agent ExiTron™ nano 12000 (Viscover™, *nanoPET Pharma* GmbH, Berlin, Germany). Upon intravenous injection, ExiTron nano 12000 circulates in the bloodstream

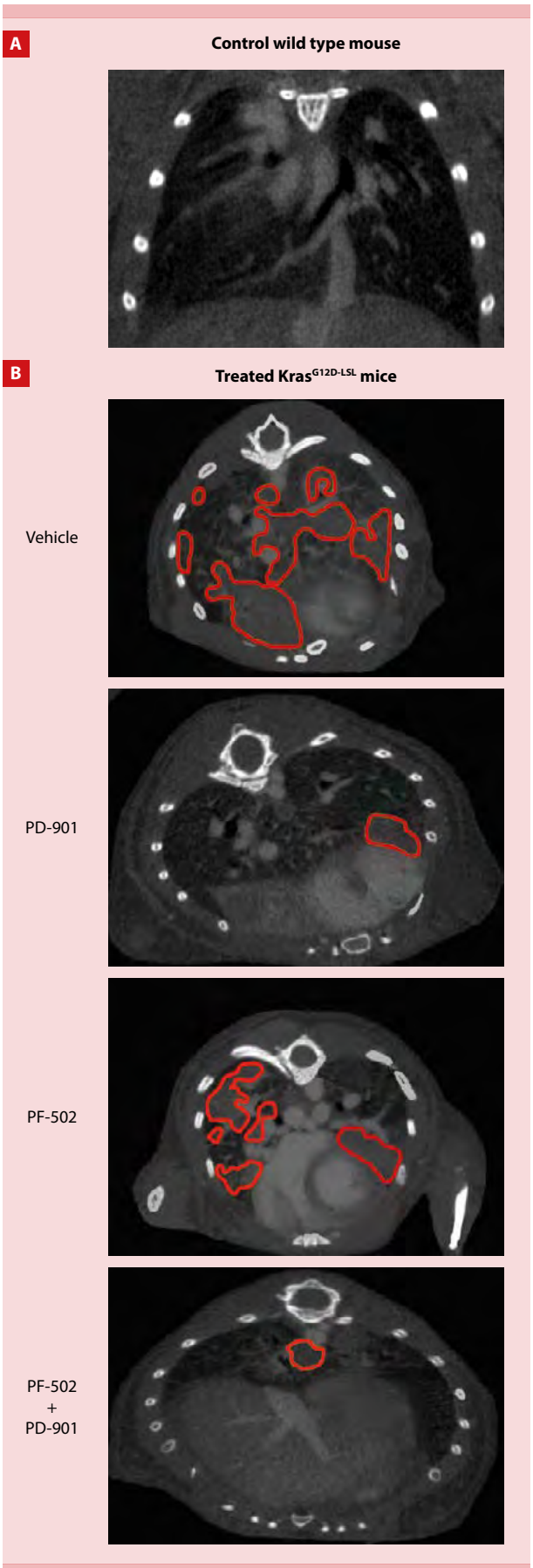

Figure 1: µCT images obtained 15 min post injection of the CT contrast agent ExiTron nano 12000. **A.** Coronal image of a healthy control wild type mouse. **B.** Transverse images of Kras$^{G12D-LSL}$ mice treated with vehicle, PD-901, PF-502, or a combination of PF-502 and PD-901, at 4 weeks after treatment initiation (tumor margins are shown in red).

and accumulates in macrophages, including those in the liver and spleen (reticulo-endothelial system, RES). Due to the enhanced permeation and retention (EPR) effect, the nanoparticulate CT contrast agent can extravasate from fenestrated blood vessels into surrounding tissue of inflamed or tumorous areas and can, thus, assist in the detection of various tumors. All CT images were acquired in standard resolution using respiratory gating (70 kVP, 114 µA, 300 ms integration time, 41 µm voxel size).

Results and discussion

To test the efficacy of PF-502 and PD-901 as single agents as well as in combination in the suppression of lung adenocarcinoma, contrast-enhanced *in vivo* µCT was performed on tumor-bearing mice 4 weeks after initiation of the therapeutic regimens.

Compared with the non-inhaled (control wild type) mice (Fig. 1A), CT images of the vehicle-treated mice clearly showed that the lung tissue is extensively covered by tumor lesions (Fig. 1B) indicating an advanced stage of tumor progression at this timepoint. CT images of mice treated with PD-901, PF-502 and combination therapy exhibited lung tissue with fewer tumors compared to vehicle, indicating that all drug treatments suppressed overall tumor growth. Quantification of the tumor volume clearly showed that while PF-502 partially inhibited tumor growth, PD-901 as single agent or in combination with PF-502 showed superior efficacy (Fig. 2).

These findings were corroborated by histological analysis (data reported elsewhere[7]), implying that the PD-901 compound, as single agent or in combination with PF-502, strongly suppresses lung adenocarcinoma progression. Furthermore, the consistency between data obtained via the two techniques indicates that µCT using ExiTron nano 12000 is a reliable method to image lung tumors *in vivo*. This is because the contrast agent allows considerable improvement in defining the tumor margins (Fig. 1B, red) due to the reduced contrast intensity of tumor relative to the surrounding blood vessels and heart but increased contrast intensity of tumor relative to air spaces in the lungs. Further work using ExiTron nano 12000 to longitudinally evaluate and quantify lung tumors verifies that this contrast agent allows accurate tumor burden estimation[8].

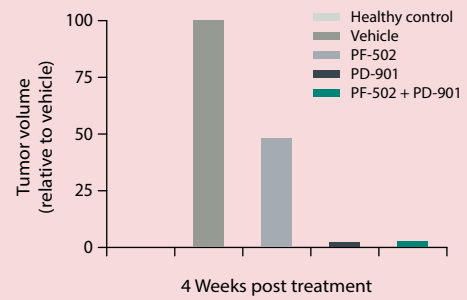

Figure 2: Histogram displaying tumor volume (relative to vehicle) in healthy control wild type mice and Kras$^{G12D-LSL}$ mice treated with vehicle, PF-502, PD-901, or a combination of PF-502 and PD-901, at 4 weeks after treatment initiation.

Conclusion

In this study, we use contrast-enhanced µCT to monitor the efficacy of two potential anti-cancer drugs in the suppression of lung adenocarcinoma in Kras$^{G12D\text{-}LSL}$ mice. The results show that the applied µCT method, involving the use of ExiTron nano 12000, enables evaluation of lung tumor progression and treatment strategies in a non-invasive manner, presenting researchers with an efficient tool for anti-cancer drug research and development.

Viscover™ Product	Order No.
ExiTron™ U, 1 x 5 injections	130-095-142
ExiTron™ U, 5 x 5 injections	130-095-143
ExiTron™ V, 1 x 5 injections	130-095-283
ExiTron™ V, 5 x 5 injections	130-095-284
ExiTron™ P, 1 x 5 injections	130-095-144
ExiTron™ P, 5 x 5 injections	130-095-145
ExiTron™ nano 6000, 1 x 5 injections	130-095-146
ExiTron™ nano 6000, 5 x 5 injections	130-095-147
ExiTron™ nano 12000, 1 x 5 injections	130-095-698
ExiTron™ nano 12000, 5 x 5 injections	130-095-700
ExiTron™ MyoC 8000, 1 x 5 injections	130-095-701
ExiTron™ MyoC 8000, 5 x 5 injections	130-095-702

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