Monitoring anticancer therapies against lung tumor in experimental models using X-ray Micro Computed Tomography

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Background
Three-dimensional micro Computed Tomography (µCT) offers the opportunity to capture non-invasively images of lung structures and lesions in mice with a high spatial resolution allowing for accurate calculation of lung lesion volume. Longitudinal imaging overcomes the limitation of single time-point imaging because it enables tracking of the natural history of disease and provides quantitative assessments of the effects of an intervention in every single mouse.

ALK (Anaplastic Lymphoma Kinase) is a transmembrane protein and a member of the family of insulin receptor tyrosine kinases. Oncogenic fusion proteins harbouring the ALK kinase domain have been identified in different cancer types. In lung cancer, the most frequent chimeric protein involves fusion of the ALK kinase domain at the C-terminus to part of the EML4 (echinoderm microtubule-associated protein-like 4) protein at the N-terminus in approximately 3-13% of human non-small cell lung cancers (NSCLC). The pivotal role of EML4-ALK in the carcinogenesis of NSCLC was demonstrated in experimental models such as the transgenic mouse model described by Soda, expressing EML4-ALK specifically in lung alveolar epithelial cells under the control of the Surfactant Protein C (SPC) promoter (1). A similar transgenic model was developed internally using the lung specific Clara Cell Secretory Protein (CCSP) promoter. A dual ALK/c-Met inhibitor (Crizotinib Xalkori®) was approved by FDA in September 2011 for non-small cell lung cancers (NSCLC) expressing ALK. Escape from Crizotinib treatment linked to mutations in the ALK kinase domain has been reported (2) and novel second generation Alk inhibitors active on Crizotinib resistant cancer are under development. Here we report the evaluation of an ALK inhibitor (hereafter compound-A) in comparison with Crizotinib, in the CCSP-EML4-ALK-TG16 transgenic mouse model using µCT.

Method
73 CCSP-EML4-ALK-TG16 transgenic mice were received at 6 weeks of age. For baseline imaging, mice were imaged twice at 1 week interval using µCT to identify growing lung tumor nodules. Three homogenous groups were randomized on the basis of the tumor size of one selected tumor nodule per mouse, and treated according to the following protocol:

<table>
<thead>
<tr>
<th>Group 1 (n=10)</th>
<th>Group 2 (n=10)</th>
<th>Group 3 (n=8)</th>
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<tbody>
<tr>
<td>Control</td>
<td>Compound-A</td>
<td>Crizotinib</td>
</tr>
<tr>
<td>(µCT Days 54, 61, 70, 77, 85)</td>
<td>(50 mg/kg; p.o. 10mL/kg) (Days 49-72)</td>
<td>(50 mg/kg; p.o. 10mL/kg) (Days 49-72)</td>
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Mice were monitored longitudinally for 5 imaging sessions of 7 min each using the Skyscan 1076 ® scanner (35um, Ti 0.025mm, 72kV; 145uA, exposure 316ms, rot step 1°). During imaging, the mice were anesthetized with Aerane® (0.2%, O₂:2L/min) and kept warm. Images
were reconstructed using NRecon ® and Regions Of Interest (ROI) were manually drawn over the selected tumor nodule using the image analysis software CTAn®. Tumor volume was calculated by stacking 2-dimensional ROI.

Statistical analysis was done on tumor volume from day 47 to day 77 using 2 way analysis of variance with repeated measures on one factor, with significance level set at \( p < 0.05 \). Partial tumor Regression (PR) corresponds to reduction of 50% of the tumor size compared to baseline and Complete tumor Regression (CR) is declared when the tumor is no more visible on the scan.

**Results**
The figure below shows longitudinal \( \mu \)CT monitoring of the selected lung nodule in one representative mouse of control (top row) and compound-A treatment (bottom row) groups.

![Results](image)

Compound-A had a significant effect on tumor nodule volume vs control from day 54 \( (p=0.0098) \) to day 77 \( (p<.0001) \) with 96% tumor growth inhibition on day 77, 3/10 CR and 10/10 PR. Crizotinib had significant effect on tumor nodule volume vs control from day 61 \( (p=0.0191) \) to day 77 \( (p=0.0295) \) with 44% tumor growth inhibition on day 77, 0/10 CR and 3/10 PR. Compound-A was found significantly more active than Crizotinib on day 61 \( (p=0.013), \) day 70 \( (p=0.0025) \) and day 77 \( (p<.0001) \).

**Conclusion**
Longitudinal monitoring of lung tumor nodules using X-ray \( \mu \)CT in CCSP-EML4-ALK-TG16 transgenic mice developing ALK+ lung tumor nodules, revealed superior activity of the Alk Inhibitor compound-A compared to Crizotinib at 50 mg/kg given daily.

**References:**