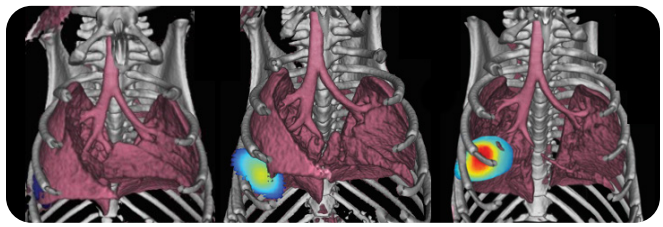
CASE STUDY

Assessment of MYC-driven progression of small cell lung cancer

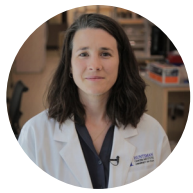
Introduction

Lung cancer is the leading cause of cancer death worldwide in both men and women. In the United States, one type of lung cancer, small cell lung cancer (SCLC), accounts for ~14% of all lung cancers[1]. SCLC has a typical survival rate of 10-12 months, with approximately 6% of patients surviving two years[1]. The primary chemotherapy treatment regimen for SCLC is etoposide plus a platinum-based drug such as cisplatin or carboplatin. Initially many patients respond well to this regimen, however tumors can quickly develop resistance to these therapy cocktails. Despite this treatment resistance, the chemotherapy regimen has not changed substantially in almost 40 years. This, paired with poor prognostic factors of this disease, highlight the urgent need to develop new therapeutic approaches[2,4].

Dr. Trudy G. Oliver is a leading lung cancer researcher and heads a team at the Huntsman Cancer Institute in Salt Lake City, Utah. Her focus is on better understanding mechanisms of drug response and resistance in lung cancer with the goal of improving treatments for patients. With the aim of developing personalized cancer treatments for lung cancer based on the unique characteristics of the tumor, such as MYC amplification, Dr. Oliver uses a myriad of techniques including human genomics and Genetically-Engineered Mouse Models (GEMMs) in her research.



Our Challenge



"Even though MYC amplification has been associated with tumor progression and treatment resistance, we needed to be able to reliably visualize tumor burden in the lung. One of the biggest challenges previously hindering our research was the inability to accurately and non-invasively quantify changes in tumor volume over the course of treatment."

- Dr. Trudy G. Oliver

This case study illustrates how Dr. Oliver and her research team used preclinical Computed Tomography (microCT) imaging to track disease progression and therapeutic response in SCLC GEMMs modified using the bacterial Cre/LoxP system.

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The challenge

Human SCLC cells are characterized as either (1) classic or (2) variant. There are many specific characteristics of the variant cells including overexpression of MYC (part of a family of MYC transcription factors that also includes MYCL and MYCN, proto-oncogenes that play a role in cell cycle progression, apoptosis and cellular transformation) as well as differential expression of a number of key transcriptional regulators[4]. MYC-driven tumors are highly aggressive, spread rapidly, and respond differently to therapy compared to tumors driven by MYCL. Being able to understand the mechanisms behind MYC-driven SCLC may lead to more targeted therapies and better treatment outcomes[3,4].

Even though MYC amplification has been associated with tumor progression and treatment resistance, *in vivo* imaging, specifically quantifying tumor volume changes post-treatment *in vivo*, has not been used to date to understand how MYC impacts these processes. There are a number of methodologies for quantifying lung cancer *ex vivo* such as histology but these require large numbers of animals in each treatment cohort and typically only provide a 'snapshot' of the disease[3].

It has been shown that MYC is a transcriptional regulator of Aurora A and B, and preclinical studies suggest that SCLC with MYC alterations are sensitive to Aurora kinase inhibitors[4]. To assess the efficacy of drugs that inhibit MYC, Dr. Oliver and colleagues analyzed etoposide, cisplatin, inhibitors of CKIε, BRD4, CDK2, as well as Alisertib (Aurora kinase A and B inhibitor) in 17 human SCLC cell lines. They found that Alisertib, Barasertib and multiple other Aurora kinase inhibitors exhibited increased efficacy in high MYC-expressing SCLC cell lines[3].

To determine the efficacy of Alisertib and various treatment combinations *in vivo*, microCT was used to image lung tumors in Genetically Engineered Mouse Models (GEMMs). GEMMs of SCLC are based on simultaneous loss of Rb1 and Trp53, and most Rb1/Trp53 GEMMs develop classic SCLC. Dr. Oliver and colleagues generated Rb1fl/fl Trp53fl/ fl MycLSL/LSL (RPM) mice, which developed the variant subtype of SCLC. Treatment combinations included either: (1) PBS vehicle control, (2) cisplatin and etoposide, (3) Alisertib, or (4) cisplatin, etoposide, plus Alisertib.

Seeing is believing

Preclinical imaging is a well established tool for monitoring cancer progression and treatment effects, as each imaging modality has different strengths based on the technique used. For example, microCT imaging can be used as the inherent density changes between lung tissue and tumor provides superior image contrast for a variety of pulmonary applications. In addition, the use of respiratory-gated scanning techniques, that reduce motion-induced imaging artifacts, make it easier to quantify the disease such as lung tumors.

MicroCT imaging was used in this study to quantify tumor growth and progression and in turn evaluate potential novel targeted treatment approaches for MYC-driven SCLC. MicroCT imaging was conducted at baseline (prior to treatment) and every four days post-treatment for up to 20 days. All mice were monitored for weight loss and mice receiving Alisertib treatment did not differ from the control group but those receiving chemotherapy exhibited a ~15–20% weight loss; chemotherapy toxicity resulted in the requirement to sacrifice one animal in each treatment group[3].

What is MicroCT and Why Use It for Lung Imaging?

"MicroCT is an excellent *in vivo* lung imaging tool by providing superior image contrast as it uses the inherent density changes between normal lung tissue and tumor. In addition, respiratory-gated scanning techniques allow the accurate quantification of functional readouts from the microCT images."

- Dr. Trudy G. Oliver

Using the images acquired on the Quantum microCT system, total tumor volume was quantified relative to total air volume. This provides a comprehensive measurement of treatment impact. Within 12 days of first detecting tumors, control animals treated with PBS demonstrated rapid tumor growth. A modest delay in tumor growth was observed in the Alisertib-treated animals. This was largely attributed to the effect of the treatment at early time points. A significant delay in tumor growth was observed in the chemotherapy-treated animals. Interestingly, tumor stasis was exhibited in the majority of animals treated with a combination of Alisertib and chemotherapy[3].

The percent change in tumor volume was calculated on day 19 (or earlier if survival was shorter than 19 days) and compared to day zero in each treatment group. Although tumor growth was initially delayed, mice treated with Alisertib alone did not exhibit an overall tumor response compared to untreated animals within the first 20 days of treatment. In the chemotherapy group, 5 of 14 animals showed signs of stable disease while the majority (9 of 14) progressed during treatment. In the chemotherapy and Alisertib group, 3 of 16 animals exhibited a > 30% reduction in disease while 10 of 16 exhibited stable disease[3].

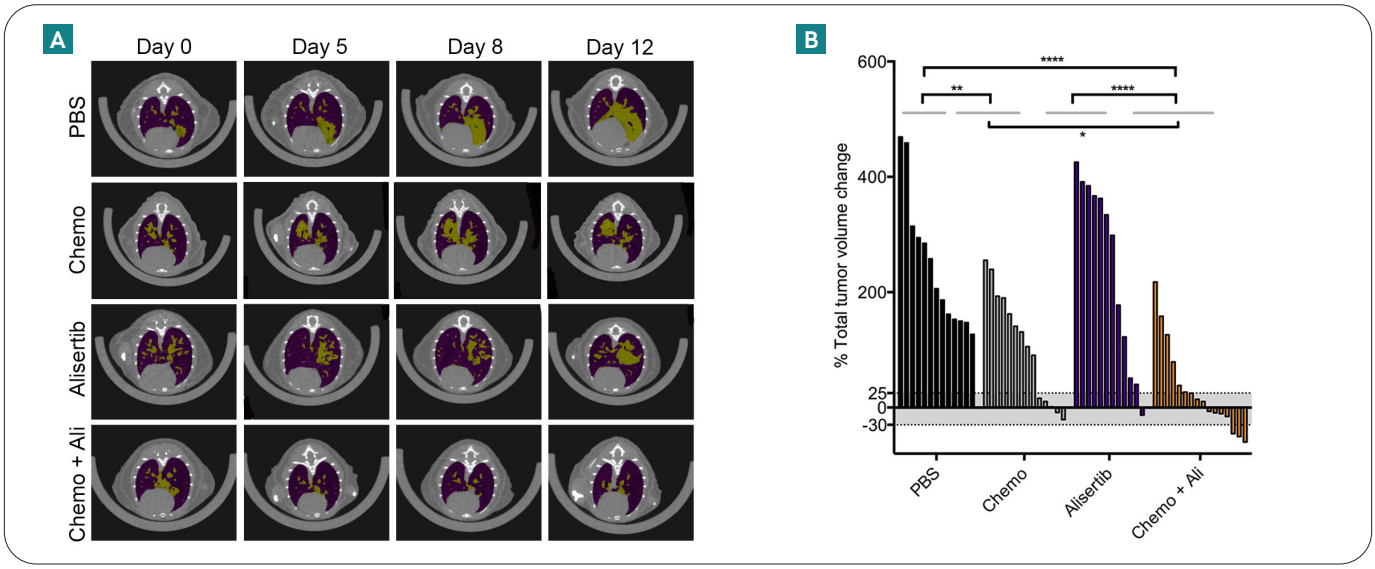

Figure 1: MicroCT imaging results for each treatment group on Day 0, 5, 8 and 12 are shown in Figure 1A. SCLC tumors and normal lung and airway are shown in yellow and purple, respectively. The waterfall plot (Figure 1B) shows the total percentage change in tumor volume from day 0-19 in each RPM mouse from each respective treatment group (for three cycles of chemotherapy). Gray shading on the graph indicates partial response and stable disease. P values were calculated with two-tailed unpaired t tests ($p < * 0.023$, $** < 0.002$, $**** < 0.0001$)[3].

The outcome

Using microCT imaging, researchers in Dr. Oliver's laboratory were able to quantify MYC-driven SCLC progression *in vivo* and throughout the course of treatment. They compared the rates of tumor progression in GEMM tumors treated with Alisertib to those receiving standard SCLC chemotherapy regimens alone. Dr. Oliver and colleagues found that mice treated with the combination of cisplatin, etoposide, plus Alisertib exhibited the greatest reduction in tumor growth compared to the other treatment protocols[3]. Importantly, a subsequent Phase II clinical trial validated these findings, demonstrating that patients with MYC-driven SCLC treated with Alisertib plus chemotherapy as second-line therapy had a greater progression-free survival compared to those treated with chemotherapy alone[5]. Dr. Oliver and her team will continue to use microCT imaging and GEM models to further elucidate key features of human SCLC and test potential treatments for MYC-driven SCLC in an effort to identify a more effective therapy for these patients.

The Future of MicroCT Imaging for Lung Cancer Research

Dr. Oliver envisages that the Quantum microCT will ultimately transform how lung cancer is quantified as it is possible to extract functional imaging parameters e.g. Functional Residual Capacity or Tidal Volume measurements using respiratory-gated scanning techniques to quantify disease states.

Conclusion

MicroCT imaging has traditionally been used for bone imaging due to its high density. However, because of the density changes between lung tissue (comprised of mostly air) and tumor, it offers superior image contrast for a variety of pulmonary applications. As seen in this case study, Dr. Oliver and her team demonstrated how the Quantum microCT system easily quantified treatment effects of various therapies in MYC-driven SCLC.

MicroCT imaging with the Quantum system offers:

- High resolution, high speed scanning
- Low dose imaging ideal for longitudinal studies
- Functional imaging using respiratory-gated scanning eliminates motion-induced artifacts and allows accurate quantification over time, making 4D imaging possible

References

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