CROWN BIOSCIENCE

High-frequency Ultrasound Guided Injection and Monitoring of Orthotopic Murine Models

A JSR Life Sciences Company

FACTSHEET

Introduction

Orthotopic tumor mouse models provide several distinct advantages compared to their subcutaneous counterparts due to their organ-specific tumor microenvironment (TME) and increased clinical relevance and predictivity. However, orthotopic implantation of cell lines can be time consuming, technically challenging and inefficient due to the laboratory and surgical procedures required to establish these tumor models, also necessitating careful consideration of animal welfare due to increased risks to the species used for study. Furthermore, the luc-tagged cell lines used to create orthotopic animal models for BLI studies are limited in number and have been shown to create additional hurdles when evaluating certain immuno-oncology therapeutics.

Crown Bioscience has evaluated the use of high-frequency (HF) ultrasound technology as a preclinical tool to overcome many of the challenges associated with orthotopic tumor mouse models. The benefits of this technology include efficient, precise and highly reproducible tumor implantation, non-invasive real-time tumor tracking, orthotopic use of non-luc-tagged cell lines, and improved animal welfare.

HF ultrasound in orthotopic mouse models is a promising tool that provides accurate, reproducible and translational insights for today's therapeutic development.

HF Ultrasound Technology Overview

Crown Bioscience has selected the Vevo 3100 HF ultrasound (Fuji Film Visual Sonics). This is a micro-ultrasound technology that offers high frequency to produce higher resolution. The frequency range of 12 – 71 MHz and 30-micron resolution are well-suited for small animal studies. The high resolution of the Vevo 3100 HF ultrasound allows for precise and less invasive orthotopic tumor implantation using ultrasound guidance, as well as accurate tumor detection and sizing in 2D and 3D imaging.

Key Advantages

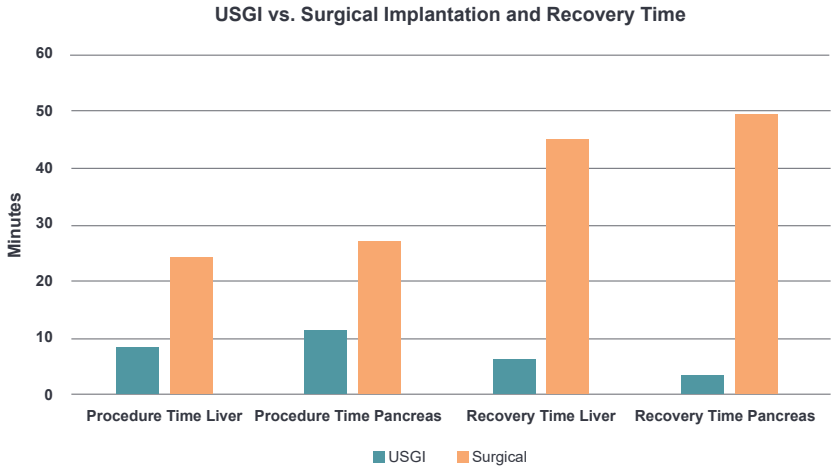
The utilization of HF ultrasound for orthotopic tumor guidance and monitoring offers many benefits. These include:

- Efficient, precise and highly reproducible tumor implantation
- High-resolution imaging and rapid tumor volume measurement
- No BLI loss-of-signal due to necrotic tumor tissue
- Applicability to many different types of cancer models, such as lung, liver, colon, brain, and pancreatic cancers
- Orthotopic imaging of non-luc-tagged cell lines
- Improved animal welfare
- Satisfies the 3 R's of Replace, Reduce, Refine

Comparison of HF Ultrasound to Surgical Implantation and Traditional Monitoring

HF ultrasound guided implantation offers higher throughput when compared to surgical implantation due to the shorter procedure time, need for fewer technicians, and faster animal recovery time. The end result is a shortened study duration when compared to surgical implantation. Results of a comparison study of ultrasound guided implantation (USGI) versus surgical implantation conducted with liver and pancreas tumors is shown in Figure 1 below.

Figure 1: HF ultrasound compared with surgical



Crown Bioscience recently completed a study that assessed the benefits of HF ultrasound in orthotopic tumor implantation and tumor monitoring. A summary of the study method and results is provided below.

HF Ultrasound Guided Orthotopic Implantation and Tumor Monitoring: Hep3B-luc Liver Model

Study Goal

Assess the benefits of replacing traditional invasive surgical orthotopic implantation of hepatic tumor cell lines with a minimally invasive ultrasound guided approach. Evaluate tumor monitoring using HF ultrasound as compared to bioluminescence imaging.

Study Methods

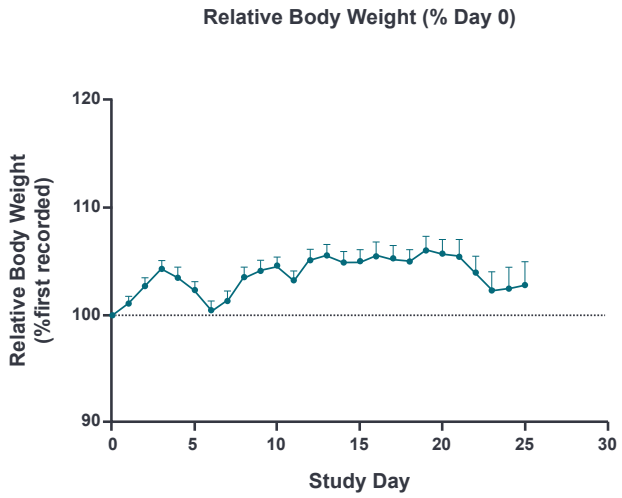
- Hep3B cells were transduced to express firefly luciferase short tandem repeat (STR) profile match to parental cell line.
- $1x10^6$ Hep3B-luc cells in 20 µL of Matrigel were injected orthotopically into the liver via ultrasound guided injection.
- In-life growth and terminal *ex vivo* assessed by bioluminescence imaging (BLI) using Spectrum BL (PerkinElmer, US) and HF ultrasound imaging using Vevo 3100 system (Visualsonics, Canada).

Study Results

In-life tumor volumes were assessed by bioluminescent imaging (BLI) using the Spectrum BL (PerkinElmer, US) and high frequency ultrasound (HFUS) using the Vevo 3100 system (Visualsonics, Canada). HFUS images were analyzed using Vevolab® software (Visualsonics, Canada). 100% of Hep3B-luc tumors implanted orthotopically using HFUS demonstrated progressive tumor growth, as measured by both tumor associated bioluminescence (TABL) (Figure 3) and HFUS (Figure 4). No significant weight loss in study animals due to the presence of these tumors was observed (Figure 3).

HFUS imaging acquisition enabled the tumor location within the organ of interest to be accurately pinpointed. The HFUS images were then analyzed using Vevolab® software (Visualsonics, Canada), enabling 3D reconstruction of these tumors, giving a tumor volume reading (Figure 4). HFUS imaging analysis confirmed the location of these tumors within the liver, and this was further validated by ex vivo BLI of the livers upon termination (Figure 4).

Figure 2: Relative body weight change after orthotopic Hep3B-luc tumor inoculation and In-life tumor growth as assessed by BLI.



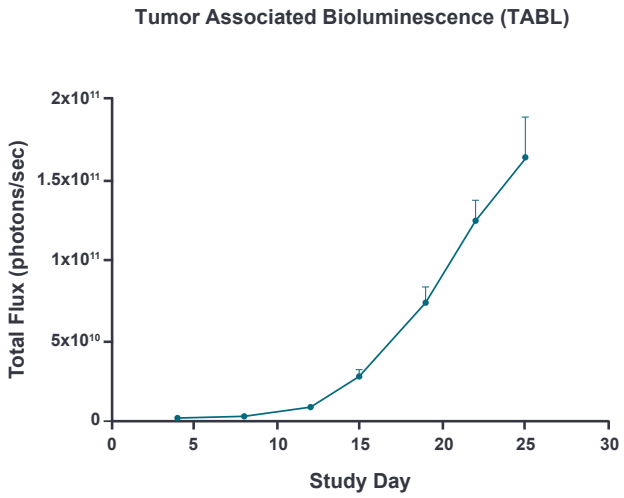
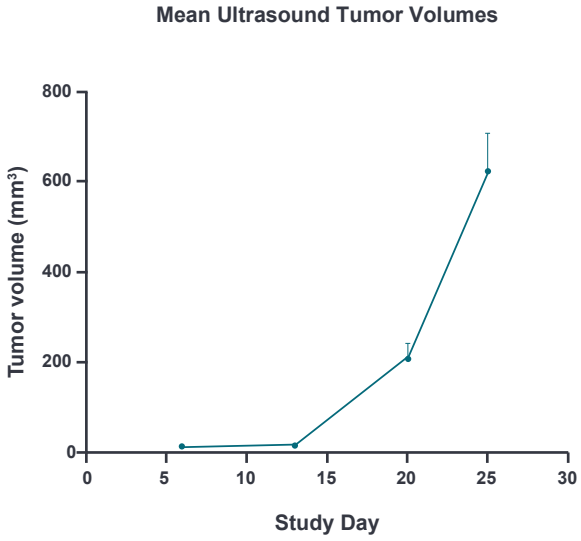
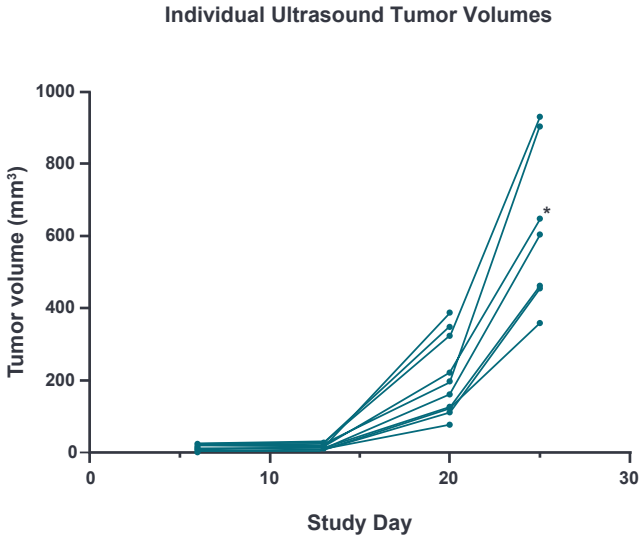
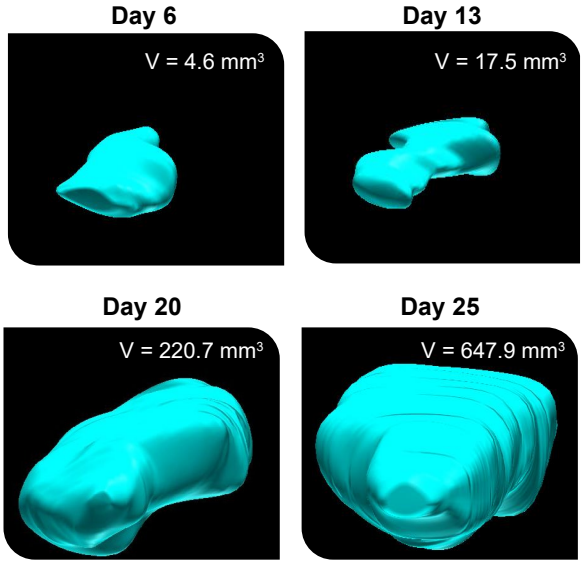


Figure 3: Tumor volumes as assessed by high frequency ultrasound and *ex vivo* BLI confirmation of tumor location within the liver



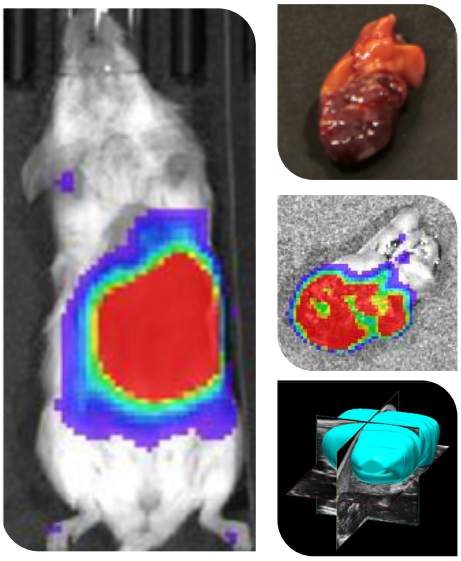


3D tumor measurements



* Example images taken from the same animal

* In- life/*ex vivo* BLI and Ultrasound

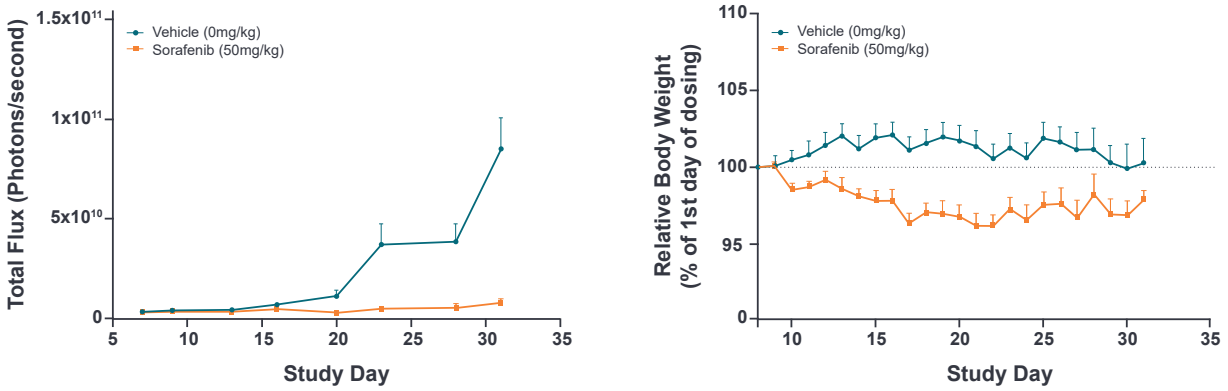


Left: Final in-life BLI image. **Right**: (top to bottom) photograph, *ex vivo* BLI and final 3D ultrasound image confirming tumor location.

Orthotopic Hep3B-luc model: SoC Validation

Mice were implanted orthotopically with Hep3B-luc cells using HF ultrasound guided injection. After 7 days of tumor growth mice were randomized and treated with 50 mg/kg daily Sorafenib or vehicle control for 21 days. Treatment with Sorafenib resulted in a statistically significant inhibition in tumor growth of HF ultrasound injected orthotopic Hep3B-luc ($p \leq 0.0001$).

Figure 4: Effects of SOC therapy on orthotopically implanted Hep3B-luc tumors as assessed by BLI and bodyweight change



Study Conclusions

- The time taken for HF ultrasound guided implantation compared to traditional surgical methods is significantly reduced, enabling a higher throughput of animals on study.
- HF ultrasound guided implantation is less traumatic than traditional surgical methods, resulting in faster recovery times and fewer surgery related complications.
- Tumor tracking and measurement by HF ultrasound aligns with BLI imaging.
- HF ultrasound is a promising tool for oncology researchers seeking accurate, reproducible and translational insights for today's therapeutic development.

Summary

HF ultrasound guided implantation of orthotopic tumors is a minimally invasive technique that reduces study time, enables accurate monitoring of tumor localization and growth, and adheres to the principles of the 3R's (reduce, refine, replace).

HF ultrasound guided orthotopic models enable tumor implantation without the need for luciferase tagging of tumor cells, resulting in shortened study timelines. HF ultrasound guided injection takes less time than traditional surgical implantation meaning that a larger number of animals can be inoculated in a shorter period of time. The use of HF ultrasound guided injection also reduces the risk of surgery related complications and potential tissue trauma and inflammation.

The use of HF ultrasound imaging of orthotopic models to track tumor progression has several advantages over traditional imaging modalities used to track bioluminescently-tagged tumors, including higher resolution and rapid, precise tumor volume measurements.

Additionally, with HF ultrasound imaging, bioluminescently-tagged tumor models are not required, eliminating the potential of loss-of-signal due to necrotic tumor tissue. Studies have found that the signal for using bioluminescence for tracking tumors *in vivo* can drop out after a certain period of time due to necrotic tumor tissue. HF ultrasound methods can continue to track even necrotic tumor masses in live animals[1,2].

HF ultrasound in orthotopic mouse models is a promising tool that provides accurate, reproducible and translational insights for today's therapeutic development.

References

1 Badr CE. Bioluminescence imaging: basics and practical limitations. Methods Mol Biol. 2014;1098:1-18. doi: 10.1007/978-1-62703-718-1_1. PMID: 24166364.

2 Shen YT, Asthana R, Peeters C, Allen C, DeAngelis C, Piquette-Miller M. Potential Limitations of Bioluminescent Xenograft Mouse Models: A Systematic Review. J Pharm Pharm Sci. 2020;23:177-199. doi: 10.18433/jpps30870. PMID: 32407285.

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