

Use of *in vivo* Fluorescence Imaging in a Mouse Model of Viral Hepatitis

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Background

- ❖ Adenoviral clearance from liver is used as a model of viral hepatitis
- ❖ Adenovirus is hepatotropic when injected intravenously
- ❖ Previous work performed in our lab assessed importance of apoptosis-inducing molecules in adenoviral clearance from liver using *in vitro* assays examining hepatic adenoviral transgene expression
 - ❖ Requires sacrifice of mice at each time point throughout adenoviral clearance
 - ❖ Peak expression only 30 fold above background

- ❖ Most *in vivo* imaging has been done in nude mice due to their translucent skin and lack of fur
 - ❖ These mice lack critical immune cells making them unsuitable for our studies

Goal

To develop a system:

- ❖ More sensitive than our previous *in vitro* system
- ❖ Requiring fewer mice than our previous *in vitro* system
- ❖ That works with mice on a C57BL/6 background which have thick, dark fur, pigmented skin, but are the most widely used mouse strain for genetic modifications

Methods

- ❖ C57BL/6 mice with various immune deficiencies were injected intravenously with Ad-CMV-GFP as measured in OPU (optical particle units)

- ❖ Correlation studies: dose ranges from 6.4×10^8 to 8×10^{10} OPU to produce range of expression levels
- ❖ Clearance study: 8×10^{10} OPU

- ❖ This virus encodes the transgene GFP (green fluorescent protein)

- ❖ The Maestro™ machine was used for imaging due to its multispectral fluorescence imaging capability which enhances sensitivity and efficiently allows for autofluorescence subtraction

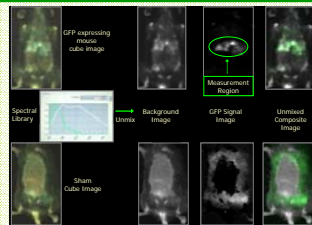
- ❖ Correlation study:

- ❖ Mice examined day 4 post-injection with pictures taken by Maestro™ machine of mouse intact, abdomen opened with liver in native conformation and showing portal vein area

- ❖ Livers weighed and homogenized, then total GFP/liver determined using spectrofluorometer set at excitation 485nm and emission 520nm

- ❖ Clearance study:

- ❖ Mice imaged *in vivo* with the Maestro™ machine at varying time points post-infection until fluorescence undetectable

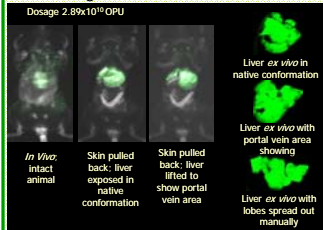


Using the Maestro™

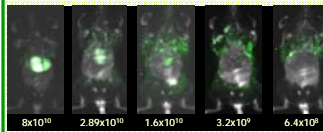
- ❖ Mouse anesthetized and fur removed from abdominal area
- ❖ Image cube created by taking picture from 500-720nm at 10nm intervals (see above)
- ❖ Spectral library created by defining background wavelength and GFP wavelength using Maestro™ software
- ❖ Same spectral library used to unmix all cube images into a background image and a GFP image
- ❖ Unmixed composite image created from background image and GFP image together
- ❖ GFP image used to obtain quantitative measurement
- ❖ Same measurement region used for a given mouse over all time points of imaging

Correlation of *In Vivo* Intact Mouse Maestro™ Imaging and *In Vitro* Spectrofluorometer Measurement

GFP expression is distributed evenly throughout all lobes of the liver



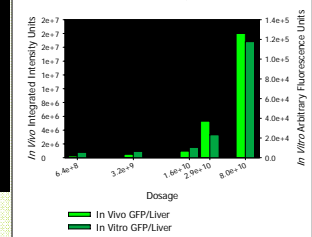
Intact mouse *in vivo* fluorescence over a range of viral doses



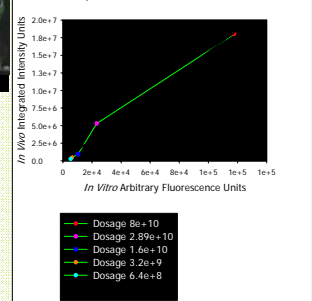
Ex vivo livers of highest and lowest dose and liver homogenates



Comparison of Maestro™ *In Vivo* Measurement with *In Vitro* GFP Measurement by Spectrofluorometer

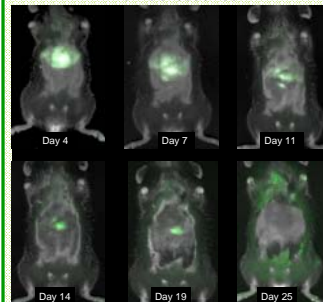


In Vivo Maestro™ Measurement vs. *In Vitro* Spectrofluorometer Measurement

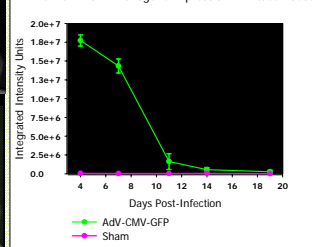


Adenoviral Clearance Over Time by Maestro™ Measurement

Adenoviral clearance measured by *in vivo* fluorescence



In Vivo Maestro™ Measurement of AdV-CMV-GFP Transgene Expression in Intact Mouse



Method Limitations

Other source of fluorescence in hepatic area: gallbladder



Potential use of IP injection of cholecystokinin to empty gallbladder prior to imaging

Summary

- ❖ Amount of virus injected must be above a certain threshold to produce linear infection *in vivo*
- ❖ Good correlation of *in vivo* with *in vitro* data
- ❖ Maestro™ system more sensitive than previous *in vitro* system
 - ❖ Peak expression averaged from multiple experiments approximately 440 fold above background versus previous 30 fold above background
- ❖ Successful technique for looking at adenoviral clearance in mice with appropriate genetic background
- ❖ Technical difficulties minimal

Conclusion

In vivo imaging can be used to measure adenoviral clearance in a single mouse with advantages over our previous system

Acknowledgements

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