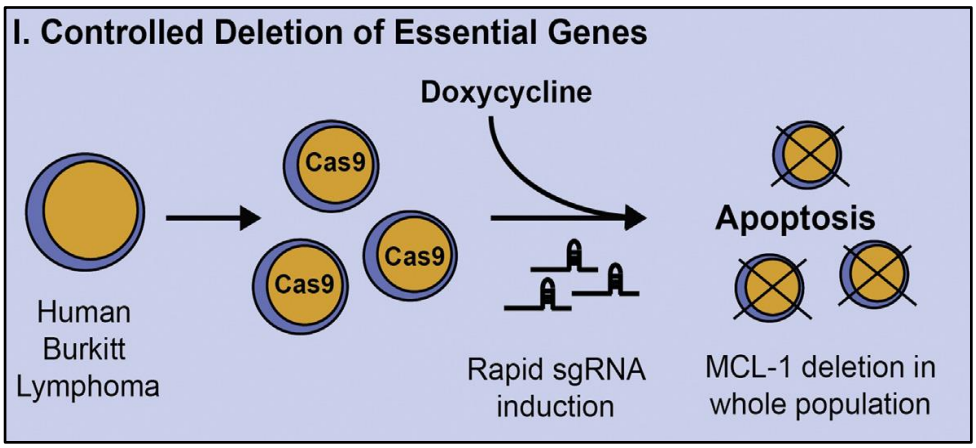
FEATURED PUBLICATION

An Inducible Lentiviral Guide RNA Platform Enables the Identification of Tumor-Essential Genes and Tumor-Promoting Mutations In Vivo

Drs. Brandon J. Aubrey, Gemma L. Kelly, Marco J.Herold and colleagues
Walter and Eliza Hall Institute of Medical Research and University of Melbourne, Victoria, AUSTRALIA

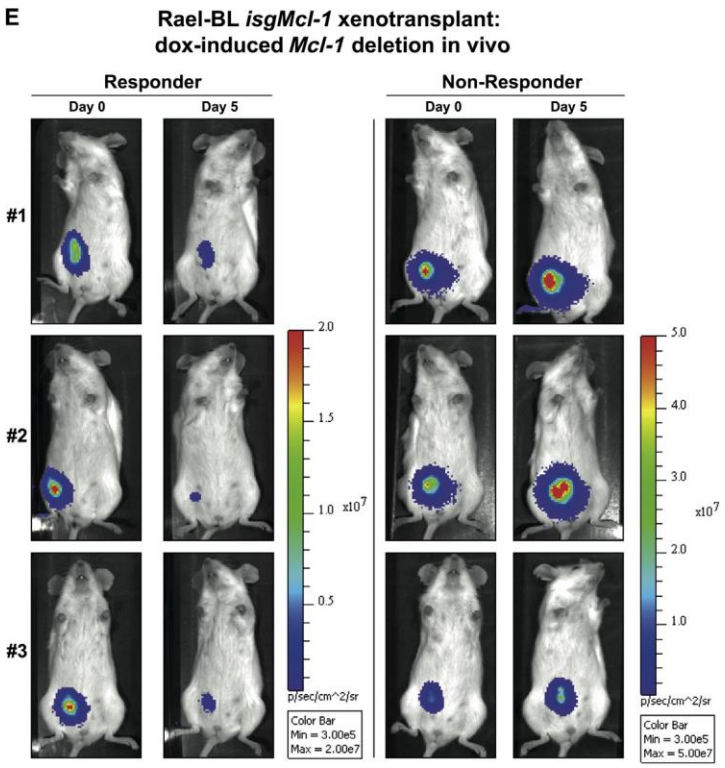
The CRISPR/Cas9 system is an exciting methodology for genetic modification. Aubrey, Kelly et al. have advanced this technology by developing an inducible lentiviral system. This platform facilitates efficient gene targeting and utilizes an imaging-based tool for phenotypic assessment following deletion of essential genes and identification of tumor-promoting mutations.



- The CRISPR/Cas9 strategy is based on the DNA- cleaving capacity of the *Streptococcus pyogenes* Cas9 endonuclease, which can be directed to specific DNA sequences in virtually any genome by an engineered small guide RNA (sgRNA).
- Authors develop a doxycycline(dox)-inducible guide RNA lentiviral vector system wherein Cas9 is constitutively expressed in all cells. Treatment with dox rapidly induces the sgRNA, which activates Cas9 and directs it to the target genomic sequence.
- Tumor cells (Burkitt lymphoma) expressing firefly luciferase are used as a model system wherein bioluminescent light production correlates with viable cell activity and tumor burden. Apoptotic cells are characterized by decreased light production due to absent ATP and O_2, required for the chemical reaction catalyzed by luciferase to produce light.

This system mediates the efficient, temporally controlled deletion of MCL-1 in human Burkitt lymphoma cell lines that require this anti-apoptotic BCL-2 protein for sustained survival and growth. Imaging reveals a decrease in tumor burden in responders, indicative of MCL-1 deletion and apoptosis, while non-responders display increased tumor burden.

Imaging may be used as an insightful phenotypic assessment tool to validate the effectiveness and broad utility of CRISPR/Cas9 approaches.



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