

p53/MCA High Mutational Load Model of Soft Tissue Sarcoma

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Reagents (Catalog #)

MEM media: Millipore Sigma (M4655)

MCA: Millipore Sigma (213942)

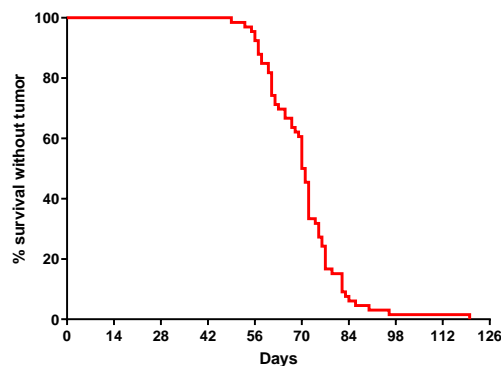
Sesame Oil: Millipore Sigma (S3547)

Ad-sgp53-Cas9 virus: VQAd5 sgp53-Cas9 (101816), ViraQuest, Inc

Ad-Cre virus: Ad5CMVCre (VVC-U of Iowa-5), University of Iowa

Tumor initiation with adenovirus (Ad-Cre or Ad-sgp53-Cas9) and MCA IM injection:

1. Prepare virus: Ad-Cre if injecting into p53^{fl/fl} mice or Ad-sgp53-Cas9 if injecting into wild type mice (or other genetic background without floxed *Trp53*)
 - a. Let virus thaw.
 - b. Add 25 ul of virus to 600 ul DMEM media, then add 3 ul 2 M CaCl₂. Look for slight color clearing at bottom of tube after CaCl₂ addition to ensure efficacy of mixture..
 - c. Let virus sit for 15 minutes. Prepped virus has 1 hr effective time from mixing (45 minutes after incubation).
2. Anesthetize mouse with 2% isoflurane and transfer to hood.
3. Inject 50 ul of precipitated virus into the gastrocnemius of the right hind leg using an insulin syringe.
4. Inject 50 ul of MCA (300 µg; resuspended at 6 mg/ml in sesame oil) into the same site after adenovirus injection. Due to the viscosity of the oil, use a 1 ml syringe without a needle on it to draw up the MCA, then add a 26 gauge needle for injection.
5. Observe for tumor formation at site of injection (see graph below for expected time to tumor reaching >75 mm³)



Reference: Mutational landscape in genetically engineered, carcinogen-induced, and radiation-induced mouse sarcoma. Lee CL, Mowery YM, Daniel AR, Zhang D, Sibley AB, Delaney JR, Wisdom AJ, Qin X, Wang X, Caraballo I, Gresham J, Luo L, Van Mater D, Owzar K, Kirsch DG. *JCI Insight*. 2019 Jul 11;4(13):e128698. doi: 10.1172/jci.insight.128698. PMID: 31112524