ICV injection of MALAT1 ASO

MALAT1

- PubMed “malat1” returns 13 papers
- MALAT1 = metastases associated lung adenocarcinoma transcript 1 (non-protein coding)
- miscRNA (non coding RNA), and is polyadenylated.
- MALAT1 ncRNA recruits splicing factors to nuclear speckles and affects phosphorylation of serine/arginine-rich splicing factor proteins (Tripathi et al. 2010). At least 2 other papers on pre-mRNA splicing and MALAT1.
- Using iCLIP (dual nucleotide-resolution ultraviolet cross-linking and immunoprecipitation), Tollervey et al. (April 2011) found the strongest interactors to TDP-43 were long UG repeats of MALAT1 (NEAT2) and NEAT1. MALAT1 expression was found increased in FTLD-TDP patient brains.
- MALAT1 commonly overexpressed in lung, kidney, breast & cervical cancers, and MALAT1 expression is prognostic for liver cancer recurrence after liver transplant.
- Absent in Allen Atlas

ASO Preparation

MALAT1
ISIS ASO#: 399462-7
Molecular Weight: 7274.28 Da
Extinction Coefficient: 194.66 mM-1 x cm-1 @ 260nm (HUH? See next slide…)

Dissolved 200 ug in 3 ml normal saline
Expect 66 ug/ml by weight
OD: 1.22 with dilution factor of 800

Gene said to expect about 70% by weight to be detected as ASO spectrophotometrically. We found about 50%.

Calculation of concentration:

From ISIS ASO protocol sheet:
The formula used to calculate the concentration is as follows:
(OD * 500 * mol wt) / (extinction coefficient * 1000) = concentration in mg/ml.
36 ug/ml

Injections:

250 ug: inject 7 ul	N=2
125 ug: inject 7 ul of 1:2 dilution of 250 ug	N=2
62.5 ug: inject 7 ul of 1:2 dilution of 125 ug	N=2
Saline alone 7 ul	N=3

Beers Law

$$A_\lambda = \varepsilon_\lambda bc$$

A_λ = Absorbance ε_λ=Extinction Coeff. b=path length c=conc.

$$A_{260} = \varepsilon_{260} bc$$

$$c = \frac{A_{260}}{\varepsilon_{260} c}$$

$\varepsilon_{260} = 194.66\ M^{-1}$

not

$\varepsilon_{260} = 194.66\ mM^{-1}$

Now, if the bozo you're working with gives you units of ε_{260} as **mM^{-1}** instead of **M^{-1}** and a generic equation where units are not described and you see your concentration is 1000 times too dilute you fight the math for two hours then call and ask for help only to find out the units were wrong…

ASO Preparation from ISIS ASO Protocol Sheet:

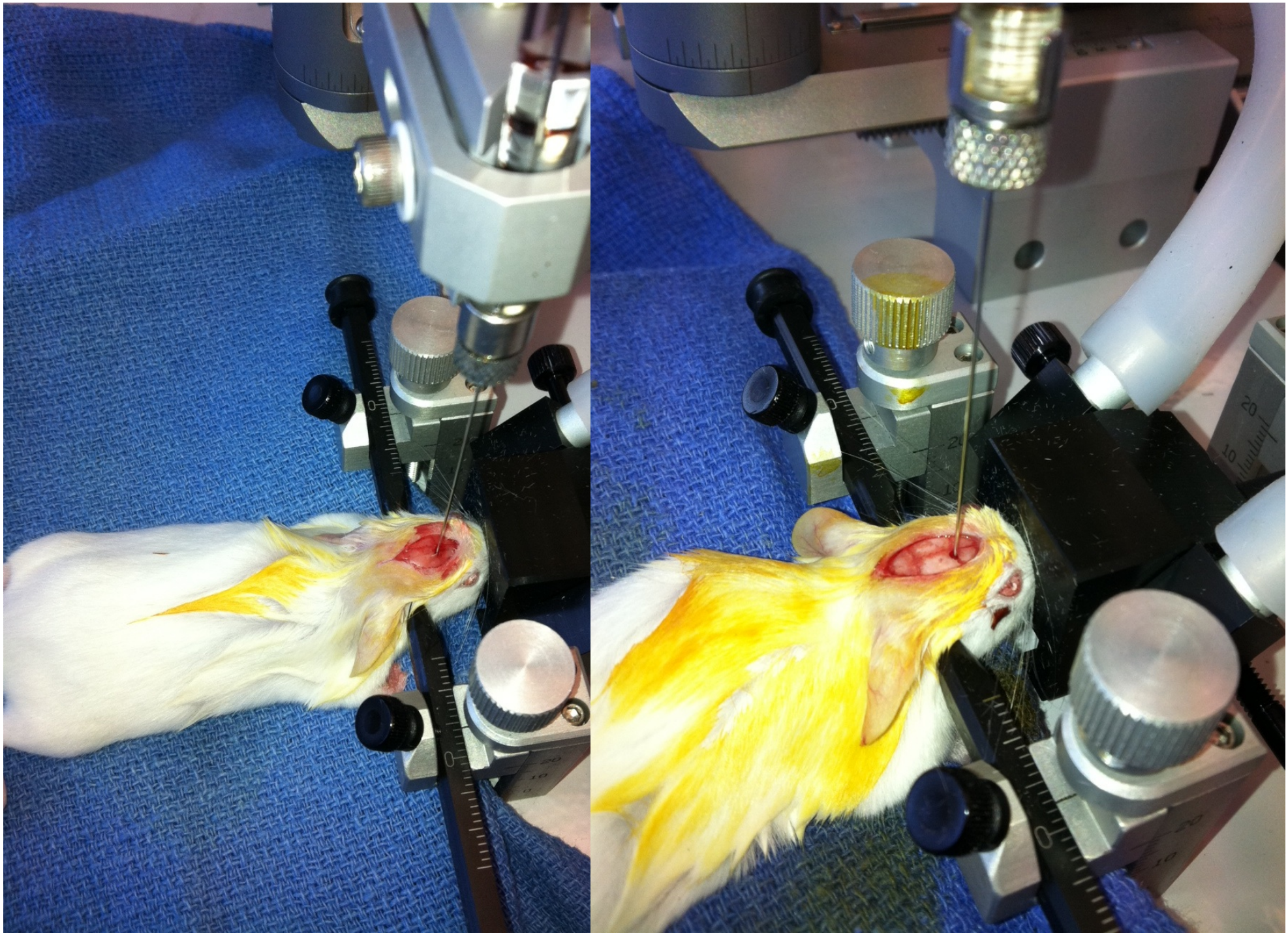
Weigh out approximate amount of ODN to be used.
Resuspend in sterile saline at about 25 mg/ml – vortex well and let sit about 15 min.
Working in sterile hood, filter though syringe filter (0.2 um; we use Gelman Acrodisc but any type of sterile filter would probably do).
Take 2 ul of filtrate and dilute in 1 ml of H2O; read OD at 260.

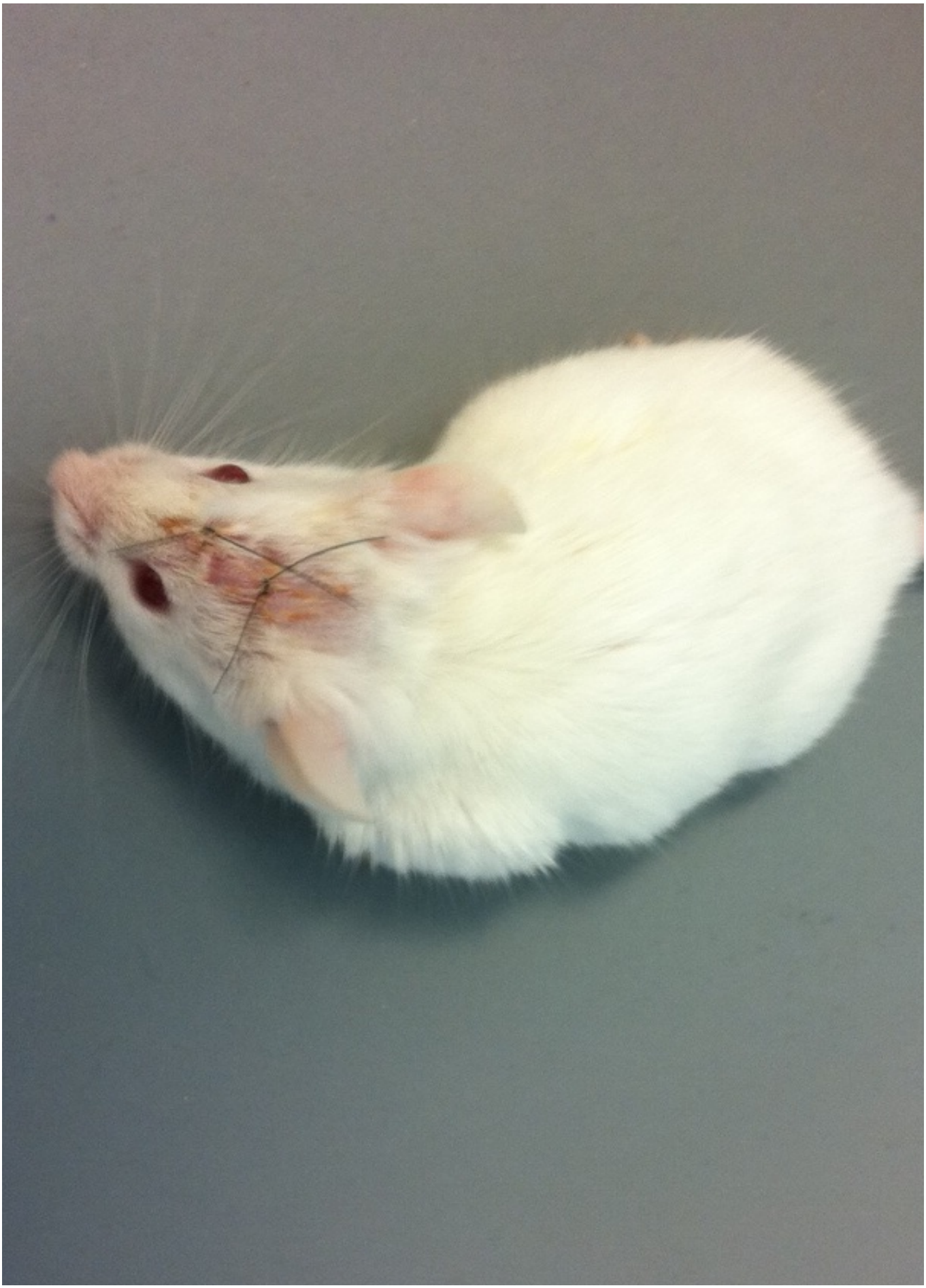
The formula used to calculate the concentration is as follows:

(OD * 500 * mol wt) / (extinction coefficient * 1000) = concentration in mg/ml.

For dosing solutions, dilute stock solution to desired concentration for injection in sterile saline and aliquot to prevent contamination. Sterile dosing solution can be stored at 4˚C for months. Store the stock is kept frozen at –20˚C or –80˚C. When defrosting stock, heat to 37˚ C, and vortex well before using.

- 10-50 mg/kg is a typical range of doses for injected oligonucleotides.
- Oligos typically dosed IP or IV.
- Oligo dose, method of delivery, and dosing schedule will depend on cells targeted, gene of interest, and assay endpoint.





Original Plan:

3 with Saline
3 with 250 ug ASO
3 with 125 ug ASO

Actual:

3 with Saline
2 with 250 ug ASO
2 with 125 ug ASO
2 with 62.5 ug ASO

One mouse injected with 250 ug was found dead the next morning.

250 ug ASO in a 28 g mouse = 9 mg/kg

Body Weights vs Day of Treatment