# 4-Hydroxytamoxifen (4OHT) topical treatment

*Reserve anesthesia machine 24hr prior to treatment

1. Put mice in the isoflurane chamber (Isoflurane ~3.5, Air flow ~3.5)
2. Once mice are anesthetized, shave hair on the back with clippers*
    a. If mice have short hair that cannot be shaved off completely, wait for ~3 days and then shave. Alternatively, hair removal cream can be used, but make sure that no residual cream is left on the skin as it causes skin irritation. Rinse off with saline solution.
3. Dip the brush in the 4OHT and paint on the back skin**. Our default concentration is 25mg/ml in DMSO. However, for highly penetrant/aggressive allele combinations (such as $Braf^{V600E}$; $Pten^{FL/FL}$) 2.5mg/ml in DMSO or lower may be used.
    a. To induce individual OR spot treatment with P2 pipette
4. Put mice back in the cage and monitor until they wake up
5. Repeat treatment the next day.

* Tail and/or ear skin as well as mucosa may also be treated with 4OHT. The commonly used driver mutations ($Braf^{V600E}$, $Nras^{Q61R}$, $Pten^{Flox}$, $Cdkn2a^{Flox}$) result in faster melanoma growth on hairy back skin than on tail/ear/mucosa, often requiring euthanasia due to back skin tumors before melanomas on other surfaces form. Thus, if melanomas on tail/ear/skin are desired it is critical to wait until the 4OHT/DMSO solution is completely absorbed before waking up the mice. The tail may also be wrapped in Saran wrap to protect other areas from becoming “cross-contaminated” with 4OHT.

** If one wishes to induce individual melanomas in highly penetrant/aggressive strains (for instance to follow individual tumors longitudinally) 4OHT induction can be performed by spot treatment: Apply 0.5-1.0ul of 25mg/ml 4OHT on 2-4 spots using a P2 pipette.

4OHT: Sigma Aldrich, Cat # H6278-50MG

# Doxycycline treatment

Doxycycline (Dox) is used to activate the Tet transactivator (rtTA3 in our mouse strains), resulting in the inducible expression of construct under the control of the TRE promoter. While cDNAs that induce a binary genetic change such as Cas9 or Cre need to be induced with Dox only for a short period of time (we have observed tumorigenesis after putting mice on Dox for 1 day), mice with inducible shRNA constructs must be maintained on a Dox diet if continuous gene silencing is desired.

Dox is administered via the feed and mice consume it ad libitum. We purchase our Dox chow from Harlan and ask for a color to be added (usually green or red) to avoid confusing it with regular chow. Two default concentrations of Dox are available (625mg/kg and 200mg/kg), but custom food can be purchased with different concentrations.

We have observed that the higher Dox concentration (625mg/kg) significantly decreases melanomagenesis in $Braf^{V600E}$; $Pten^{FL/FL}$; Tyr-CreERt2 mice, while the lower concentration (200mg/kg) has only a minor effect. Thus, it is imperative to choose the right Dox concentration:

- Cas9 or Cre: We recommend using 200mg/kg for 14 days or 625mg/kg for 7 days.
- cDNAs: One must weigh the expression levels that are desired with the negative effects of Dox. The highest dose of Dox (625mg/kg) may not only decrease melanoma growth, but also result in superphysiological overexpression levels. Other labs have gone as low as 50mg/kg Dox, but the optimal dose should be determined based on expression level of the endogenous and ectopic transcript/protein.
- shRNA: Highly dependent on the efficiency of shRNA knockdown and expression level of the target gene. The lowest Dox concentration that results in efficient gene silencing should be used. This concentration needs to be determined for every shRNA/target gene.