**Epidermodysplasia verruciformis** (EV) is a rare autosomal recessive skin disease that leads to a high susceptibility to dermal infection by human papillomavirus (HPV), specifically the β-strains.¹ These infections lead to persistent scaly wart-like lesions, macular lesions, cutaneous horns, and a high risk of skin carcinoma in areas that are exposed to UV-radiation.² Although the majority of the population has cell-mediated immunity to HPV, patients with EV lack this immunity.¹ EV is most commonly (75%) caused by a mutation in **TMC6** or TMC8.¹ **TMC6** is known to have a role in zinc-transport through its interaction with ZnT-11,3, is known to have a role in neutrophil degranulation, and is known to form a complex with calcium- and integrin- binding protein-1 (CIB1).4 *It is unknown how TMC6 aides in innate immunity to* β-*HPVs.*

The **primary goal** of this project is to understand the role TMC6 plays in innate immunity to HPV and how this consequently can lead to an increase in cell proliferation. The **long-term goal** of this project is to fully understand how **TMC6** affects the innate immune system, and how that leads to cell-mediated β-HPV immunity. This understanding could potentially lead to novel therapeutic options for patients with EV. *Mus musculus* will be used as a **model** organism because it can easily be used to model skin carcinomas as well as papillomavirus infection.5 My **hypothesis** is that mutations in **TMC6** will lead to a decrease in neutrophil activation, reducing innate immunity, and a dysregulation of proteins involved in cellular-proliferation, explaining the high prevalence of cancer.

**AIM 1: Determine conserved amino acids between TMC6 homologs**

**Approach:** I will first NCBI BLAST to identify homologs of TMC6, and then Clustal Omega will be used to align homolog protein sequences in order to identify well-conserved amino acids regions in the TMC6 gene. I will look for amino acids that are conserved between all organisms with skin, such as mice, and have differences in organisms with scales, such as danio rerio. Using CRISPR-Cas9 I will mutate these amino acids to an amino acid with different biochemical properties and introduce mouse papillomavirus (MmuPV1) to these mice. I will use a blood test to assay for neutrophil levels and will use quantitative-PCR (qPCR) to determine the relative amount of viral-DNA in the dermis of each group. **Hypothesis:** I hypothesize that the mutant mice will show a decrease in neutrophil levels and an increase in relative dermal viral-DNA compared to control. This is because conserved amino acids among skin-having organisms are possibly crucial to skin-cell-mediated immunity. **Rationale:** By mutating these conserved amino acids we can see which regions are important for neutrophil activation, and thus innate immunity. By testing for relative viral-DNA compared to controls (level of infection) I can show that neutrophil activation plays a role in cell-mediated immunity.

**AIM 2: Characterize differentially expressed genes in response to different amino acids mutations**

**Approach:** The mutant mice created earlier will be used. Skin biopsies will be taken from uninfected mutants, infected mutants, an uninfected control, infected mutants and control exposed to blue-light, and an infected control. These biopsies will then undergo RNA-sequencing to determine gene expression in these lines. The relative gene expression of each uninfected line will be compared the control groups, and the relative gene expression of each infected line will be compared to both the uninfected mutant of that group and the control groups. The relative gene expression of the blue-light groups will be compared to the infected mutant of that group and the infected control. I will also use Gene Ontology to determine the biological process that each differentially expressed gene plays a role in. **Hypothesis:** I hypothesize that the uninfected mutant mice will show a decrease in expression of neutrophil activating genes or immune system genes compared to control. The infected mutant mice will also show dysregulation of cellular proliferation genes, with extreme dysregulation of cellular-proliferation occurring in the blue-light groups. **Rationale:** RNA-sequencing will show which mutations are crucial for different aspects of innate immunity and cellular proliferation in response to both infection and blue-light.

**AIM 3: Determine and quantify protein expression involved in neutrophil activity and cellular-proliferation**

**Approach:** The same mutants and groups from before will be used. Dermal biopsies of the warts or skin-carcinoma appearing in each group will be taken and be subjected to TMT10plex tagging. The tagged samples from each group will then be subjected to liquid chromatography – mass spectrometry (LC-MS), quantified, and then sorted by biological processes using Gene Ontology. The infected groups will be compared to their respective uninfected mutant control and the WT-control, while the blue-light groups will be compared to their respective infected mutants, the infected control, and the blue-light infected control. Proteins seen throughout groups that are involved in neutrophil activity or cellular-proliferation will then be knocked-out using CRISPR-CAS9. These mice will then be subjected to Mmu-PV1 infection and assayed for neutrophil levels, relative infection rates, and tumor size. **Hypothesis:** TMC6 mutations coupled with infections will lead to a decrease in proteins involved in neutrophil degranulation, neutrophil activation, and immune system response. This may be coupled with a difference in proteins related to cellular-proliferation, such as a decrease in cellular-proliferation inhibitors. The blue-light groups will show a significant change in proteins that results in increased cellular-proliferation. When knocked-out, proteins important to innate immunity and cellular-proliferation will cause similar phenotypes to EV. **Rationale:** The relative protein levels in these groups can elucidate how TMC6 affects other protein expression levels and which regions are responsible for this. By knocking these proteins out, new proteins that are critical in EV development can be discovered. By analyzing the differing protein levels in the blue-light group it can show which proteins are important in skin-cell carcinoma development.

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**Epidermodysplasia verruciformis** (EV) is a rare autosomal recessive skin disease that leads to a high susceptibility to dermal infection by human papillomavirus (HPV), specifically the β-strains.¹ These infections lead to persistent scaly wart-like lesions, macular lesions, a high risk of skin carcinoma, and possibly cutaneous horns.² Although the majority of the population has cell-mediated immunity to HPV, patients with EV lack this immunity.¹ EV is most commonly (75%) caused by a mutation in **TMC6** or TMC8;¹ however, *the role TMC6 plays in innate immunity to β-HPV is unknown.*

The **long-term goal** of this project is to fully understand the role and to what extent TMC6 plays in the CIB1-TMC6-TMC8 complex, and how this leads to cell-mediated immunity to β-HPVs. This understanding could possibly lead to a novel therapeutic treatment option. The **primary goal** of this project is to understand the role TMC6 plays in stabilizing the complex, and whether or not TMC6 interacts directly with β-HPVs. Mus musculus will used as a **model** because it can be used to model skin carcinomas and has the components for a CIB1-TMC6-TMC8 complex4.My hypothesis is that mutations in specific regions or motifs of TMC6 decrease the stabilization of the CIB1-TMC6-TMC8 by not properly interacting with CIB1 domains.