Ultraviolet (UV) irradiation is the major cause of non-melanoma skin cancer, the most common form of cancer in the United States (1). UV irradiation activates Erbb2 through an indirect mechanism involving reactive oxygen species (2). Erbb2 is a receptor tyrosine kinase member of the epidermal growth receptor family and has been found to play a role in the pathogenesis of breast and other cancers (3). In order to determine the influence of Erbb2 on the response of the skin to UV, Erbb2 mutant mice were developed that lack Erbb2 expression in the epithelium of the skin. Because of the role of Erbb2 in growth regulation, we hypothesized that decreased proliferation upon genetic ablation of Erbb2 will result in fewer mutations after UV exposure by allowing more time for repair of DNA damage (Figure 1). To test this hypothesis, mice lacking Erbb2 expression in the skin and intermediate controls were repeatedly exposed to UV or sham-irradiated and skin samples removed at multiple time points following 43 UV exposures for analysis.

DNA damage was quantified by immunofluorescence for cyclobutane dimers, a signature adduct of UV irradiation, in the skin. UV-induced damage was repaired by 36 hours in UV-exposed Erbb2 mutant skin, but not until 72 hours in UV-exposed control mice (Figure 2). If DNA damage repair is not completed, mutations result. Mutations in p53 are the most common mutations detected after UV irradiation. Assessment of p53 mutations by immunofluorescence demonstrated that p53-positive foci were significantly smaller in UV-exposed Erbb2 mutant skin than in UV-exposed control mice (Figure 3). Taken together, these results demonstrate that Erbb2 suppresses DNA damage repair and increases mutagenesis after UV irradiation.

In order to confirm these results, Erbb2 mutant and control mice were exposed 5 times to UV and samples removed at several time points for analysis. Similar analysis was done using immunofluorescence for cyclobutane dimers, proliferation markers, and p53 mutant cells. Proliferation markers were used in order to determine whether Erbb2 has an affect on p53 mutant cells. Although the cyclobutane dimer analysis is ongoing, our analysis of p53 immunofluorescence following five exposures produced similar results as in the 43 exposure experiment.
Methods

**Animals**
Skin-targeted Erbb2 mutant and control mice were used. Mice were shaved one day before treatment. Mice were exposed to a cumulative 5 exposures of UVA/B or sham-irradiated and euthanized at 0, 18, and 36 hours following UV.

**Immunofluorescence**
Sections of skin fixed in ethanol or neutral buffered formalin were incubated with Anti-thymine dimer, anti-p53, and Proliferating cell nuclear antigen (PCNA) antibodies and secondary antibodies conjugated to a fluorochrome. The results were photographed, cells quantified, and signal intensity of cyclobutane dimers quantified using Image J software.

Results and Discussion

*Cyclobutane dimer immunofluorescence and quantification as a measure of DNA damage repair in Erbb2 control and mutant mice*

In the 26 kJ/m² experiment (Figure 2), we found that DNA damage in the form of cyclobutane dimers had been repaired by 36 hours after UV, a significant difference from that of the wild type mice. In the 5x UV experiment (Figure 3), we could not determine the time at which DNA damage had been repaired because the intensity of the cyclobutane dimers steadily increased. The standard error for this data set was large, and thus we will optimize the quantification procedure to minimize these errors. Also, we will attempt to quantify samples from later time points in order to get a better estimate of the time at which repair had been completed for the Erbb2 mutants after only 5 exposures to UV.

![Sham](image1.png)

12 h

72 h

![Figure 2. Cyclobutane dimer staining and quantification using Image J software following 43 UV exposures.](image2.png)
Immunofluorescence and quantification of p53 mutations

In our previous experiment (Figure 4a and 4b), we found a general decrease in the number of p53 positive foci per skin length for skin lacking Erbb2 expression (although not statistically significant) and a significant decrease in the number of p53 positive cells per focus in skin lacking Erbb2. The total number of p53 positive cells was significantly decreased from those of the previous experiment, which was expected due to the decreased amount of UV exposure (not shown). Pooled data from both the 18 and 36 hour time points after 5 UV exposures show that there is a significant decrease for Erbb2 mutants in both the number of p53 positive foci per skin length and the number of p53 positive cells per focus compared to that of control mice (Figure 5). These results further confirm our hypothesis that Erbb2 increases mutagenesis after exposure to UV.
Figure 4b. p53 Analysis of p53 mutations following 43 UV exposures. (* indicates a significant difference in the number of p53 positive cells per focus between Erbb2 control and mutant mice.)

Figure 5. Analysis of p53 mutations following 5 UV exposures.

Immunofluorescence and quantification of p53 mutations and proliferation

In order to determine whether Erb2 has a specific affect on proliferation of p53 mutant cells, we worked out conditions for double-staining of p53 and PCNA (Figure 6). Double staining was performed on samples from both the 72 hour after 43 UV exposure experiment and all three time points (0, 18, and 36 hours) after 5 UV exposures. The number of p53 positive cells (green) per focus was quantified as well as the number of p53 positive cells that were also PCNA positive (red). Results for quantification of samples exposed 5 times to UV are shown in Figure 7. Although these results are preliminary, there seems to be a trend that the percentage of proliferating p53 positive cells (those that are both PCNA and p53 positive) are lower in the Erbb2 mutant mice.
Conclusion

Our results support the hypothesis that genetic ablation of Erbb2 accelerates the repair of UV-induced DNA damage, as measured by cyclobutane dimer immunofluorescence. Based on analysis of p53 following 43 exposures to UV, we assumed that genetic ablation of Erbb2 does not affect the number of p53-positive foci, but leads to smaller p53-positive foci following UV-irradiation. However, after analysis of p53 following 5 exposures to UV, we have evidence to suggest that Erbb2 affects both the number of p53 positive foci and the size of each focus. Although results from p53 proliferation analysis are preliminary, there seems to be the trend that the percentage of p53 positive cells that are proliferating are lower in the Erbb2 mutant mice. This suggests that Erbb2 may increase proliferation of p53 positive cells after UV.
References

