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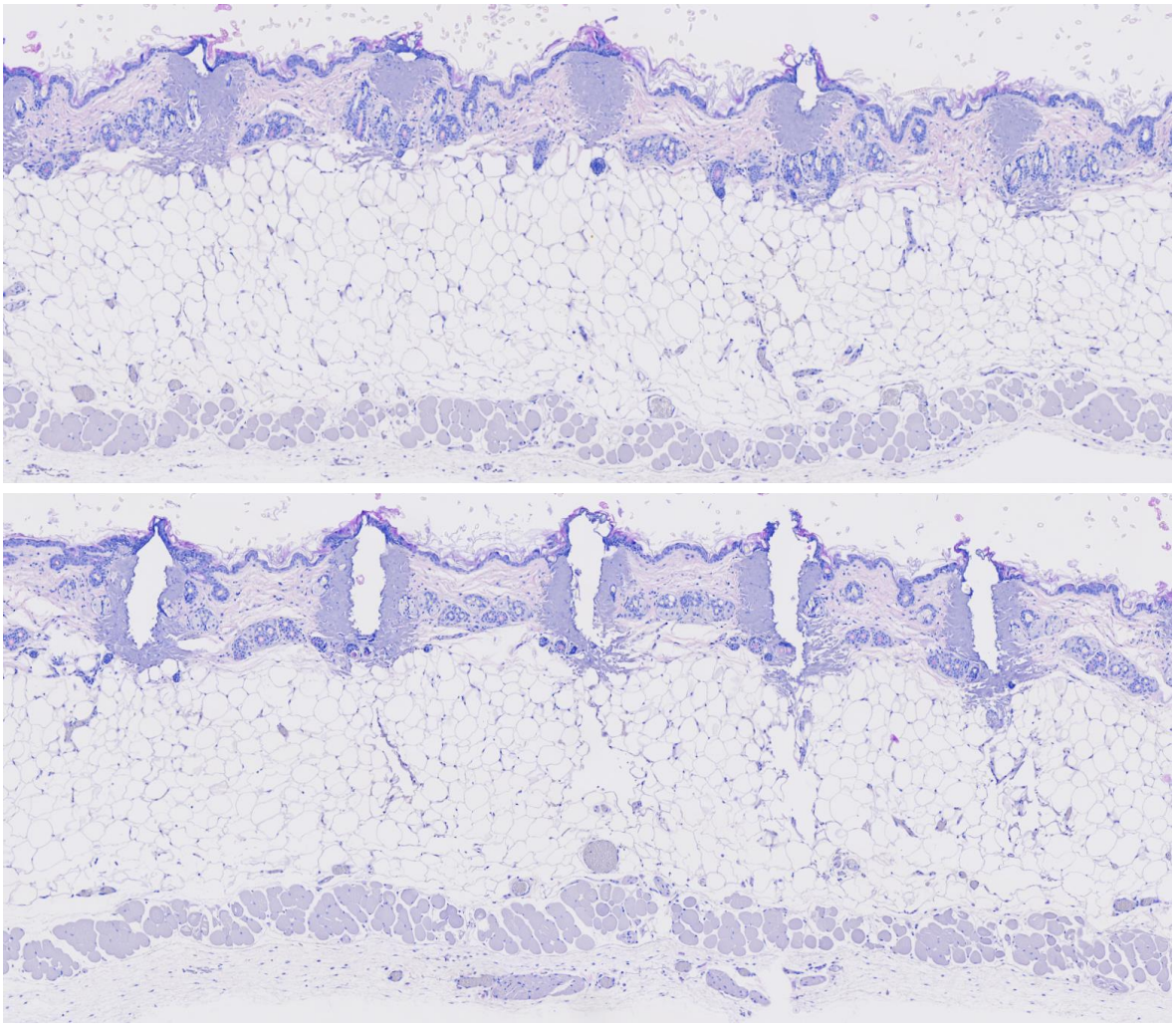

PhD Thesis

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# Exploring hedgehog inhibition in basal cell carcinomas using local, targeted treatments – topical vismodegib and ablative fractional $CO_2$ laser

*This thesis has been submitted to the Graduate School of Health and Medical Sciences, University of Copenhagen. February 15th, 2024*

# List of papers

This thesis is based on the following three papers:

**I** Pedersen KK, Høyer-Hansen MH, Litman T, Hædersdal M, Olesen UH. *Topical delivery of hedgehog inhibitors: current status and perspectives.* IJMS. 2022;23(22):14191. doi:10.3390/ijms232214191

**II** Pedersen KK, Granborg JR, Lerche CM, Litman T, Olesen UH, Hædersdal M. *Ablative fractional laser treatment reduces hedgehog pathway gene expression in murine basal cell carcinomas*. Lasers Med Sci. 2024;39(1):55. doi:10.1007/s10103-024-03997-1.

**III** Pedersen KK, Hædersdal M, Olesen UH, Litman T. *Transcriptomic analysis of early-stage basal cell carcinomas in murine skin following topical treatments with ablative fractional laser and vismodegib*. Submitted February 2024.

# Summary

Systemic administration of hedgehog inhibitors is an efficacious treatment for basal cell carcinomas (BCCs), but it is associated with multiple adverse effects. Although localized BCC treatment with topically applied hedgehog inhibitors may avoid these adverse, an efficacious topical hedgehog inhibitor treatment is currently not available. The aim of this thesis was to (i) obtain an overview of existing literature in the field of topical hedgehog inhibitor delivery and (ii) to explore the effect of local, targeted treatment with ablative fractional laser and the hedgehog inhibitor vismodegib on early-stage BCCs in a murine model. To achieve these objectives, we conducted three studies: a literature review (Study I) and two experimental studies (Study II and III).

The literature review (Study I) revealed that pre-clinical studies achieved promising cutaneous hedgehog inhibitor uptake by utilizing formulations that increase cutaneous uptake and including pre-treatments that perforate the skin barrier. Clinical trials showed that topical application of hedgehog inhibitors successfully reduced *GLI1* levels and tumor size in patients, although the percentage of complete responders was low. Furthermore, none of the clinical trials included pre-treatments and only few studies investigated drug uptake and biological response using the same experimental setup.

The first experimental study (Study II) determined cutaneous vismodegib uptake and biological responses in murine skin with early-stage BCCs. This study showed that a vismodegib microemulsion specifically developed for topical use, led to considerable cutaneous vismodegib concentrations in murine skin. Accordingly, the vismodegib treatment resulted in significant reduction of expression of the three hedgehog signaling genes *Gli1*, *Gli2*, and *Ptch1*, which indicate biological response to the treatment. Interestingly, AFL treatment alone, which is the pre-treatment that is used to perforate the skin barrier, was also able to reduce expression of the three hedgehog genes, albeit to a slightly lower degree.

The second experimental study (Study III) further characterized the effects of AFL and vismodegib treatments through RNA sequencing. The transcriptomic analysis showed that AFL and vismodegib treatments were able to reverse the gene expression pattern that follows tumor induction and elicit an immune response in murine skin with early-stage BCCs. Comparison of the two studies showed that vismodegib treatment reversed the gene expression pattern to a greater degree, while AFL treatment elicited a much more potent immune response.

Altogether, the results of this PhD thesis provide a compelling rationale for further research into AFL and vismodegib treatments for BCCs. Their significant effects on early-stage BCCs holds great promise for the development of an efficacious local, targeted BCC treatment, especially in combination with other novel or established topical BCC treatments.

# Danish summary | Dansk resumé

Systemisk behandling med hedgehog inhibitorer er et effektivt middel mod basalcelle karcinomer, men behandlingen er forbundet med betydelige bivirkninger. Lokal anvendelse af hedgehog inhibitorer på basalcelle karcinomer kunne undgå de bivirkninger, der er forbundet med systemisk brug, men der er i øjeblikket ingen tilgængelige behandling, der benytter lokal påsmøring af hedgehog inhibitorer. Formålet med denne ph.d.-afhandling var at (i) skabe et overblik over den eksisterende litteratur på området for lokal levering af hedgehog inhibitorer, og (ii) at undersøge effekten af lokal, målrettet behandling med ablativ fraktioneret laser (AFL) og hedgehog inhibitoren vismodegib på tidlige stadier af basalcelle karcinomer i mus. For at opnå disse mål udførte vi tre studier: en litteraturgennemgang (Studie I) og to eksperimentelle undersøgelser (Studie II og III).

Litteraturgennemgangen (Studie I) viste, at man i prækliniske studier kunne levere høje koncentrationer af hedgehog inhibitorer i huden ved at inkludere forbehandlinger, der perforerer hudbarrieren og ved at forbedre de formuleringer, hedgehog inhibitorerne blev leveret med. Kliniske forsøg viste, at påsmurte hedgehog inhibitorer var i stand til at reducere *GLI1*-niveauer og tumorstørrelse, men at procentdelen af patienter med komplet respons var lav. Ingen af de kliniske forsøg inkluderede forbehandlinger, og kun få af studierne undersøgte lægemiddeloptagelse samt biologisk respons i samme forsøg.

Det første eksperimentelle studie (Studie II) kombinerede undersøgelse af vismodegib optag og biologisk respons i hud fra mus, der indeholder tidlige stadier af basalcelle karcinomer. Dette studie viste, at en specielt udviklet vismodegib formulering kunne levere en høj koncentration af vismodegib i musens hud. Tilsvarende viste studiet også, at vismodegib behandlingen reducerede genekspressionen af de tre hedgehog signalgener *Gli1*, *Gli2* og *Ptch1*, hvilket indikerede biologisk respons. Studiet viste også, at AFL-behandlingen i sig selv var i stand til at reducere genekspressionen af de tre hedgehog signalgener, hvilket overraskede os.

Det andet eksperimentelle studie (Studie III) karakteriserede yderligere effekterne af AFL- og vismodegibbehandlingerne ved at analysere vores prøver med RNA-sekventering. RNA-sekventeringsanalysen viste, at AFL- og vismodegibbehandlingerne var i stand til at tilbageforandre de genekspressionsmønstre, der fulgte basalcelle karcinomernes udvikling, samt fremkalde et immunrespons i musehuden. Sammenligning af de to behandlinger viste, at behandling med vismodegib var bedst til at tilbageforandre genudtryksmønsteret, mens AFL-behandlingen var bedst til at fremkalde et immunrespons i huden.

Alt i alt viser denne ph.d.-afhandling, at AFL- og vismodegibbehandling har en stor effekt på basalcelle karcinomer. Dette resultat lægger op til yderligere forsøg med de to behandlinger, hvilket forhåbentligt betyder, at vi i fremtiden får udviklet en lokal, målrettet og effektiv behandling mod basalcellekarcinomer.