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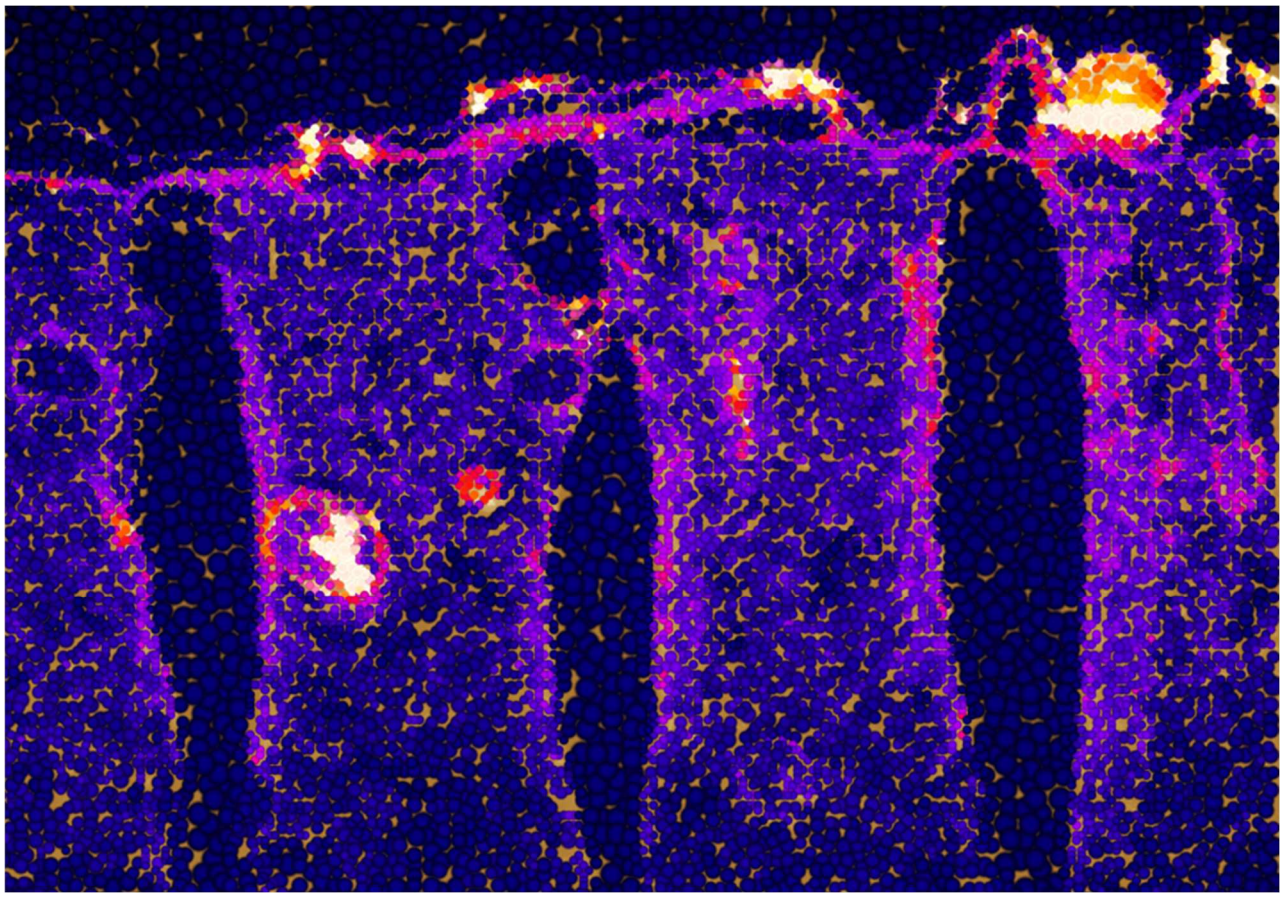

PhD Thesis

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Potential roles of the ablative fractional CO_2 laser in combination with PD-1 inhibitors

This thesis has been submitted to the Graduate School of Health and Medical Sciences, University of Copenhagen. June 30th, 2023

List of papers

This thesis is based on following three papers:

I Christensen RL, Omland SH, Persson DP, Husted S, Haedersdal M, Olesen UH. *Topical Delivery of Nivolumab, a Therapeutic Antibody, by Fractional Laser and Pneumatic Injection*. Lasers Surg Med. 2021 Jan. 53(1):154-161. doi: 10.1002/lsm.23322

II Christensen RL, Hendel KK, Persson DP, Husted S, Olesen UH, Haedersdal M. *Topical delivery of PD-1 inhibitors with laser-assisted passive diffusion and active intradermal injection: Investigation of cutaneous pharmacokinetics and biodistribution patterns*. Lasers Surg Med. 2022 Jan. 54(1):170-181. doi: 10.1002/lsm.23504

III Christensen RL, Wiinberg M, Lerche CM, Demehri S, Olesen UH, Haedersdal M. *Anti-PD-1 immunotherapy with adjuvant ablative fractional laser displays increased tumor clearance of squamous cell carcinoma, a murine study*. Submitted June 15, 2023

Throughout the thesis, the above-listed articles and manuscript will be referred to as study I, II, and III.

English Summary

The introduction of the immune checkpoint inhibitor cemiplimab has advanced the treatment landscape for keratinocyte cancers. Cemiplimab, a programmed cell death-1 (PD-1) inhibitor has gained approval as systemic treatment for both locally advanced and metastatic cutaneous squamous cell carcinomas (cSCC) and basal cell carcinoma (BCC). Though this novel treatment provides hope for patients who are ineligible for other therapies, there is a need to further enhance the anti-tumor response resulting from anti-PD-1 (aPD-1) treatment and to reduce the risk of side effects. To address these challenges, the potential roles of ablative fractional laser (AFL) could emerge as a possible technique that could augment tumor response to PD-1 inhibitors while mitigating the associated side effects. Therefore, the aim of this thesis was to elucidate the potential of AFL as a local delivery technique for aPD-1 antibodies and as potential adjuvant to aPD-1 therapy, with a focus on evaluating impact on tumor response in a cSCC mouse model.

- AFL-delivery of aPD-1 antibody revealed enhanced uptake in upper to mid dermis
- Higher laser density appeared important for AFL-assisted aPD-1 antibody delivery
- A homogenous band-shaped dispersion of aPD-1 was detected following AFL-delivery
- Tumor response was improved by combining adjuvant AFL and aPD-1 therapy in a mouse cSCC model

AFL-assisted delivery of aPD-1 antibodies was shown feasible in study I and study II. The cutaneous uptake and spatial distribution were assessed quantitatively and qualitatively in both studies. Study I was an *in vitro* study design, where porcine skin was exposed to an ablative fractional CO_2 laser and electronically-controlled pneumatic injection (EPI) delivering aPD-1. Highest AFL-assisted uptake was quantified in mid dermis corresponding to a tissue depth of 500 μm. Correspondently, EPI, the active delivery method, revealed highest uptake in mid dermis. In study II, the temporal delivery was explored for AFL-assisted uptake and the active intradermal injection in an *in vivo* setup. Uptake by AFL was dependent on higher laser density and increased over time peaking at maximum topical administration time of 4 hours and was almost non-detectable after 2 days. Intradermal injection showed an immediate high concentration that decreased over time, but still detectable 2 days after administration.

The cutaneous aPD-1 biodistribution was displayed by two imaging techniques following each delivery method both *in vitro* and *in vivo*. Two distinct distribution profiles were observed. Uptake via AFL could be seen as a homogeneous band-shaped dispersion located in upper to mid dermis,

while EPI and intradermal injection revealed a concentrated deposition dispersing from the side of injection that extended deep into dermis. *In vivo*, the aPD-1 antibodies diffused from the coagulation zone, where they had accumulated to some degree.

Mouse cSCCs exposed to AFL and systemic aPD-1 antibody treatment, revealed improved tumor response. This combination showed substantial higher tumor clearance, significantly reduced tumor growth rate and enhanced survival compared with non-treated cSCCs. Moreover, the level of cleared tumors was not as high for the monotherapies.

The investigations on the potential roles of AFL provided insight into its dual function in aPD-1 therapy. With the encouraging results, AFL-exposure could potentially be adjusted to each individual cSCC, where the main target of AFL-assisted delivery could be superficial tumors. AFL in itself can induce an anti-tumor response as well as enhance the overall treatment efficacy of aPD-1 therapy.

Danish summary | Dansk resumé

Introduktionen af immuncheckpoint-hæmmere har udvidet behandlingsregimet for patienter med keratinocyt hudkræft med en helt ny behandlingsform. Immuncheckpoint-hæmmeren Cemiplimab, er en "programmed cell death-1" (PD-1) hæmmer, godkendt til systemisk behandling af lokal fremskreden og metastatisk kutant planocellulært karcinom og basalcellekarcinom. Selvom denne nye behandlingsform giver håb for mange patienter, som ellers ikke er kvalificeret til andre behandlingsmuligheder, er der stadig behov for forbedring af det anti-tumorrespons, der ses ved PD-1 behandlingen, ligesom en høj bivirkningsprofil ved systemisk PD-1 hæmmere begrænser brugen. Til at adressere disse udfordringer har ablativ fraktioneret laser (AFL) potentiale til både at øge tumorresponset af PD-1 hæmmere og samtidig reducere associerede bivirkninger. Formålet med denne afhandling var derfor at belyse potentialet for AFL som en lokal leveringsteknik af anti-PD-1 antistoffer og som adjuvans til anti-PD-1 behandling med fokus på evaluering af tumorresponset i en præklinisk musemodel.

- AFL-assisteret levering af aPD-1 antistoffer viste et øget optag i det øvre samt midterste hudlag
- En højere laser densitet så ud til at være en vigtig parameter i AFL-assisteret levering af anti-PD-1 antistoffer
- Den kutane levering af aPD-1 via AFL, kunne detekteres som et homogent bånd-formet optag der fordelte sig horisontalt ned igennem huden
- Kombinationsbehandling med AFL adjuvans og aPD-1 i en musemodel viste et forbedret tumorrespons med øget fjernelse af tumorer

Resultaterne i studie I og II viste, at det var muligt at levere aPD-1 antistoffer ved brug af AFL. Det kutane optag og den rumlige fordeling i huden blev evalueret kvantitativt og kvalitativt i begge studier. Studie I var et *in vitro* studie, hvor grisehud blev behandlet med en ablativ fraktioneret CO_2 laser og lufttrykbaseret injektion (EPI) der begge faciliterede kutan levering af aPD-1. Det højeste optag blev kvantificeret i det midterste hudlag svarende til en dybde på 500 µm. På tilsvarende vis, viste EPI, der blev brugt som en positiv kontrol leveringsmetode, også at levere højest optag i det midterste hudlag. I studie II, blev optaget undersøgt over tid i en *in vivo* opsætning, hvor AFL og intradermal injektion blev undersøgt som leveringsmetoder. Optaget via AFL var afhængig af højere laserdensitet og steg over tid med den højeste koncentration ved den maximale topikale administrationstid på 4

timer og var stort set ikke målbar efter 2 dage. Intradermal injektion viste øjeblikkelig høj koncentration som fald hen over tid, men var stadig målbar 2 dage efter administration.

For hver leveringsmetode, både i studie I og II, blev den kutane fordeling af aPD-1 vist med to forskellige billeddannelsesteknikker. To særskilte fordelingsmønstre blev observeret. Optag med AFL kunne ses som et homogent bånd-formet optag, der fordelte sig horisontalt ned igennem det øvre samt midterste hudlag, hvorimod EPI og intradermal injektion viste en meget koncentreret deponering, som spredte sig fra injektionsstedet og ned i de dybe hudlag. Fra billeddannelses-analysen i *in vivo* studiet kunne aPD-1 antistofferne ses diffundere ud fra koagulationszonen, hvori de havde akkumuleret.

Tumorer på en planocellulær karcinom musemodel behandlet med AFL og systemisk aPD-1 antistof behandling resulterede i et forbedret tumorrespons. Denne kombinationsbehandling medførte at signifikant flere tumorer forvandt, lavere tumor væksthastighedsrate og forlænget overlevelse sammenlignet med ikke-behandlede kontroltumorer. Behandling med enten AFL eller aPD-1 alene medførte at færre tumorer forsvandt sammenlignet med kombinationsbehandlingen.

Undersøgelserne af AFLs potentielle roller har givet indsigt i dens dobbelte funktion i PD-1 behandling. Med disse lovende resultater, kan AFL tænkes tilpasset hver enkel tumor, hvor facilitering af aPD-1 antistoffer vil kunne bruges til tumorer der ligger superficielt. Derudover kan AFL inducere et anti-tumorrespons samt forbedre effekten ved aPD-1 behandling.