



Published in final edited form as:

FASEB J. 2020 March ; 34(3): 3485–3500. doi:10.1096/fj.201902164R.

Laser adjuvant for vaccination

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Abstract

The use of an immunologic adjuvant to augment the immune response is essential for modern vaccines which are relatively ineffective on their own. In the past decade, researchers have been consistently reporting that skin treatment with a physical parameter, namely laser light, augments the immune response to vaccine and functions as an immunologic adjuvant. This “laser adjuvant” has numerous advantages over the conventional chemical or biological agents; it is free from cold-chain storage, hypodermic needles, biohazardous sharp waste, irreversible formulation with vaccine antigen, undesirable biodistribution in vital organs or unknown long-term toxicity. Since vaccine formulations are given to healthy populations, these characteristics render the “laser adjuvant” significant advantages for clinical use and open a new developmental path for a safe and effective vaccine. In addition, laser technology has been used in the clinic for more than three decades and is therefore technically matured and has been proved to be safe. Currently, four classes of laser adjuvant have been reported; ultra-short pulsed, non-pulsed, non-ablative fractional and ablative fractional lasers. Since each class of the laser adjuvant shows a distinct mechanism of action, a proper choice is necessary to craft an effective vaccine formulation toward a desired clinical benefit for a clinical vaccine to maximize protection. In addition, data also suggest that further improvement in the efficacy is possible when a laser adjuvant is combined with chemical or biological adjuvant(s). To realize these goals, further efforts to uncover the molecular mechanisms of action of the laser adjuvants is warranted. This review provides a summary and comments of the recent updates in the laser adjuvant technology.

Keywords

laser; skin; adjuvant; vaccine

Introduction

An immunologic adjuvant is a component that accelerates, prolongs, or enhances adaptive immune responses to an antigen toward a desired clinical benefit (1–4). Since modern vaccine formulations in a form of highly-targeted recombinant molecules are inefficient in

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Potential conflict of interest

All authors: No reported conflicts.

inducing protective immunity, they require an immunologic adjuvant to achieve clinical significance (5, 6). Currently, most of clinical or experimental adjuvants are chemical or biological substances (5, 6). These conventional adjuvants are known to often induce undesirable local reactogenicity or systemic toxicity (7–10). Due to the safety concerns, only a limited number of adjuvants have been used with clinically approved vaccines (1, 11, 12).

In the past decade, studies have consistently demonstrated that laser light functions as an immunologic adjuvant. Since laser is a physical parameter, it does not remain in exposed tissues and has less chance to induce side effects in comparison to either chemical or biological adjuvants. This new and safe strategy has been logically combined with skin vaccination to maximize the efficacy of vaccines and induce protection. The skin is enriched with professional antigen presenting dendritic cells (DCs) and has been considered to be an ideal vaccination site to initiate adaptative immune responses and protection when DCs are provided with a proper cue (13). Skin-based vaccination to target antigen-presenting cells (APCs) has been consistently shown to be superior to the conventional intramuscular route (14–16) and offer dose sparing (17–19). In response to these advantages, various technologies are now in use or under development for skin delivery of vaccines (17, 20).

To date, there are four major categories of the “laser adjuvant” that have been classified based on the nature of the laser treatment: (i) ultrashort pulsed lasers, (ii) non-pulsed lasers, (iii) non-ablative fractional lasers, and (iv) ablative fractional lasers. In this review, the initial studies as well as the most recent updates on this technology are summarized and commented.

1. Ultra-short pulsed laser adjuvant

Ultra-short pulsed laser (UPL) adjuvants are laser pulses with durations in the nanosecond range or shorter that cover the exposed tissue to enhance immune responses. These ultra-short pulses are delivered at repetition rates ranging from 10 to 1,000 Hertz over several minutes. Typically, a dose chosen for this approach causes no frank tissue damage (Table 1).

UPL adjuvant was first reported by Russian scientists using copper vapor lasers emitting nanosecond pulses of yellow 510 nm and green 578 nm light with kilohertz repetition rates at a power density between 1–6 W/cm² applied on circular skin exposures of about 5–10 mm in diameter over 1–3 min (21–23). Onikienko *et al.* treated human subjects or mice with UPL followed by intradermal injection of a commercial prophylactic influenza or hepatitis B vaccine. The group found that the UPL treatment resulted in significant increases of antigen-specific antibody titers both in human and mice. The same Russian investigators also reported the potentiating effects of the UPL adjuvant on experimental therapeutic vaccines for chronic hepatitis B and cancer in human subjects (22). The group observed that the UPL treatment induced rapid release of heat shock protein 70 (HSP70) by skin fibroblasts and keratinocytes and concluded that this release enhanced immune responses via recruitment and activation of Langerhans cells (21).

Consistently, Chen *et al.* demonstrated that a Q-switched neodymium-doped yttrium aluminum garnet (Q-Nd:YAG) laser emitting 532 nm light at a repetition rate of 10 Hz (Spectra-Physics Inc., Mountain View, CA) possessed the adjuvant effect. The group found

that the UPL treatment of mouse skin followed by intradermal vaccination significantly increased humoral immune responses to model and inactivated influenza vaccines without inducing any tissue damage or inflammation (24). Interestingly, this approach could augment humoral and antigen-specific CD4⁺ and CD8⁺ T cell responses to intramuscular immunization using a hair-like optical fiber emitting a long-pulsed potassium titanyl phosphate (KTP)/532 nm laser (Laserscope, San Jose, CA). The group demonstrated that the mechanisms of action involved increased motility and migration and enhanced antigen uptake and presentation of MHC class II-positive APCs in skin after the UPL treatment. Chen *et al.* consistently showed the adjuvant effect of the UPL adjuvant on an intradermal nicotine vaccine augmenting production of antibody against nicotine (25). Interestingly, the adjuvant effect of the UPL adjuvant was further augmented if it was combined with a T_H1 adjuvant monophosphoryl lipid A (MPL) without inducing significant local reaction in the skin.

Chen *et al.* further demonstrated that this approach can be used to augment the efficacy of DC vaccine against solid tumors. UPL adjuvant treatment enhanced migration and maturation of APCs in the skin as well as therapeutic DCs following intradermal administration of the cells, ultimately resulting in expansion of cytotoxic CD8⁺ T cells and enhancement of anti-tumor immunity (25). In this study, the group observed enlarged perforations in the basement membrane of the lymphatics and disarray of the extracellular matrix in the dermis upon the UPL treatment, which was considered to be responsible for the enhanced migration of APCs and DCs in the skin and lymphatic vessels. The green light pulsed laser used in this study was featured with a pulse peak power over 2,500 times greater as compared to the UPL used by Russian researchers, which could have contributed to this ultrastructural change.

Our group demonstrated that pre-treatment with a Q-switched neodymium-doped yttrium orthovanadate (Q-Nd:YVO₄) laser, emitting either 532 nm or 1064 nm light in 7 nanosecond pulses at a repetition rate of 10 kHz, at an average irradiances of 1 for and 5 W/cm² for 532 and 1064 nm over 1–4 min showed the adjuvant effect on intradermal vaccination with model and influenza vaccines (26). In this study, the laser parameter for 532 nm was closer to what the Russian investigators used compared to those used by Chen *et al.*

Zhou *et al.* expanded the use of this laser parameter showing that UPL of 532 nm at 1 J/cm² increased microvascular permeability in the exposed skin tissue and translocation of intradermally-administered radiation-attenuated sporozoites (RAS) to liver (27). Since hemoglobin and oxyhemoglobin have peak absorbance from 540 nm to several parameters of 578 nm, 532 nm Nd:YAG laser and a 595 nm pulsed dye laser (Vbeam, Candela, MA) were tested if they increased the microvascular permeability and the efficacy of the malaria vaccine. Remarkably, the UPL treatment significantly augmented sporozoite-specific antibody and CD8⁺ T cell responses and conferred protection against malaria challenge. Interestingly, confocal microscopic analysis revealed that many sporozoites were recruited close to blood vessels in laser-treated skin in this study, which suggests a possible involvement of a chemotactic cue released by the UPL treatment. However, the exact molecular pathways ultimately leading to the adjuvant effect with the UPL adjuvant remains largely elusive.

Collectively, these studies show that UPL adjuvants create immunostimulatory milieu for APCs in the skin and augments the immune response to vaccine administered to the exposed skin without inducing pain or tissue damage (Figure 1). Each UPL could be further tailored with other strategies including additional chemical or biological adjuvant to achieve a particular goal of vaccination of interest.

2. Non-pulsed laser adjuvant

Non-pulsed laser (NPL) adjuvant is featured by continuous wave laser light to stimulate immune responses. Phototherapy with continuous wave light has been used to treat skin diseases for more than 3,000 years (28). Concentrated light has been well established to be effective to treat lupus vulgaris (29), suggesting that light can modulate host-pathogen interactions. Accordingly, a concept of laser immunotherapy combining photothermal therapy and immunostimulation is proposed by Dr. Wei R. Chen in 1997 (30). A series of work demonstrated that photothermal effect primarily drives the induction of robust anti-cancer immune response (31). Typically, this approach combines laser exposures with photoabsorber indocyanine green (ICG) and immunologic adjuvant (such as glycated chitosan, GC) to induce dye-enhanced thermal interaction and immune stimulation. It has been demonstrated that this approach is mainly mediated by photothermal effect, but photochemical effect could contribute to the formation of the robust anti-cancer immunity. Zhou *et al.* showed that mice treated with photothermal therapy (PTT) alone had a significant smaller tumor burden than that of the control mice. A 10-min treatment with 980 nm laser at a density of 0.85 W/cm² (510 J/cm² in total) increased the temperature of tumor tissue, which caused cell death in mouse models of pancreatic cancer and melanoma (32). In this approach, heat generation in tissues is considered to induce immunologic cell death with release of heat shock proteins (HSPs), calreticulin (CRT), high-mobility group box 1 (HMGB1) protein and adenosine triphosphate (ATP) (33). In response to this, DCs are stimulated and produce IFN- γ and increase their expression of MHCII and CD80 and recruitment of DCs and T cells in tumor microenvironment. Consistently, a 10-min treatment with 805–808 nm light at an irradiance of 1–1.5 W/cm² along with topical imiquimod application has been shown to induce release of damage-associated molecular patterns (DAMPs) (HSP70, HSP90 and HMGB1) suppressed tumor growth in mammary tumor (34) and skin squamous cell carcinoma in humans and mice (35), and melanoma (36) and refractory cutaneous warts in humans (37). In this approach, immunogenic cell death also provides sources of tumor neoantigens, creating an in situ autologous cancer vaccine.

Wang *et al.* recently showed that a personalized cancer vaccine encapsulating JQ1, a bromodomain-containing protein 4 (BRD4) inhibitor, and ICG along with tumor cells in a hydrogel matrix inhibited the tumor relapse by promoting the maturation of dendritic cells and eliciting tumor infiltration of cytotoxic T lymphocyte (CTL) upon 808 nm laser treatment (Changchun New Industries Optoelectronics Tech. Co., Ltd, Changchun, China) for 2 min at an irradiance of 2.0 W/cm² (38). In this system, temperature increase in the treated area with 808 nm near-infrared (NIR) laser triggered on-demand release of tumor-specific antigens and cytokine expression, subsequently facilitating the activation of dendritic cells. Interestingly, since the group did not observe the comparable DC maturation with heat generation only, the immune response seems to have been caused by increased

release of tumor antigens, not photothermal effect. The difference in laser parameters used in these studies may explain the difference in the effects of lasers used.

Currently, this concept has been further expanded to photoimmunotherapy which uses monoclonal antibody tagged with the fluorophore and efficiently mounts anti-cancer immunity with heat generated upon light exposure (39, 40). These results suggest that laser immunotherapy is mainly mediated by photothermal effect, but photochemical effect may contribute to the formation of strong anti-cancer immunity.

All the reports on NPL adjuvant to date use a NIR range of laser light (1061 – 1301 nm) (Table 1). A dose chosen for this approach has been reported to cause no thermal damage nor inflammation in the exposed tissue.

Our group reported for the first time the adjuvant effect of a continuous wave Nd:YVO₄ laser (RMI Laser, Lafayette, CO) emitting 1064 nm light (26). We showed that a one-minute exposure at a 5 W/cm² irradiance of the skin on a 5 mm spot to the continuous wave 1064 nm laser augmented antibody titers to intradermal model and influenza vaccines and conferred better protection in an influenza lethal challenge. The dose and irradiance used in this study were confirmed to be non-tissue damaging in mice and below the level of pain or tissue damage in human subjects. The 1064 nm laser was reported to induce the expression of selected cytokines and chemokines including CCL2 and CCL20 and functional and migrational changes in DCs in the skin in this study. This immunostimulatory milieu with the laser adjuvant led to a mixed T_H1-T_H2 immune response to the whole inactivated influenza vaccination. Kimizuka *et al.* expanded this finding showing that economical semiconductor laser diodes have adjuvant effect (41). A Gallium arsenide (GaAs) diode laser emitting 1061 nm (Axcel Photonics, Marlborough, MA) at an average irradiances of 5 W/cm² or Indium phosphide (InP) diode lasers emitting 1258 (Innolume, Dortmund, Germany) or 1301 nm (SemiNex Co., Peabody, MA) at an average irradiances of 1–2 W/cm² over 1 min replicated the adjuvant effect of the large diode-pumped solid-state Nd:YVO₄ laser system on the intradermal influenza vaccine without inducing damage or inflammation in the exposed tissue. These low-cost handheld laser devices are 10–100 times less expensive than high-frequency, ultrashort pulsed lasers, which establish the feasibility of the laser adjuvant approach for use in the clinic. It should be noted that the representative adjuvant including alum and MF59, but no laser adjuvant induced allergenic IgE response to the vaccination. Morse *et al.* further demonstrated that the same 1064 nm laser adjuvant modulated migratory DCs in the skin, specifically activating the Lang⁺ and CD11b⁻Lang⁻ subsets and recruited Ly6C⁺ monocytes (42). Consistently, and the adjuvant effect was dependent on DC subsets expressing Langerin and CCR2. In this study, it has been suggested that thermal effect played a minimal role as the same dose of 1064 nm laser with a pulsed wave showed limited effects on the DC subsets and immune responses. Kimizuka *et al.* further revealed that continuous wave 1064 nm laser induced generation of reactive oxygen species (ROS) in the exposed skin and transiently stimulated mast cells, leading to expression of a defined set of chemokines including CCL2 and CCL20 that ultimately induced CCL21 expression in the lymphatics without overt inflammation (43). Our group further demonstrated that the immunostimulatory milieu established with the laser exposure induced migrational changes of skin-resident migratory CD103⁺ DCs, which played a

pivotal role in augmentation of the adaptive immune response to the intradermal influenza vaccination (Figure 1). High-fluence low-power laser irradiation (HF-LPLI) has been consistently reported to target cytochrome c oxidase (COX) in electron transport chain (ETC) in mitochondria and induce generation of ROS. A 10-min exposure with 633nm He-Ne laser at an irradiance 0.2 W/cm^2 (the fluence of 120 J/cm^2) induced ROS generation, mitochondrial permeability transition (MPT) and apoptosis in cultured human lung adenocarcinoma cells (ASTC-a-1) (44, 45). Consistently, 635 nm continuous wave semiconductor laser for 10 min at a fluence rate of $50\text{--}200 \text{ mW/cm}^2$ inhibit enzymatic activity of COX and cause respiratory chain superoxide anion (O_2^-) burst and tumor killing in cultured human lung and mammary cancer cell lines. It has been further shown that exposure of xenografts in vivo with 635 nm laser at an irradiance of 500 mW/cm^2 for 40 min significantly suppresses tumor growth (46). Although the laser vaccine adjuvant uses a smaller dosage than the laser immunotherapy, the mode of action of laser immunotherapy targeting mitochondrial ETC to augment the immune response is similar to that of the laser vaccine adjuvant. Further study is necessary to understand specific photochemical events in response to laser-tissue interactions between a laser parameter and target tissue to evoke desirable responses for a therapeutic purpose.

Gelfand *et al.* performed clinical testing to determine if the effect of the NPL adjuvant was consistent between mice and humans, as the skin structure of humans is quite different from that of mice (47). The group treated human subjects with continuous wave 1064 nm laser from a modified handheld diode laser device (IPG Photonics, Oxford, MA) for 1 min on 5 mm^2 areas of the lower back skin at an irradiance of 5 W/cm^2 . All subjects tolerated this dose of the laser with no evidence of skin damage. Immunohistochemical analysis on skin biopsy samples taken at 4 h revealed reductions in the number of CD1a^+ Langerhans cells and CD11c^+ dermal DCs in the dermis, indicating emigration of these cell types out of the exposed skin. Consistently, gene expression analysis revealed increases in chemokine expression including CCL17 and CCL20, which were also observed in the mouse skin exposed to continuous wave 1064 nm laser. These results confirm that the responses of the skin cells to the NPL adjuvant in mice are similar to that in humans.

In short, these studies show that NPL adjuvant is a safe and effective to augment the efficacy of vaccine. These results collectively show that the use of continuous wave NIR laser with distinct wavelength and power is an effective tool to reproducibly modulate innate programs in the skin and can be explored for the broader applications such as the treatment of immune-related skin diseases. On a note, unlike other classes of the laser adjuvants, a combination approach of the NPL adjuvant with other adjuvant has never been tested for the potential synergistical effect in the context of vaccination.

3. Non-ablative fractional laser adjuvant

Fractional lasers are small diameter laser beams to create controlled damage to the skin and generate wound-healing responses. Fractional laser devices are therefore employed in skin rejuvenation and treating skin conditions including scarring (48). The treatment typically takes seconds creating an array of vertical columns of damaged or ablated skin within the

epidermis and dermis. Power is optimized to induce minimal damage outside the treated tissue and pain while maximizing the wound healing response.

Non-ablative fractional laser (NAFL) induces controlled coagulation of tissue to produce micro-sterile inflammation and self-renewable microthermal zones (MTZs) in the skin. These laser devices emit microbeam pulses in the range of 1410 to 1550 nm to coagulate a series of vertical columns of tissue. The resultant dead cells in MTZs release nucleic acid and activate nucleic acid sensing pathways including the cGAS/STING pathway to induce sterile inflammatory responses and skin tissue regeneration. Therefore, NAFL has been successfully used for skin rejuvenation.

A series of studies have shown that NAFL can also function as an immunologic adjuvant for skin vaccination (Table 1). Wang *et al.* used a US Food and Drug Administration- (FDA-) approved NAFL devices PaloVia® Skin Renewing Laser developed by Palomar emitting a 1410 nm laser microbeam to generate an array of MTZ in the mouse skin or Fraxel SR-1500 laser by Solta Medical in the pig skin (49). The NAFL treatment followed by intradermal vaccination augmented humoral immune responses to influenza vaccine. The group further showed that topical application of imiquimod, which acts on toll-like receptor 7 (TLR7) and activates innate and adaptive immune responses, synergistically augmented humoral and T cell responses with the NAFL treatment. Topical imiquimod is FDA-approved for the treatment of anogenital warts, actinic keratosis, and superficial basal cell carcinomas (50) and a feasible choice for a combination approach. This particular combination approach in this study conferred cross-protection in a lethal challenge murine model of influenza. The mechanisms of action involve DAMPs released from MTZs, which recruit a large number of APCs to ultimately augment CD4⁺ and CD8⁺ T cell responses. In this sequence, the transient expression of a selective set of cytokines including TNF- α released from plasmacytoid DCs (pDCs) play a pivotal role in the strong adjuvant effect and T cell responses via IFN regulatory factor7 (IRF7). Wang *et al.* further showed that the NAFL treatment combined with microneedle vaccination augmented anti-influenza immunity while reducing local reactogenicity (51). The NAFL treatment enhanced the uptake and transportation of antigens by APCs and immune responses to vaccine packaged in the microneedle. This enhancement was mediated by dsDNA sensing STING pathway and subsequent interferon upregulation, leading to APC recruitment and migration (Figure 2). The group further demonstrated that this approach induced cross-protective immunity against influenza infection in mice and pigs.

Remarkably, Lopez *et al.* demonstrated that this approach can be used to induce protective effector memory (T_{EM}) and tissue-resident (T_{RM}) CD8⁺ T cell responses against herpes virus in the local mucosa (52). In this study, the treatment of the skin with NAFL (1410-nm PaloVia Laser) along with the local application of imiquimod cream followed by intradermal vaccination of peptide vaccine containing the immunodominant herpes simplex virus 2 (HSV-2) induced long-term memory CD8⁺ T cell responses and synergistically augmented protective immunity against genital herpes infection challenge. Interestingly, this study examined direct effect of NAFL on cultured DCs. An exposure of immature DCs to laser induced phenotypic maturation and promoted production of proinflammatory cytokines including IL-12 and TNF- α . The NAFL treatment was confirmed to induce accumulation of

functionally matured DCs in the treated skin *in vivo*, which seems to be the central mechanism of action by which laser adjuvant enhances protective T-cell responses with NAFL. This is the first to demonstrate that laser-based adjuvant has potential to promote generation of T_{RM} which play a key role in protection against viral infection.

Kim *et al.* combined transdermal vaccination using hyaluronan (HA) nanocarriers with NAFL to enhance the efficacy of transdermal vaccination (53). HA is a natural macromolecule with intrinsic high permeability into the skin and was used as a carrier of a model antigen for non-invasive vaccination on the skin in this study. Conjugates of ovalbumin (OVA) and HA also facilitated endocytosis via HA receptor and induced maturation of DCs *in vitro*, providing an additional adjuvant-like effect. The conjugates were confirmed to penetrate into the mouse and porcine skin more efficiently. Consistently, NAFL treatment using 1410-nm PaloVia Laser device significantly augmented humoral responses to OVA after non-invasive transdermal application of the conjugates.

Li *et al.* took a different approach to augment intradermal influenza vaccination using the NAFL adjuvant. The group combined 1410 nm NAFL treatment using 1410-nm PaloVia Laser device with topical application of all-*trans* retinoic acid (ATRA, a vitamin A derivative) to synergistically augment immune responses (54). ATRA is an established pleiotropic modulator of innate and adaptive immunity (55). In addition, topical application of ATRA is approved by the FDA for acne treatment (56) and represents a feasible choice for a combination strategy. In this study, ATRA stimulated upregulation of cytosolic nucleic acid sensors and their downstream factors, leading to enhancement of type I interferon expression caused by release of DAMPs upon the NAFL treatment. The combination approach significantly enhanced antibody responses against an inactivated influenza virus vaccine by 10-fold. The group also observed this approach induced cross-reactive neutralizing antibody and conferred cross-protection against infection with heterogeneous viral strains. Since ATRA alone induced a T_H2 -biased immune response, the combination approach was proved to be essential to induce T_H1 - T_H2 -balanced immune responses.

These results consistently demonstrate that NAFL from a clinically approved device is a feasible and effective approach to augment the efficacy of vaccine. Most of studies also show its synergistic effect with chemical adjuvants to induce robust T cell responses and protection. Further complementary development of skin vaccination technology is desired to fully take advantage of this approach.

4. Ablative fractional laser adjuvant

Ablative fractional laser (AFL) typically uses 2790, 2940 or 10600 nm laser to create micro-channel arrays (50–150 μm in diameter with a depth of less than one millimeter) in the skin (Table 1). These wavelengths show high absorption by water and cause explosive superheating of the aqueous content of the tissue column, leading to progressive ablation of the narrow columns of tissue. Each micro-channel is surrounded by a narrow (around 5 μm -thick) layer of coagulated dead tissue. AFL treatment promotes collagen formation and retraction of the dermis and epidermis to tighten the skin and is therefore used for skin resurfacing (57). In addition, AFL has been investigated for a safe method to enhance transcutaneous delivery of drugs (58, 59). Since the skin is enriched with APCs, the

literature indicates that the delivery of vaccine to the skin is more effective vaccination than the conventional intramuscular or subcutaneous delivery (14–16, 19, 60). It appears that AFL not only facilitates delivery of antigens to APCs in the skin, but also acts as an adjuvant augmenting the immune response to intradermal or transdermal vaccination.

Chen *et al.* used AFL treatment of the skin to generate microchannel arrays in the epidermis to facilitate vaccine delivery (61). An UltraPulse® Fractional CO₂ Laser emitting 10600 nm (Lumenis Inc., Yorkneam, Israel) was used to create microchannels. This approach was proved to deliver a model protein vaccine (OVA) efficiently with gauze skin patches onto the skin and enhance antigen take-up by APCs in the skin augmenting the immunogenicity of the vaccine compared to the conventional tape-stripping method. Weiss *et al.* used the P.L.E.A.S.E.® (Precise Laser Epidermal System) device developed by Pantec Biosolutions AG (Ruggell, Liechtenstein) to improve vaccine delivery, which is based on a 2940 nm diode-pumped, erbium:yttrium-aluminium-garnet (ER:YAG) laser (62). A model antigen application after AFL treatment enhanced antigen uptake by skin DCs and induced significantly higher antigen-specific T cell responses compared to the subcutaneous injection. However, compared to the subcutaneous injection of the same allergen, this approach rather led to the induction of a T_H2-biased response. Interestingly, this response was significantly redirected toward T_H1 responses by co-application of a TLR agonist CpG oligodeoxynucleotides (ODN) with their T_H1-promoting immunomodulatory capacity (63). These results show that AFL treatment enhances transdermal delivery of vaccine antigen to APC populations in the skin and at the same time strongly influences on the magnitude and polarization of T cells, which could be further optimized by an addition of a chemical adjuvant.

Scheiblhofer *et al.* further advanced this approach to elicit high antibody titers by exploring an optimal laser parameter and adjuvant to be combined (64). The group found that antibody responses against transcutaneously administered hepatitis B surface antigen vaccine were dependent on micropore depth and peaked at a laser power density of 8.4 J/cm², while being independent of micropore density. Having optimized the laser parameter, the group further tested combination of AFL with 5 representative adjuvants of MPL, heat labile enterotoxin (LT)-B subunit from *E. coli*, CpG-ODN 1826, alum, CRM197 (a nontoxic diphtheria toxin derivative) to have found that alum significantly reduced antibody titers and other adjuvants induced marginal changes only. LT-B and CpG shifted the polarization of the immune response toward T_H1, suggesting that a laser parameter and choice of an adjuvant to be combined should be carefully selected depending on a specific goal of immunization.

Chen *et al.* used an approach to combine topical application of powder vaccine-coated array patches with AFL treatment of the skin to generate micro-channel arrays in the epidermis to adjuvant vaccine (65). The UltraPulse® Fractional CO₂ Laser was used to create microchannels. This approach was proved to deliver vaccine into the skin efficiently and able to maintain the same immunogenicity as the intradermal injection of live-attenuated or chemically-adjuvanted inactivated vaccine formulation, while reducing local inflammation that causes permanent scars generally found in the intradermal administration of these vaccines. These results suggest that the limited inflammation around microchannels may be sufficient to augment the immunogenicity of the intradermal vaccine.

Terhorst *et al.* tested the effect of AFL on a vaccibody, which is a homodimeric chimeric protein consisting of a XC-chemokine XCL1, a dimerization unit made of the hinge and CH3 domain of human IgG3, and moiety of antigen for the targeted delivery of vaccine antigen to target XCR1⁺ dermal DCs (66). The XCR1⁺ DC subset is increasingly recognized as being critical for formation of cytotoxic T cell and T_H1 responses, long-term adaptive memory responses (67–71) and early and long-term antibody responses (72). In order to facilitate the delivery of the vaccibody, this group employed the P.L.E.A.S.E.® device. The treatment of skin generated an array of several hundred micropores of approximately 150 µm diameter over a 14 mm² spot. Interestingly, the combination of the AFL treatment with the targeted delivery of a model cancer antigen on prophylactic or therapeutic cancer vaccine to dermal XCR1⁺ DCs significantly augmented antigen-specific CD8⁺ and CD4⁺ T cell responses and protection against melanoma. The mild inflammatory milieu created by dead keratinocytes upon the AFL treatment was considered to have contributed to the adjuvant effect of AFL. Hervé *et al.* recently advanced this approach further. The group used the P.L.E.A.S.E.® system to create laser-induced epidermis-limited perforation to augment the efficacy of epicutaneous vaccination (73) using a non-invasive and needle-free skin patch Viaskin® which has been successfully used for transcutaneous delivery of food allergen for immunotherapy (74) and genetically-inactivated recombinant pertussis toxin (75) and respiratory syncytial virus (RSV) N-nanorings (76) for vaccination. This approach induced limited skin inflammation with upregulation of IL-1β and IL-33, enhanced Langerhans cell activation and facilitated migration of antigen-positive DCs in skin to draining lymph nodes, ultimately inducing a rapid increase in antibody titers against a model vaccine and recombinant pertussis toxin.

Hessenberger *et al.* used this methodology to improve immunotherapy for allergy with the skin patch (77). Allergen-specific immunotherapy involves gradual administration of allergen using various routes to induce the reinstatement of immunologic tolerance toward allergens (78). Compared to the conventional subcutaneous immunotherapy (SCIT) involving subcutaneous injection of allergen using the hypodermic needle, transcutaneous application, which is called epicutaneous immunotherapy (EPIT), has been consistently found to be safe but with the modest efficacy and needs improvement (78). The transcutaneous immunization using skin patch with a recombinant glass pollen allergen Phl p 5 via laser-generated micropores led to induction of an allergenic T_H2-biased response compared with the subcutaneous injection of the same allergen. Interestingly, this response was significantly suppressed by co-application of a TLR agonist CpG-ODN with the T_H1-promoting immunomodulatory capacity (63) in these pre-sensitized mice but not by SCIT. Kumar *et al.* similarly treated mice with AFL before application of a model allergen (79). The group treated the mouse skin with P.L.E.A.S.E.® laser to generate 75 micropores per cm² (50–75 µm in base diameter and 20–30 µm in depth). The AFL treatment with topically applied OVA using the skin patch facilitated the delivery of the allergen into the skin and take-up by APCs with no inflammation. Since a combination of CpG-ODN and 1,25-dihydroxyvitamin D3 (VD3) was found to profoundly increase TGF-β and IL-10, two primary cytokines for regulatory T cell (Treg) and subsequent immunological tolerance induction, and IL-12 and IL-18 for a T_H1 response while suppressing IL-4 for a T_H2 response in the skin among other tested combinations of adjuvants, the group then applied

OVA mixed with CpG-ODN with VD3 onto each micropore in OVA-sensitized mice. Consistently, the combination therapy significantly enhanced Tregs, suppressed IgE response and alleviated allergic airway responses in OVA-sensitized mice while the conventional SCIT failed to do so. Machado *et al.* used mannan-neoglycoconjugates which binds C-type lectin receptors and targets CD14⁺ DCs and Langerhans cells to avoid T_H2-biased immune responses and induce proper modulation of the immune response upon EPIT (80). The group pre-treated the skin with the AFL prior to the transcutaneous application of a pollen allergen-mannan neoglycoconjugates to facilitate the transdermal delivery. This approach synergistically augmented T_H1/T_H17-biased humoral and cellular immune responses and suppressed IgE responses to the pollen allergen in mice. The suppression of IgE responses with AFL was found to be superior to intradermal injection of the allergen. Consistently, the expression of proinflammatory cytokines and chemokines was increased upon with the AFL treatment in the skin, being responsible for the synergistic effect. Interestingly, in contrast to NPL, depletion of mast cells (MCs) had no substantial effect on adaptive immune responses in the context of AFL-mediated epicutaneous immunization (81). These results show that AFL treatment could offer a strong immunomodulatory effect to skin-based immunotherapy.

Further investigation is warranted to find a tailored combination of AFL and other adjuvants to maximize the efficacy of the current and candidate vaccine and immunotherapy.

Discussion

A series of studies clearly demonstrates that all the four classes of the laser adjuvants are able to function as an immunologic adjuvant in preclinical models and clinical studies. Contrary to the conventional chemical or biological adjuvants, all the preclinical and clinical safety studies indicate that the laser adjuvant is safe and induces no significant local or systemic side effects. The laser adjuvants show a number of advantages over the conventional adjuvants. i) Laser is a physical parameter and does not persist in the tissues, reducing risk of side effects. ii) The laser adjuvant presents no requirement of irreversible formulation with vaccine nor poses risk of alteration to the vaccine antigen. iii) The laser device is free from cold-chain storage and suitable for long-term storage. iv) The laser application requires no hypodermic needles and produces no biohazardous sharp waste. Although no clinical trial has been conducted to evaluate the efficacy of the laser adjuvant to date, further efforts on rigorous preclinical safety and toxicology would open a path for the laser adjuvant to be used in the clinic.

It is challenging to directly compare the efficacy of a class of the laser adjuvant with each other because of the variability of the immunization models used in the literature. For example, co-application of imiquimod adjuvant with NAFL treatment was essential to induce T cell responses to a peptide herpes vaccine and protection in a preclinical model of herpes infection (52) and to inactivated influenza vaccine and protection in a preclinical model of influenza infection (49). On the other hand, NAFL treatment alone was sufficient to induce T cell responses and confer protection when combined with a PR8 model influenza vaccine-packaged, biodegradable microneedle array (51). UPL adjuvant alone was also sufficient to confer protection in a mouse model of influenza (21, 22), induce CD8⁺ T

cell response to a sporozoite vaccine and protection in a malaria challenge model (27) and CD8⁺ T cell response to vaccinia virus-based vaccine and protection in a vaccinia challenge model (65), while the other laser adjuvants require an addition of other adjuvants to induce T cell responses. Since immunologic adjuvants receive regulatory approval only with specific vaccines, further mechanistic investigation to fully characterize the mode of action of each laser adjuvant is warranted to achieve clinical significance.

Although limited, the literature shows that each class of the laser adjuvant has the distinct molecular mechanisms of action depending on the type of laser and parameters used (Table 2). There seems to be common mechanisms across the classes, while some are very specific to a certain class. Disrupting the stratum corneum in the epidermis has been shown to induce the secretion of pro-inflammatory cytokines, chemokines and release of DAMPs, acting as an inherent adjuvant (82). In this process, damaged keratinocytes release cytokines such as IL-25, IL-33 and TSLP and subsequently activate other skin-resident immune cells (83). In this regard, the mechanisms of action involved in the NAFL adjuvant are relatively clear as they create controlled damages, induce DAMPs release and activation of dsDNA sensing STING pathway, and then evoke limited inflammation around the damaged tissue in the treated skin, ultimately stimulating APC migration and function and augmenting adaptive immune responses (51). The mechanisms of action involved in the AFL adjuvant appear to be similar to those of NAFL. Upon AFL treatment, the mild inflammatory milieu is created by dead keratinocytes surrounding columns, which is considered to stimulate the immune response in a similar way as NAFL. These fractional laser-based approaches generally lead to the induction of T_H2-biased responses (62) and often need T_H1 adjuvant to redirect immune responses to confer protection (52). In comparison, the molecular pathways involved in the UPL or NPL remain relatively unclear. High-frequency UPL induces HSP70 release while lower frequency UPL creates disarray of the extracellular matrix, but like other adjuvants used in licensed vaccines, both eventually activate key Langerhans cells (21) and APCs in skin (24). On the other hand, NPL adjuvant induces ROS generation and activation of innate programs including up-regulation of a selective set of chemokines, which were not seen in UPL adjuvant (24), ultimately activating migratory DCs (42, 43). In contrast to the AFP or NAFL adjuvant, no tissue damage has been detected by histological examination after the UPL or NPL treatment in the human and mouse skin (24, 26, 47). The photoreception mechanisms, exact molecular identity of photoreceptors, and subsequent signaling pathways involved in the adjuvant effect of the UPL or NPL adjuvant remain elusive. In order to direct the current and candidate vaccines with the laser adjuvant toward the desired clinical benefits with minimize side effects, it is imperative to advance basic research on the molecular mechanisms of action of the laser adjuvant.

Each class of the laser adjuvants shows distinct characteristics as an immunologic adjuvant, suggesting that the judicious use of the laser adjuvant is needed to achieve the specific goals of a particular vaccine or immunotherapeutic. It appears that the laser adjuvant generally leads to the induction of a T_H2-biased response. As such, in order to confer protection with vaccination or reverse allergenic responses, combination strategies have been extensively tested (Table 3). Interestingly, in some cases, the use of chemical adjuvant is necessary to achieve the goals. For example, co-application of additional adjuvant with its T_H1-promoting immunomodulatory capacity was essential to confer protection (49, 52) or

suppress allergenic responses (62, 77), while majority of studies employ it to further augment therapeutic efficacy. Since the use of additional adjuvant would pose a significant regulatory hurdle, most of the studies focus on licensed chemical or biological adjuvants. In order to take advantages of non-invasive nature of the laser adjuvant, topical application of additional adjuvant is desirable for a combination approach. Not surprisingly, combination strategies to date employed FDA-approved topical agents including imiquimod and ATRA (Table 3).

With the proven safety and efficacy, AFL adjuvant has reached clinical development. A group at Medical University of Vienna sponsored by Pantec Biosolutions AG completed Phase I clinical trial of “EPIMMUN Influenza” comparing laser-assisted epidermal using the P.L.E.A.S.E.® system to intradermal administration of Sanofi Pasteur/MSD seasonal influenza vaccine INTANZA 15µg (NCT02988739, the result of the study is not available yet). The other laser adjuvants have not yet reached advanced preclinical development. It should be noted that the first demonstration of the effect of the laser adjuvant was performed in cancer patients in Russia (21–23). A recent expanded list of intradermal vaccines along with the matured medical laser industry is expected to boost initiation of the clinical translation of this technology into intradermal or transdermal vaccination. In addition, the recent studies demonstrate that the laser adjuvant could be used to augment the efficacy of immunotherapy for allergy. Exploration of broader applications of this approach to the treatment of immune-related diseases is expected to follow this path in the near future.

Acknowledgments

Funding

This work was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award number R01AI105131 (S.K.) and R21AI144103 (S.K.) and Massachusetts General Hospital Executive Committee On Research (ECOR) Interim Support Funding (S.K.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Non-standard abbreviations

AFL	ablative fractional laser
ATP	adenosine triphosphate
ATRA	all- <i>trans</i> retinoic acid
APC	antigen-presenting cell
BRD4	bromodomain-containing protein 4
CRT	calreticulin
CpG-ODN	oligodeoxynucleotides
COX	cytochrome c oxidase
CTL	cytotoxic T lymphocyte
DC	dendritic cells

ETC	electron transport chain
EPIT	epicutaneous immunotherapy
GaAs	Gallium arsenide
GC	glycated chitosan
LT	heat labile enterotoxin
HSP	heat shock protein
HSV	herpes simplex virus
HF-LPLI	high-fluence low-power laser irradiation
HMGB1	high-mobility group box 1
HA	hyaluronan
InP	Indium phosphide
ICG	indocyanine green
MTZ	microthermal zones
MPT	mitochondrial permeability transition
MPL	monophosphoryl lipid A
NIR	near-infrared
NAFL	non-ablative fractional laser
NPL	non-pulsed laser
OVA	ovalbumin
PTT	photothermal therapy
KTP	potassium titanyl phosphate
Q-Nd:YAG	Q-switched neodymium-doped yttrium aluminum garnet
Q-Nd:YVO₄	Q-switched neodymium-doped yttrium orthovanadate
RAS	radiation-attenuated sporozoites
ROS	reactive oxygen species
RSV	respiratory syncytial virus
SCIT	subcutaneous immunotherapy
TLR	toll-like receptor
UPL	ultra-short pulsed laser

VD3 1,25-dihydroxyvitamin D3**References**

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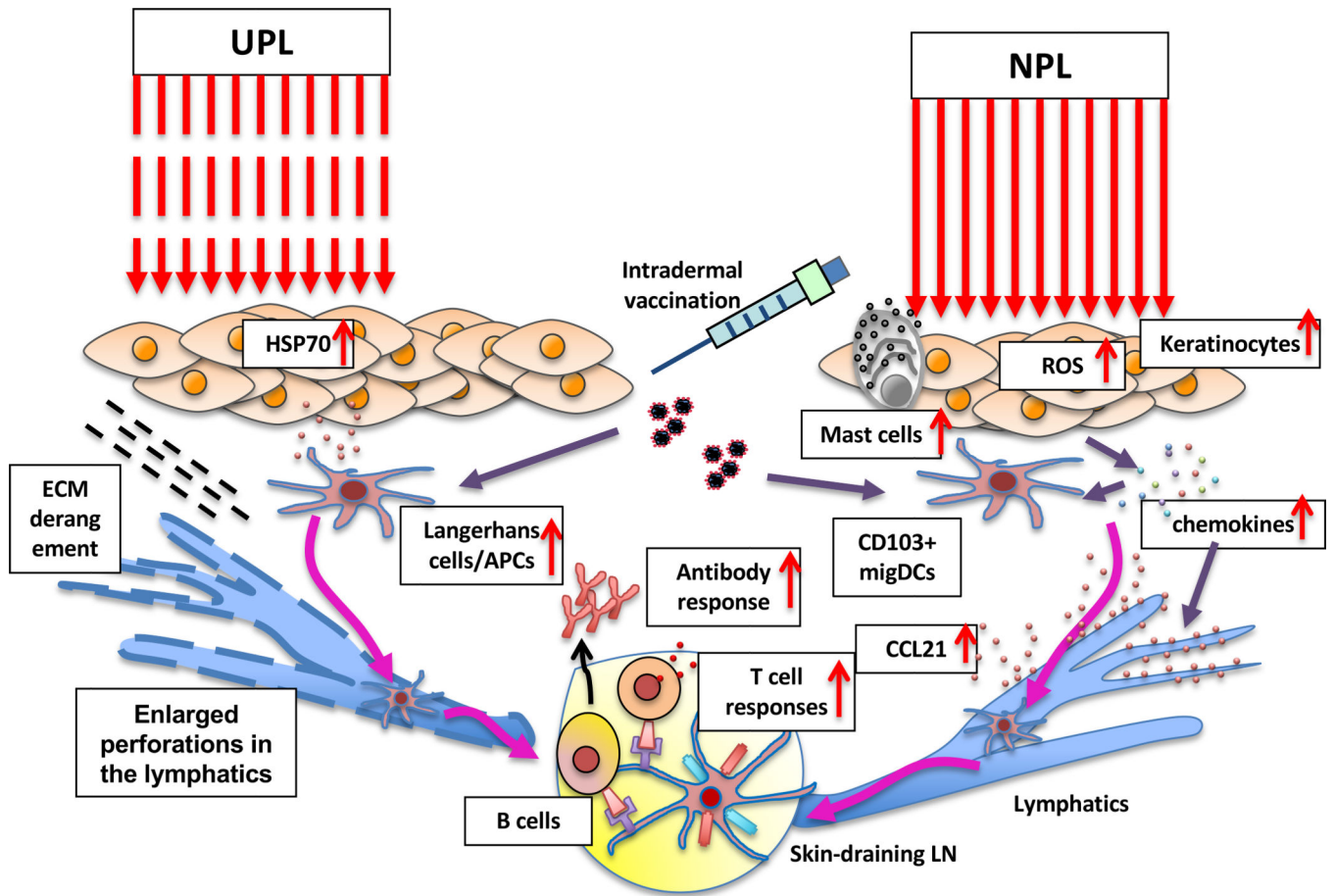


Figure 1. The mechanisms of action of the UPL and NPL adjuvants.

Ultrashort-pulsed laser (UPL) induces extracellular release of HSP70 and disarray of extracellular matrices activating Langerhans cell and facilitating migration of DCs, respectively. Non-pulsed laser (NPL) a near-infrared (NIR) range stimulates skin cells including keratinocytes and mast cells via reactive oxygen species (ROS) and induces the expression of a defined set of chemokines including CCL2 and CCL20 that ultimately induce CCL21 expression in the lymphatics and migrational changes of skin-resident migratory CD103⁺ and Lang⁻CD11b⁻ DCs.

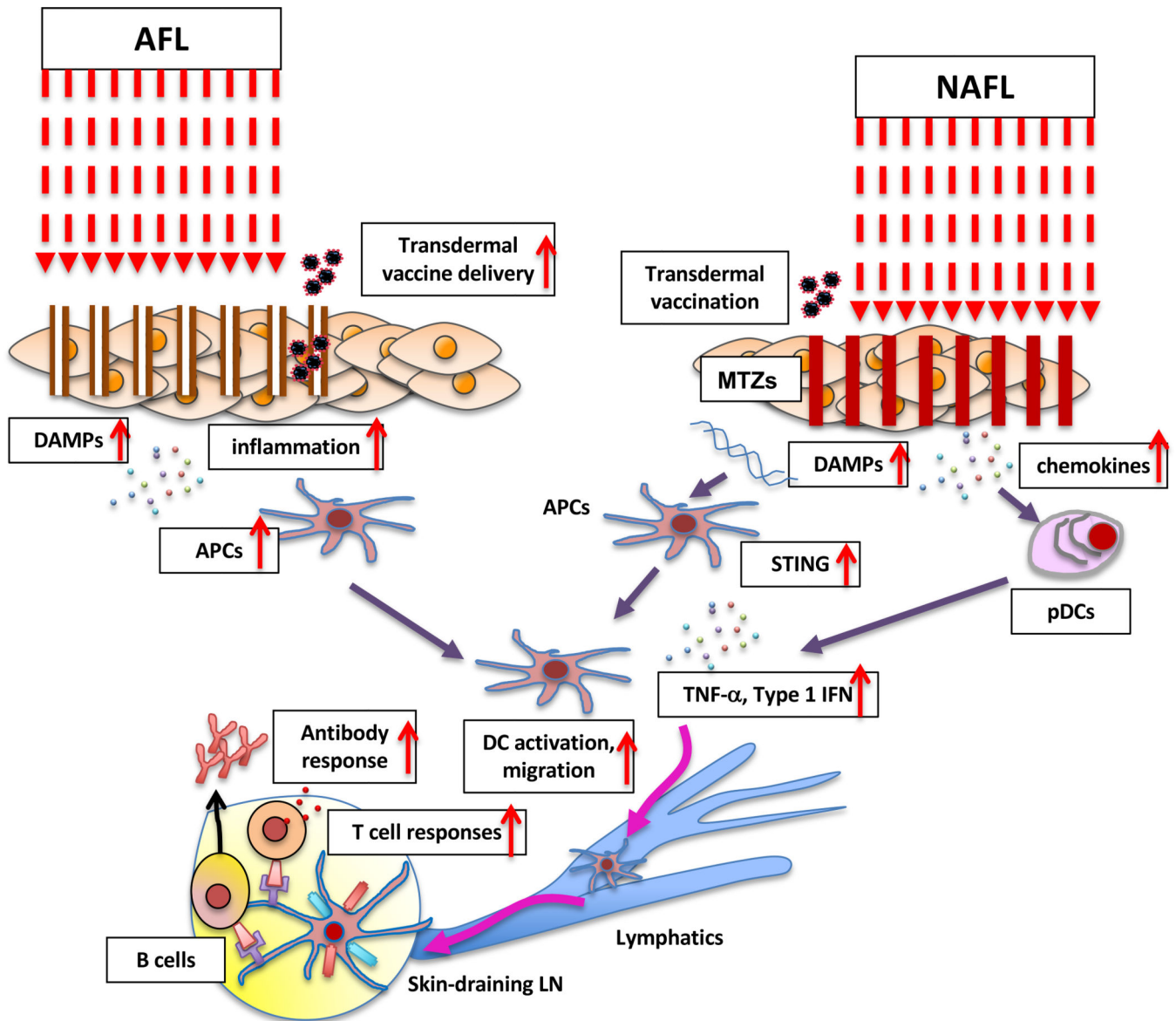


Figure 2. The mechanism of action of the AFL and NAFL adjuvants.

AFL skin treatment induces the mild inflammatory milieu created by a narrow layer of coagulated dead tissue around each micro-channel. The efficient delivery of transdermal vaccine to skin DCs is also facilitated by the micro-channels. The NAFL treatment induces skin cell death (called microthermal treatment zones, MTZs) releasing damage-associated molecular patterns (DAMPs) including dsDNA and micro-sterile inflammation array. dsDNA is taken up by antigen presenting cells (APCs) and recognized by intracellular pattern recognition receptors (PRRs). PRRs then transduce signals to STING activating IRF3 and NF- κ B. Type I interferons, proinflammatory cytokines and chemokines are produced to enhance the maturation and migration of APCs. AFL treatment also induces expression of chemokines in the skin. These chemokines recruit plasmacytoid dendritic cells

(pDCs) from circulation system into the skin. The pDCs release a number of factors to enhance the maturation and migration of APCs.

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Table 1.

Four classes of the laser adjuvant.

Class	Laser type	Wavelength	Pathway Activated	Immune responses stimulated	Tested in	References
Ultra-short pulsed laser (UPL)	Copper vapor	511/578 nm	Extracellular release of HSP70 to enhance Langerhans cell activation and antigen presentation	Ab against influenza and hepatitis B Augment cancer vaccines	Mice, humans	(21–23)
	Q switched Nd:YAG	532 nm	Migration and activation of APCs in the skin	Ab against influenza Ab and T cell responses against OVA	Mice	(24)
	Potassium titanyl phosphate (KTP)	532 nm	n.d.	Ab and T cell responses against OVA upon intramuscular injection	Mice	(24)
	Q switched Nd:YAG	532 nm	Disarray of the extracellular matrix in the dermis facilitates migration of DCs	Cytotoxic T cell response against solid tumor	Mice	(84)
	Q switched Nd:YAG	532 nm	n.d.	Ab against nicotine	Mice	(25)
	Q switched Nd:YVO ₄	532 or 1064 nm	A selective upregulation of chemokines and cytokines in the skin leading to APCs activation and migration	Ab against influenza and OVA	Mice	(26)
	Q switched Nd:YAG (532) Pulsed dye laser (595)	532 and 595 nm	Increase in vascular permeability increases translocation of sporozoite to liver	Sporozoite-specific antibody and CD8+ T cell responses	Mice	(27)
Non-pulsed laser (NPL)	Semiconductor laser	980 nm	Photothermal effect induced cancer cell death and subsequent DC and CTL activation and recruitment in tumor microenvironment	CTL response against pancreatic cancer and melanoma	Mice	(32)
	n.d.	808 nm	Laser along with imiquimod induced intracellular expression of DAMPs	Suppression of tumor growth of skin SCC	Mice and Humans	(35)
	Diode laser	805 nm	n.d.	Synergistic effects between laser immunotherapy and immune checkpoint inhibitor on tumor growth of melanoma	Humans	(36)
	Diode laser	808 nm	Laser along with imiquimod treatment induced lymphocyte infiltration into warts	Suppression of tumor growth of refractory warts	Humans	(37)
	Diode laser	980 nm	Photothermal effect induced cancer cell death and subsequent DC and CTL activation and recruitment in tumor microenvironment	Induction of tumor-specific CTLs in breast cancer model	Mice	(34)
	n.d.	808 nm	Photothermal effect induced thermal lysis of the tumor cell membrane and release	Maturation of DCs and induction of tumor infiltration of CTLs in breast cancer model	Mice	(38)

Class	Laser type	Wavelength	Pathway Activated	Immune responses stimulated	Tested in	References
			of tumor-specific antigens			
	CW Nd:YVO ₄	1064 nm	Photochemical reaction in skin tissue leading to immunostimulatory microenvironment for APCs via expression of a selective chemokines	Ab and T cell responses against influenza and OVA	Mice, human (no immunogenicity study)	(26)
	GaAs (1061) InP (1258, 1301), CW Nd:YVO ₄ (1064)	1061, 1258, 1301 nm	n.d.	Ab response against influenza	Mice	(41)
	CW Nd:YVO ₄	1064 nm	Selective activation of Lang+ and Lang-CD11b- skin DCs and recruitment of Ly6C+ monocytes	Ab response against influenza	Mice	(42)
	CW Nd:YVO ₄	1064 nm	Photochemical reaction in mast cells and keratinocytes leading to ROS generation and chemokine/cytokine expression and immunostimulatory microenvironment for skin DCs	Ab response against influenza	Mice	(43)
	Custom diode laser	1064 nm	n.d.	Induction of migration response of skin DCs	Human (no immunogenicity study)	(47)
Non-ablative fractional laser (NAFL)	Fractional ER:Glass (PaloVia laser, mice; Fraxel SR-1500, pigs)	1410 nm (mice) 1540 nm (pigs)	Sterile inflammation caused by an array of MTZs in skin recruits pDCs	Ab and T cell responses against influenza with imiquimod	Mice, pigs	(49)
	Fractional ER:Glass (PaloVia laser, mice; Fraxel SR-1500, pigs)	1410 nm (mice) 1540 nm (pigs)	Activation of dsDNA sensing pathway and subsequent interferon upregulation by MTZs in skin facilitates migration of DCs	Ab and T cell responses against influenza using microneedles	Mice, pigs	(51)
	Fractional ER:Glass (PaloVia laser)	1410 nm	Adjuvant effect of micro-injury in skin	Ab against OVA using hyaluronan nanocarriers	Mice, pigs (no immunogenicity study)	(53)
	Fractional ER:Glass (PaloVia laser)	1410 nm	Direct activation of skin-resident DCs	T _{EM} and T _{RM} CD8+ T cell responses against herpes virus with imiquimod	Mice	(52)
	Fractional ER:Glass (PaloVia laser)	1410 nm	ATRA stimulated upregulation of cytosolic nucleic acid sensors and their downstream factors and enhanced type I interferon expression with NAFL	Ab against influenza with ATRA	Mice	(54)
Ablative fractional laser (AFL)	Fractional CO ₂ (UltraPulse device)	10600 nm	Efficient delivery of vaccine to skin DCs	Ab against OVA	Mice	(61)
	Fractional CO ₂ (UltraPulse device)	10600 nm	Efficient delivery of vaccine to skin DCs	Ab against OVA and T cell responses against OVA-expressing vaccinia virus	Mice, pigs (no immunogenicity study), humans (no	(65)

Class	Laser type	Wavelength	Pathway Activated	Immune responses stimulated	Tested in	References
					immunogenicity study)	
	Fractional ER:YAG (P.L.E.A.S.E. device)	2940 nm	The mild inflammatory milieu created in the dermis activates skin-resident DCs and induces infiltration of APCs	T cell response against melanoma using a vaccibody targeting XCR1+ DCs	Mice	(66)
	Fractional ER:YAG (P.L.E.A.S.E. device)	2940 nm	The limited inflammatory response activates Langerhans cells and facilitates DC migration	Ab response to OVA and recombinant pertussis toxin using Viaskin®	Mice	(73)
	Fractional ER:YAG (P.L.E.A.S.E. device)	2940 nm	The limited inflammatory response induced by laser microporation activates DC	T _H 1/T _H 17-biased Ab and T cell responses using pollen allergen-mannan neoglycoconjugates	Mice, human skin explant (no immunogenicity study)	(80)
	Fractional ER:YAG (P.L.E.A.S.E. device)	2940 nm	Suppressing allergenic responses if AFL and T _H 1-chemical adjuvant are combined in immunotherapy for pre-sensitized mice	Suppressed allergenic IgE and T cell responses to the grass pollen allergen Phl p 5 with CpG-ODN	Mice	(77)
	Fractional ER:YAG (P.L.E.A.S.E. device)	2940 nm	Efficient antigen delivery to distinct APC population in the skin	T _H 2-biased Ab and T cell responses to model vaccines with CpG-ODN	Mice, Pig ear skin preparation (no immunogenicity study)	(62)
	Fractional ER:YAG (P.L.E.A.S.E. device)	2940 nm	n.d.	Ab responses against HBsAg is dependent on a laser parameter and adjuvant to be combined	Mice	(64)
	Fractional ER:YAG (P.L.E.A.S.E. device)	2940 nm	Increasing cytokine expression for TH1/Treg induction while suppressing TH2 response.	Enhanced Tregs, suppressed IgE response against OVA in combination with chemical adjuvants	Mice	(79)
	Fractional ER:YAG (P.L.E.A.S.E. device)	2940 nm	Increasing cytokine expression	The resultant immune responses were largely not affected by the presence of $\gamma\delta$ T cells or MCs.	Mice	(81)

Ab, antibody; APC, antigen presenting cell; ATRA, all-*trans* retinoic acid; CpG-ODN, CpG-oligodeoxynucleotides; CW, continuous wave; CTLs, cytotoxic T lymphocytes; DC, dendritic cell; DAMPs, damage-associated molecular patterns; ER:YAG, erbium:yttrium-aluminium-garnet; MCs, mast cells; MTZ, microthermal zone; Nd:YAG, neodymium-doped yttrium aluminum garnet; Nd:YVO₄, neodymium-doped yttrium orthovanadate; n.d., Not described; KTP, potassium titanyl phosphate; SCC, squamous cell carcinoma; T_{EM}, effector memory; T_{RM}, tissue-resident.

Table 2.

Mechanisms of action of the laser adjuvants.

Molecular pathways activated	Laser type	Wavelength	Immunological consequences	References
Extracellular release of HSP70	UPL	532 nm	Langerhans cell activation and antigen presentation	(21–23)
Disarray of the extracellular matrix in the dermis	UPL	532 nm	facilitates migration of DCs	(84)
Release of DAMPs (HSP70, HSP90 and HMGB1)	NPL	980 nm	Activation of DCs	(32) (34–37)
Release of tumor-specific antigens	NPL	808 nm	Maturation of DCs	(38)
ROS generation	NPL	1064 nm	Selective activation skin DCs	(43)
Release of DAMPs Activation of nucleic acid sensing pathways including the cGAS/STING pathway	NAFL	1410 – 1540 nm	Recruitment of pDCs	(49, 51, 54)
Direct physical effect of laser	NAFL	1410 nm	Direct activation of skin-resident DCs	(52)
Physical enhancement in vaccine delivery	AFL	2940 – 10600 nm	Enhanced antigen take-up by skin APCs	(61, 62, 65)
Mild inflammatory milieu created in the dermis	AFL	2940 nm	Activation of skin-resident DCs	(66, 73, 80, 81)

AFL, ablative fractional laser; APCs, antigen presenting cells; cGAS, cyclic GMP-AMP synthase; DAMPs, damage-associated molecular patterns, DCs, dendritic cells; HMGB1, High Mobility Group Box 1; HSP70, heat shock protein 70; NAFL, non-ablative fractional laser; NPL, non-pulsed laser; pDCs, plasmacytoid DCs; ROS, reactive oxygen species; STING, stimulator of interferon genes; UPL, ultra-short pulsed laser.

Table 3.

Combination approaches with the laser adjuvants.

Chemical adjuvant	FDA approval	Laser type	Wavelength	Distinct effect of combination	References
MPL	Used in a licensed vaccine as adjuvant	UPL	532 nm	Further augmentation of ab response to nicotine	(25)
Imiquimod	Treatment for anogenital warts, actinic keratosis, and superficial basal cell carcinomas (topical application)	NPL	808 nm	Synergistic suppressive effect on tumor growth	(35, 37)
		NAFL	1410 – 1540 nm	Synergistic augmentation of humoral and T cell responses	(49, 51)
		NAFL	1410 nm	Addition of imiquimod is necessary for the laser adjuvant to show the effect	(52)
ATRA	Acne treatment (topical application)	NAFL	1410 nm	Further augmentation of ab response to influence vaccine	(54)
CpG-ODN	No	AFL	2940 nm	Suppressing allergenic responses if AFL and the chemical adjuvant are combined	(62, 77)
CpG-ODN + VD3	No	AFL	2940 nm	Increasing cytokine expression for T _H 1/ Treg induction while suppressing T _H 2 response when combined with AFL	(79)

AFL, ablative fractional laser; ATRA, all-*trans* retinoic acid; CpG-ODN, CpG-oligodeoxynucleotides; MPL, monophosphoryl lipid A; NAFL, non-ablative fractional laser; NPL, non-pulsed laser; UPL, ultra-short pulsed laser; VD3, 1,25-dihydroxyvitamin D3.