New horizons in cancer immunotherapy: The evolving role of R848 and R837 (Review)

KRUPA R. BHALIYA, MUNEERA ANWER, ALAN MUNN and MING Q. WEI

Menzies Health Institute, School of Medical Science, Griffith University, Southport, Queensland 4215, Australia

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Abstract. Therapeutic approaches that increase the efficacy and safety of cancer treatments and improve disease outcomes have been developed worldwide. Immunotherapy uses the body's immune system to inhibit cancerous growth in tissues and organs. Various approaches have been developed to effectively control and inhibit cancerous growth, including checkpoint inhibitors, T-cell transfer therapy, monoclonal antibodies, vaccines and immunomodulators. Toll-like receptors (TLRs) target malignant cells by equipping the immune response. In addition, TLR agonists serve a key role in promoting the innate immune system and initiating antigen-specific T-cell responses. Notably, TLRs and TLR agonists have been utilized as monotherapies or in combination for the treatment of cancer. The present study aimed to review the use of R848 and R837 as TLR agonists, and outline their use as key immunomodulators in cancer therapy.

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Correspondence to: Ms. Krupa R. Bhaliya, Menzies Health Institute, School of Medical Science, Griffith University, 1 Parklands Drive, Southport, Queensland 4215, Australia
E-mail: krupa.bhaliya@griffithuni.edu.au

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1. Introduction

Immunotherapy utilizes the body's immune system to act as a natural defense against cancer cells, which is notably different to traditional cancer treatments, including chemotherapy and radiotherapy. Advancements in immunotherapy have revolutionized disease management and cancer immunology. Results of clinical trials demonstrated that several types of immunotherapies, such as adoptive cellular therapy and immune checkpoint inhibitors (ICIs), exhibit potential in the treatment of cancer; however, efficacy may vary between patients and benefits may only be observed in certain groups (1).

Receptors, such as Toll-like receptors (TLRs), bridge the gap between innate and adaptive immunity, highlighting them as potential targets for therapeutic interventions. TLRs contribute to the first line of defense against pathogens, triggering signaling pathways that initiate immune and inflammatory responses (2).

The limited immunogenicity of cancer cells in combination with an immunosuppressive microenvironment is a pivotal protective factor that allows evasion of immune surveillance. Immune cells do not distinctly recognize tumor cells, and the tumor microenvironment (TME) notably hinders the infiltration or survival of immune cells (3).

TLRs are evolutionarily-conserved receptors that play a vital role in immune responses, particularly in recognizing microbes. TLRs belong to the family of pattern recognition receptors and are directly involved in regulating inflammatory responses, as well as activating innate or adaptive immune responses to eliminate infectious microorganisms and debris derived from cancer cells.

TLRs expressed on the cell membrane form an effective group with those located on the endosomal plasma membrane. These include TLR1, TLR2, TLR4, TLR5, TLR6, TLR10, TLR3, TLR7, TLR8 and TLR9. In cancer immunotherapy, the modulation of TLRs is employed to enhance the immune response against cancer cells. Thus, specific drugs or agents are designed to activate specific TLRs, thereby initiating an immune response that targets cancer. This strategy is commonly referred to as TLR agonism.

2. An overview of R848 and R837

R848 and R837 are synthetic compounds that modulate the immune response against cancer cells, acting as agonists

for TLRs. R848, also known as Resiquimod, activates TLR7/8 receptors in various immune cells. R848 induces the anti-tumor response; thus, exhibiting potential as an immunotherapeutic agent. Notably, dendritic cells are actively involved in initiating and regulating immune responses, and these are stimulated by R848. However, immunotherapies are only effective in a specific TME, and R848 remodels the TME to aid in immunotherapy (4,5).

R837, also known as Imiquimod, also activates TLR7/8 receptors in various immune cells. R837 is well-established as a combination therapy with chemotherapy and photochemical therapy, and is used to improve immunotherapeutic efficacy (6).

3. Mechanisms of action

R848 and R837 are imidazoquinolines that act as agonists for TLR7 or TLR8. Their drug-receptor interaction activates immune cells and creates an environment that stimulates T helper cell (Th1) immune responses. In humans, R848 functions as an agonist for both TLR7 and TLR8; however, in mice, it acts as a preferential agonist to TLR7. R837 is a selective antagonist of TLR7 only. The Myd88-dependent signaling pathway is utilized by R848 and R837 to activate TLR7/TLR8, which in turn activates transcription factors; namely, NF-κB and JNK. Ultimately, this pathway leads to the production of Th1 cytokines, such as type I interferon (IFN I), interleukin (IL)-6, IL-12, IFN-γ and tumor necrosis factor-α (TNF-α), while suppressing the expression and secretion of Th2 cytokine, IL-4. Moreover, Th1 cytokines trigger the release of several cytokines that facilitate innate and acquired immunity, through activation of monocytes, macrophages, and plasmacytoid dendritic cells via TLR7/8. In addition, the activation of TLRs stimulates the activity of antigen-presenting cells (APCs). APCs present antigens to T-cells, thereby initiating an immune response. This, in turn, triggers tumor-associated macrophages (TAMs) that modulate the TME through activating innate immune responses. Thus, tumor growth is inhibited and the TME is disrupted via R848- and R837-mediated activation of TAMs (Fig. 1) (7).

4. Preclinical studies in cancer immunotherapy using R848 and R837

Several preclinical studies have previously been conducted to study the role and effects of R848 and R837 in cancer immunotherapy. Ye *et al* (8) evaluated the impact of intravenous R848 and stereotactic body radiation therapy (SBRT) on tumor growth and immune response in primary pancreatic tumors, using murine models of orthotopic pancreatic ductal adenocarcinoma (PDAC). Results of this previous study demonstrated that the combination of R848 and SBRT significantly activated the pancreatic TME, increased levels of IFN-γ, granzyme B and chemokine ligand 5, and decreased levels of IL-4, IL-6 and IL-10. In addition, R848 and SBRT increased tumor antigen-specific CD8+ T-cells, reduced the number of regulatory T-cells and improved antigen-presenting cell maturation (8). Moreover, Mottas *et al* (9) utilized amphiphilic polymer and monoblock peptide nanoparticles to encapsulate and deliver R848 and R837 to human and murine cancer cell lines. Results of this previous study revealed that R848 and R837 targeted blood-borne macrophages and promoted tumor regression in experimental gliomas without adaptive immunity. In a further study, nanoparticles consisting of polyglycolic acid (PLGA) combined with immunostimulant granulocyte-colony stimulating factor (ICG) were used as a carrier for R848 via near-infrared (NIR). In both human and murine cell lines for prostate cancer, a favorable immunohistochemical response was observed. The combination of nanoparticles containing cancer cell membranes derived from surgically excised cancer cells and mesoporous polydopamine carrying R848 were also studied, and the results revealed that this nano vaccine exhibited potent phagocytosis, isotype targeting and tumor-specific immune responses when exposed to NIR. In addition, this combination promoted photothermal immunotherapy at the tumor site (10). Results of a further previous study revealed that R837 in combination with hematoporphyrin monomethyl ether arrested primary tumor progression and prevented lung metastasis, when administered via biomedical ultrasound (7,11-13). Thus, the results of preclinical studies have led to the development of clinical trials that aimed to determine the efficacy and safety of R837 and R848 in healthy human volunteers.

5. Clinical trials

R837 and R848, initially explored for their potential in the treatment of herpes simplex virus (HSV), exhibited limitations in clinical trials due to insufficient efficacy and notable side effects. Despite evidence suggesting success in the treatment of acute and chronic HSV lesions, off-label usage became common (14-16). For example, R837 demonstrated potential in the treatment of vaginal or cervical human papillomavirus (HPV) lesions, and the effects were more apparent in combination with photodynamic therapy or HPV therapeutic vaccines for HPV-16-mediated vulvar intraepithelial neoplasia (17). However, significant side effects, such as severe vulval erythema, and unsuccessful trials in patients with cervical intraepithelial neoplasia were reported (18).

In a clinical trial, R837 demonstrated disease clearance rates exceeding 50%, highlighted by increased levels of immune cells in biopsied lesions. Approval from the Food and Drug Administration (FDA) was granted for the treatment of basal cell carcinoma in 2004, with high disease clearance rates observed in Phase III clinical trials (19). Notably, Th1 polarization, enhanced IFN I signaling and immune cell recruitment were observed in skin biopsies. R848 also demonstrated high levels of efficacy in cutaneous T-cell lymphoma treatment, inducing significant improvements in patients (20). Notably, clinical trials that aim to explore novel combinations and delivery strategies of R837 and R848 are ongoing (Table I).

The SINS study (trial no. NCT00066872) was a multi-centric, randomized study including 500 patients with basal skin cell cancer, who participated for 18 months. Patients were categorized depending on the presence of superficial or nodular lesions and the participating center. Patients were randomly assigned to two treatment arms, and those with superficial lesions were treated for 6 weeks, while patients with nodular lesions were treated for 12 weeks. Patients in treatment arm one received topical Imiquimod, and this was

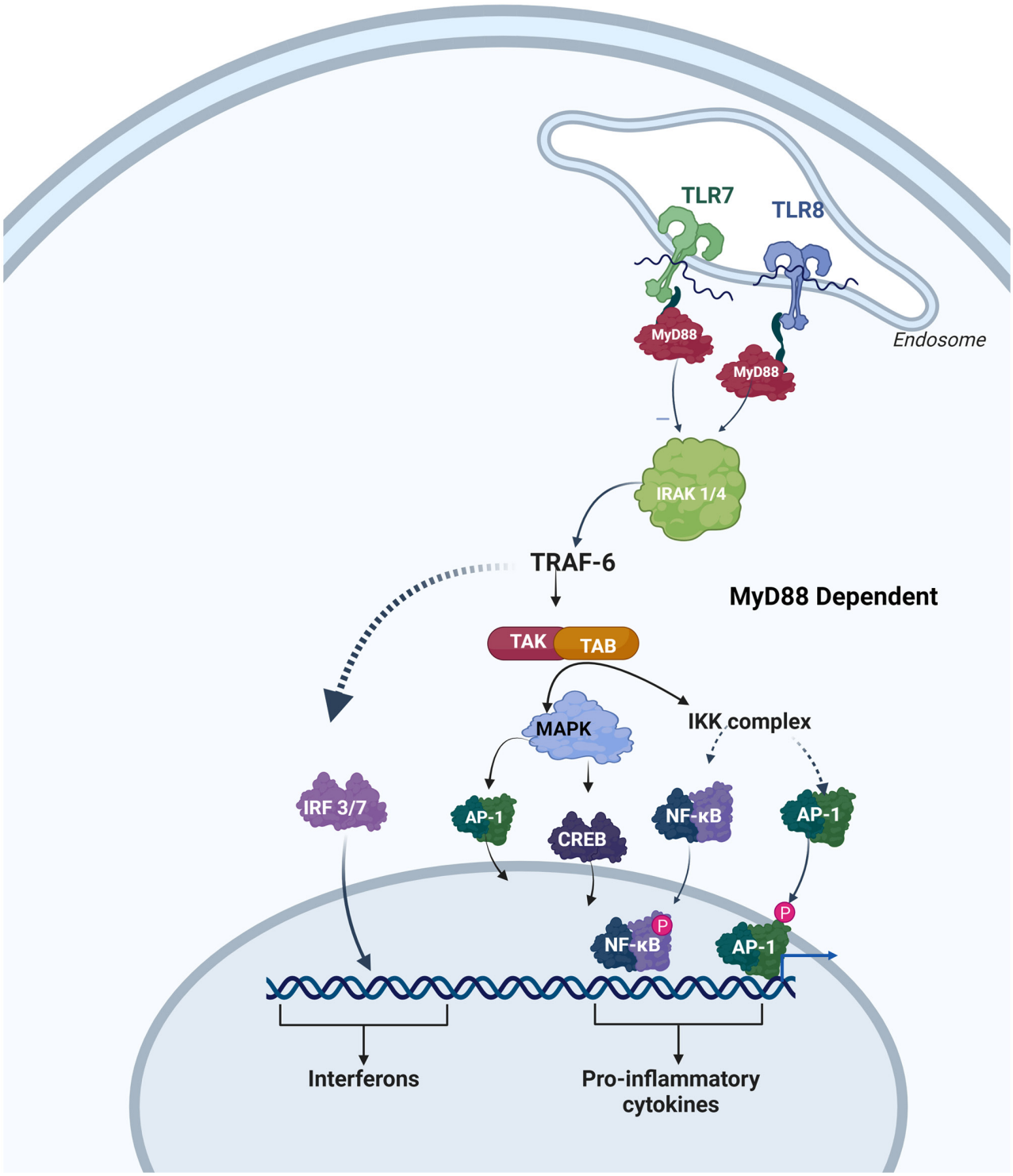

Figure 1. Mechanism of action of R848 and R837.

applied to a single lesion once daily. Surgical excision was offered to patients who exhibited recurrence or failed early treatment. Patients in treatment arm two underwent surgical excision. Follow-up was carried out at 6, 12 and 18 weeks, every 6 months for two years, and every 5 years. Results of this previous study revealed that Imiquimod was effective in ~90% of patients with large superficial basal cell carcinoma (diameter, >4 cm). Notably, Imiquimod was considered the preferred course of treatment; however, it was only marginally less effective than excisional surgery due to the associated cosmetic outcomes (21).

Females who are yet to reach menopause are primarily affected by vulval intraepithelial neoplasia, a pre-malignant disease. This condition is symptomatic and invasive, leading to challenges in clinical practice. Treatment goals include symptom relief and inhibition of disease spread. Surgery is frequently used to cure this malignant condition; however, it may be deformative and exhibits a high recurrence rate. Recent

Table I. Registered clinical trials that aimed to determine the safety and efficacy of Imiquimod and Resiquimod in the treatment of various cancers.

No.	Date of registration	Status of recruitment	Scientific title	Study type	Study design	Phase of trial	Sample size	Trial ID for reference
1	08-03-2002	Complete	Phase II topical immunomodulatory therapy with Imiquimod for the chemoprevention of recurrent and high-grade cervical intraepithelial neoplasia (CIN)	Interventional	Open label study	Phase 2	57	NCT00031759
2	06-08-2003	Complete	A randomized controlled trial of excisional surgery vs. Imiquimod 5% cream for nodular and superficial basal cell carcinoma	Interventional	Randomized, open label study	Phase 3	500	NCT00066872
3	08-03-2004	Complete	Double-blind, vehicle-controlled study to evaluate apoptosis in basal cell carcinoma treated with Aldara™ (Imiquimod) cream, 5% applied once or twice a day	Interventional	Randomized, double-blind study	Phase 1	48	NCT00079300
4	07-01-2006	Completed	Evaluation of the impact of adjuvants accompanying peptide immunization in high-risk melanoma	Interventional	Randomized, parallelly assigned, open label study	Phase 2	104	NCT00273910
5	27-03-2007	Completed	Laser and TLR-agonist immunotherapy: a novel autologous melanoma vaccine study	Interventional	Open label study	Phase 1	11	NCT00453050
6	26-07-2007	Recruiting	Three non-invasive treatment options for superficial basal cell carcinoma: PDT vs. Imiquimod vs. 5-fluorouracil-treatment of SBCC	Interventional	Single blinded masking used, uncontrolled, treatment	Phase 4	600	NL-OMON31670
7	25-09-2007	Not recruiting	A pilot study of topical imiquimod therapy for the treatment of recurrent extramammary Paget's disease	Interventional	Single allocation, open label study	N/A	8	NCT00504023
8	25-09-2007	Completed	A randomised phase II multi-centre trial of topical treatment in women with vulvar intraepithelial neoplasia (tr3-vin)	Interventional	A randomized phase II multi-center trial (treatment)	2	204	ISRCTN34420460

Table I. Continued.

No.	Date of registration	Status of recruitment	Scientific title	Study type	Study design	Phase of trial	Sample size	Trial ID for reference
9	25-06-2008	Not recruiting	Combination Therapy with Imiquimod Cream 5% and Tazarotene Cream 0.1% for the treatment of Lentigo Maligna	Interventional	Randomized, parallelly assigned, open label study	N/A	90	NCT00707174
10	01-09-2009	Recruiting	Phase I study to determining toxicity and immunity of the p53 synthetic long peptides vaccine combined with interferon-alfa in patients treated for colorectal cancer-p53-slp vaccine in combination with IFNA	Interventional	Open uncontrolled TRIAL	1	10	NL-OMON33981
11	07-11-2008	Completed	A phase I efficacy and safety study of hpv16-specific therapeutic DNA-vaccinia vaccination in combination with topical Imiquimod, in patients with hpv16+ high grade cervical dysplasia (CIN3)	Interventional	Non-randomized, parallelly assigned. open label study	Phase 1	75	NCT00788164
12	26-11-2008	Active, not recruiting	Vaccination of patients with ovarian cancer with dendritic cell/tumor fusions with GM-CSF and Imiquimod	Interventional	Randomized, parallelly assigned, open label study	Phase 2	23	NCT00799110
13	05-01-2009	Authorized	Clinical and immunological effects of Imiquimod and HPV-vaccination compared to Imiquimod alone in female patients with usual type vin	Interventional	Controlled, randomized, double blind: parallelly assigned study	Phase 2	N/A	EUCTR2008-008251-42-NL
14	13-01-2009	Completed	Phase II study of topical Imiquimod and weekly Abraxane for the treatment of breast cancer cutaneous metastases	Interventional	Single group assignment, open label STUDY	Phase 2	15	NCT00821964
15	11-05-2009	Completed	Phase II evaluation of Imiquimod, a topical toll-like receptor 7 (tlr7) agonist in breast cancer patients with chest wall recurrence or skin metastases	Interventional	Open label, single group assignment	Phase 2	10	NCT00899574

Table I. Continued.

No.	Date of registration	Status of recruitment	Scientific title	Study type	Study design	Phase of trial	Sample size	Trial ID for reference
16	01-10-2009	Not recruiting	A pilot study of pngvl4a-crt/e7 (detox) for the treatment of patients with hpv16+ cervical intraepithelial neoplasia 2/3 (cin2/3)	Interventional	Non-randomized. Intervention model, parallel assignment, open label	Phase 1	132	NCT00988559
17	28-04-2010	Not recruiting	Topical Imiquimod in treating patients with persistent HPV-infection after surgical or radiation treatment of cervical cancer-tactiq (treatment after cervical cancer with topical Imiquimod)-trial	Interventional	Controlled, randomized, open labelled, parallel assigned study	Phase 3	Na	EUCTR2010-019657-18-AT
18	09-06-2010	Not recruiting	Phase I/II study of peptide vaccination associated with tumoral immunomodulation with proinflammatory cytokines and Imiquimod in patients with advanced metastatic melanoma	Interventional	Open label, single group assignment	Phase 2	21	EUCTR2010-020435-40-BE
19	19-07-2011	Not recruiting	Imiquimod/BTIC lysate-based vaccine immunotherapy for diffuse intrinsic pontine glioma in children and young adults	Interventional	Single group assignment, open label STUDY	Phase 1	8	NCT01400672
20	17-08-2011	Not recruiting	Phase I/II study of tlr7 agonist Imiquimod, cyclophosphamide, and radiotherapy in breast cancer patients with chest wall recurrence or skin metastases	Interventional	Non-randomized, parallelly assigned, open label study	Phase 1/ phase 2	32	NCT01421017
21	30-04-2013	Authorized-recruitment may be ongoing or finished	Risk of skin cancer on skin areas treated with Ingenol mebutate gel, 0.015% and Imiquimod cream, 5%	Interventional	Randomized, open label, parallelly assigned study	Phase 4	480	EUCTR2012-003112-31-GB
22	19-08-2013	Complete	A phase 4 trial comparing the cumulative incidence of SCC after treatment with Ingenol mebutate and Imiquimod for multiple actinic keratoses on face and scalp. A multi-centre, randomised, two-arm, open label, active-controlled, parallel group, 36-month trial	Interventional	Randomized, parallelly assigned, open label study	Phase 4	485	NCT01926496

Table I. Continued.

No.	Date of registration	Status of recruitment	Scientific title	Study type	Study design	Phase of trial	Sample size	Trial ID for reference
23	28-08-2013	Complete	Phase II clinical trial of combination of personalized peptide vaccination, cyclophosphamide and Imiquimod for advanced pancreatic cancer patients who failed standard therapy-Phase II study of peptide vaccination for advanced pancreaticcancer patients	Interventional	Parallel, randomized study	Phase 2	66	JPRN-UMIN000011593
24	11-08-2014	Terminated	Phase I/II study of low- dose cyclophosphamide, tumor associated peptide antigen-pulsed dendritic cell therapyand Imiquimod, in patients with progressive and/or refractory solid malignancies	Interventional	Single group assignment, open label STUDY	Phase 1/ phase 2	3	NCT02224599
25	15-09-2014	Active: not recruiting	Surgical excision vs. combined treatment with curettage and Imiquimod for nodular basal cell carcinoma: an open, non-inferiority, randomized controlled trial	Interventional	Randomized, parallelly assigned, open label study	Phase 3	145	NCT02242929
26	10-03-2015	Completed	A randomised controlled multicentre trial of Imiquimod vs. radiotherapy for lentigo maligna (LM) when staged surgical excision with 5 mm margins is not possible, is refused, or fails	Interventional	Randomized, parallelly assigned, open label study	Phase 3	126	NCT02394132
27	20-03-2015	Active, not recruiting	A randomised controlled multicentre trial to	Interventional	Randomized, controlled trial, open	Phase 3	266	ACTRN12615000266561

Table I. Continued.

No.	Date of registration	Status of recruitment	Scientific title	Study type	Study design	Phase of trial	Sample size	Trial ID for reference
			evaluate the effect of Imiquimod vs. radiotherapy on treatment failure at 6 months in patients with lentigo maligna (LM) for which staged surgical excision with 5 mm margins is not possible, is refused, or fails		masking parallelly assigned study			
28	01-01-2016	Recruiting	Randomized trial of treatment of vaginal intraepithelial neoplasia (vain): laser vaporization and Imiquimod	Interventional	Single centric, randomized interventional trial	N/a	60	ISRCTN23349576
29	21-06-2017	Active, not recruiting	A feasibility trial of alternating intravaginal application of 5-fluorouracil and Imiquimod for treatment of high-grade cervical squamous intraepithelial lesions	Interventional	Pilot prospective study	N/a	13	NCT03196180
30	06-07-2017	Completed	Randomized clinical trial evaluating the efficacy of topical Imiquimod in high grade cervical intraepithelial lesions	Interventional	Randomized. parallelly assigned, single blinded study	Phase 2	90	NCT03233412
31	20-07-2022	Open to recruitment	Assessing effectiveness of combined therapy of antioxidant and topical 5% Imiquimod in management of oral leucoplakia-A randomised clinical trial	Interventional	Randomized, parallel group, active controlled trial	Phase 3/ phase 4	60	CTRI/2022/07/044182
32	04-03-2024	Not yet recruiting	Immunophenotyping, microbiome, clinical outcome and biomarkers for predicting immunological response in patients with high-grade cervical	Interventional	Randomized, open labelled, parallelly assigned	Phase 4	96	NCT06356012

Table I. Continued.

No.	Date of registration	Status of recruitment	Scientific title	Study type	Study design	Phase of trial	Sample size	Trial ID for reference
			intraepithelial lesions treated with Imiquimod					
33	16-09-2010	Not recruiting	A phase II clinical trial evaluating autologous dendritic cells pulsed with tumor lysate antigen +/- toll-like receptor agonists for the treatment of malignant glioma	Interventional	Randomized, parallelly assigned, open label study	Phase 2	60	NCT01204684
34	15-07-2012	Completed	A phase I/IIA, dose-ranging safety and efficacy study of topical Resiquimod for the treatment of early stage cutaneous t cell lymphoma	Interventional	Non-randomized, single group assignment, open label study	Phase 1/ phase 2	13	NCT01676831
35	29-10-2020	Not recruiting	Clinical trial exit interview study in cutaneous t-cell lymphoma (CTCL) to capture meaningful treatment benefit from a patient's perspective	Interventional	Randomized, single blinded study	Phase 3/ phase 4	30	EUCTR2020-001992-34-DE

pilot and small-scale studies using novel topical therapies with Imiquimod as a non-surgical alternative have demonstrated positive outcomes.

A randomized Phase II multi-centric clinical trial (trial no. ISRCTN34420460) included 204 patients. Patients received topical treatment of Imiquimod or Cidofovir for up to 24 weeks. Every 6 weeks, patients were reviewed and evaluated, and treatment was administered for up to 24 weeks if there was no full response. Notably, a full response was established histologically at 30 weeks following therapy initiation. In total, 156 patients (87%) followed the treatment plan for >6 weeks (78 in each group). Prior to the initial 6-week safety evaluation, 5 patients in the Imiquimod group and 7 patients in the Cidofovir group stopped responding to follow-up or withdrew from the study. Of the 84 patients assigned to the Cidofovir treatment group, 31 (37%) experienced adverse events of grade 3 or above, whereas 39 (46%) of the 84 patients assigned to the Imiquimod treatment group experienced adverse events of grade 3 or above. Notably, the most common grade 3 and 4 events were headache, pruritus, pain in the vulva and exhaustion. Further investigations into the use of Cidofovir and Imiquimod are required, as these reagents demonstrate high levels of efficacy and safety, and may exhibit potential as an alternative to surgical intervention in the treatment of vulval intraepithelial neoplasia (22).

An open, blinded, non-controlled clinical trial (trial no. NL-OMON3398) aimed to determine whether Imiquimod, granulocyte-macrophage colony-stimulating factor, IL-2 and IFN-α induce peritumoral responses when used in conjunction with a peptide vaccination. The type of human leukocyte antigen and gene expression of the tumor determined whether the MAGE-3.A1 peptide, NA17.A2 peptide or both peptide vaccines would be used. If both antigens were expressed, the patient was treated with both peptide vaccines. This combination triggers cytolytic T-cell (CTL) responses, which were evaluated through comparing the frequency of either anti-MAGE-3. A1 or anti-NA17.A2 CTLp in the pre- and post-immune blood of patients. This trial exhibited limitations, as there were a small number of subjects, and technical problems led to unreliable data. Thus, the trial was subsequently terminated (23).

NCT03196180 was an early Phase I, open-label clinical trial involving 13 patients with high-grade cervical intraepithelial neoplasia, who were treated with topical fluorouracil and Imiquimod. In patients with precancerous cervical lesions, topical fluorouracil and Imiquimod ointment may lead to positive outcomes due to fewer associated adverse effects. For the treatment of high-grade cervical squamous intraepithelial lesions, a once-weekly intravaginal application of 5-fluorouracil was used, and this was alternated with

a once-weekly application of Imiquimod. For 8-16 weeks, 5-fluorouracil and Imiquimod treatments were alternated. The median age of participants was 27±2.9 years. There were no grade 3/4 adverse events, six (46.15%) grade 2 adverse events and all individuals reported grade 1 adverse events. In total, 3 patients (23.08%) reported specific adverse events. Notably, 10 patients (90.91%) exhibited histologic regression to cervical intraepithelial neoplasia (CIN), and 7 patients (63.63%) tested negative for hr-HPV following the study. Further investigations are required for the use of topical treatments in CIN 2/3 as alternatives or adjuncts to surgical therapy (24).

The NCT01676831 Phase I clinical trial was an open-label, dose-ranging trial including patients with early-stage (IA-IIA) cutaneous T-cell lymphoma (CTCL). This study investigated the safety and efficacy of Resiquimod gel at concentrations of 0.06 and 0.03%, administered to individuals with early-stage cutaneous T-cell lymphoma lesions. In 75% of patients, considerable improvements were observed in treated lesions, and 30% of patients experienced total clearance of lesions. In addition, Resiquimod also induced the regression of untreated lesions. According to the modified Severity-Weighted Assessment Tool, 92% of patients exhibited a ≥50% improvement in body surface area involved, and 2 patients experienced full clearance of the disease. In total, 4 out of 5 patients with follicotropic disease demonstrated improvements. In addition, 90% of patients exhibited reduced clonal malignant T-cells, and 30% exhibited completely eradicated malignant T-cells, according to T-cell receptor sequencing and flow cytometry analysis of treated lesions. Elevated responses were associated with enhanced cutaneous T-cell effector functions, enhanced natural killer cell functions, and the recruitment and growth of benign T-cell clones in treated skin. Cytokines were also associated with persistent clinical inflammation in patients with malignant T-cells that had been almost completely eliminated. A systemic response to medication was associated with the enhanced activation of circulating dendritic cells in 50% of patients. Further Phase II trials using topical Resiquimod in early-stage CTCL are required (23). Diseases that may be targeted by R837 and R848 are summarized in Fig. 2.

6. Combination therapy as an advanced therapeutic approach

ICIs. Previous research has focused on the combination of the TLR7/8 agonist, R848, with ICIs in cancer cells. Hänel *et al* (24) demonstrated that the combination of R848 with poly(I: C) was more effective in eliciting an immunostimulatory response than when R848 was used alone. This combination induces the reprogramming of macrophages into M1 hot-antitumor effectors through signal transducer and activator of transcription 1 activation (9). This study included patients with lung cancer resistant to immune checkpoint blockade (ICB) (25).

TLRs. In a preclinical study using a humanized mouse model, Poly(I: C) combined with R848 increased the expression of various cytokines, compared with Poly(I: C) or R848 administered alone (26). In addition, the synergistic use of Poly(I: C) and R848 with E7 DNA promoted the regression of tumors, and increased the levels of antigen-specific IFN-γ and non-specific intertumoral IL-12 (27). Results of a previous study also demonstrated that the combination of TLR agonists with prostaglandins and TLR3 Poly(I: C)/R848/PGE2 aided dendritic cells in the mobilization and secretion of cytokines, including IL-12 (p70) (28).

In a murine model, treatment with biocompatible anchors for the membrane (BAM), R848, Poly(I: C) and lipoteichoic acid led to an 83% eradication of advanced progressive melanoma and reduced tumor recurrence, when in contact with the surface of tumor cells (29).

Chemotherapy and radiotherapy. TLR7/8 agonist, R848, plays a crucial role in the action of oxaliplatin. Oxaliplatin is a chemotherapeutic drug that promotes the maturation of myeloid-derived suppressor cells (MDSCs) into M1-like macrophages, which play key roles in various immune reactions (30). A combination of Resiquimod with chemotherapeutic agents exhibits potential in the treatment of cancer, as dosage can be altered depending on patient outcomes. At present, research is focused on the use of chemotherapeutic drugs in combination with TLR-mediated immunotherapy (31). Notably, reduced tumor growth was observed following treatment with R837 in combination with γ-ionizing radiation (IR). This combination of treatment increased autophagy and the number of CD8+ T-cells, and decreased Tregs and MDSCs (31).

Adjuvant therapy. In the presence of R837-containing nanoparticles as adjuvants, PLGA-ICG-R837 nanoparticles function as vaccines. PLGA-ICG-R837 nanoparticles may trigger NIR laser-induced photothermal ablation of primary tumors and tumor-associated antigens. In mice, PLGA-ICG-R837 nanoparticles target tumor cells; thus, limiting metastasis. This strategy led to the establishment of a robust immune memory effect, potentially protecting against tumor recurrence following initial elimination (32).

Dual-functional nanoparticles combining partial thromboplastin time and immunotherapy components (PLGA-ICG-R848 NPs) promoted the maturation of dendritic cells, as evidenced by increased proportions of CD11c+CD86+ and CD11c+CD80+ cells. This strategy exhibits potential for improving the immune response against tumor cells (33).

TLR7/8 agonist-containing nanoparticles have also been developed for systemic administration. Results of a previous study described semiconducting polymer nano adjuvant (SPNIIR) for second near-infrared (NIR-II) photothermal immunotherapy. When exposed to NIR-II light, SPNIIR generated heat to erode tumors, induced immunogenic cell death and released R848, enhancing the maturation of dendritic cells. This approach effectively inhibited the growth of primary and distant tumors, and eliminated lung metastasis in a murine tumor model (34).

Zhang *et al* (35) explored intravenous TLR7/8 agonist administration using thermosensitive liposomes (TSLs) to enhance CD8+ T-cell infiltration. Mice treated with R848-loaded TSLs combined with local hyperthermia and αPD-1 exhibited a 3-fold increase in median survival, compared with controls. Mice treated with R848-TSLs and αPD-1 developed immunity to NDL cells, and remained tumor-free when NDL tumors were reintroduced. Results of a previous study

Figure 2. Imiquimod (R837) and Resiquimod (R848) target numerous diseases.

demonstrated that polydopamine-based PDA-PEG-R848-CD nanoparticles impacted 4T1 breast tumors under NIR laser irradiation, generated tumor-associated antigens and significantly improved the efficacy of PD-L1 checkpoint blockade therapy, activating innate and adaptive immune systems *in vivo* (36,37).

7. Limitations of R837 and R848

Applications of R837 and R848 may be limited due to low levels of efficacy and associated levels of toxicity in cells. R837 and R848 may exhibit potential in promoting immune responses; however, this may lead to the development of specific antibodies. Notably, this process is known as an antibody-drug reaction (ADR). ADRs may reduce the efficacy of the drug and cause undesirable adverse effects. Thus, to moderate the risk of ADRs, further *in vitro* investigations are required to establish and eliminate immunogenic epitopes (38).

Results of previous studies have demonstrated the potential of these drugs as cancer therapies. However, systemic administration of these TLR7/8 agonists remains challenging. Although TLR7 tolerance has been previously demonstrated, the tolerance induction mechanism remains unclear. Thus, further investigations are required to determine the efficacy of R837 and R848 in cancer. In addition, the association between TLR expression, agonist activity and circadian rhythm are yet to be explored in the context of TLR7/8 agonism.

TLRs play a complex, multifaceted role in the TME. Immune cell-expressed TLRs may support immunosurveillance through promoting and activating both innate and adaptive immune effectors. However, myeloid-derived

suppressor cells are recruited by TLR7 expressed by malignant cells; thus, promoting the growth and spread of tumors. The pharmacokinetic characteristics of the small-molecule adjuvants cause substantial systemic inflammation, which may impact their use in clinical practice. However, results of a previous study revealed that the covalent attachment of TLR7/8 agonists to polymer scaffolds may modify the associated safety profile and increase levels of clearance. When encapsulated in nanoparticles, R837 promoted the development of DCs. However, this previous study did not investigate drug reactions or ADRs (39,40).

Moreover, TLRs produced by tumor cells may interact with stimuli to promote the growth of tumors. Imidazoquinolines-based small molecules stimulate immune responses; however, their pharmacokinetic profile leads to systemic inflammation, ultimately impacting their use in clinical practice. Tumor immunotherapy exhibits limitations in practice due to the absence of biomarkers. Existing biomarkers, such as microsatellite instability, PD-L1, genetic mutations, and tumor mutational burden also exhibit limitations, and these are impacted by the type of cancer and cancer heterogeneity (41). The aforementioned biomarkers may not be present in all patients; thus, further investigations are required to establish reliable biomarkers of tumor immunotherapy (42). Integrative biomarker frameworks consolidating safety profiling, clinical history and prescient calculations may aid in the development of effective treatment options (10).

Patient selection and stratification are crucial in clinical trials. Notably, patients may be grouped according to age, cancer type and stage. Clinical trials including patients that are well-suited to treatments may lead to the development of customized treatment plans, and further demonstrate the integrity of the trial (43).

Clinical trials involving R848 and R837 should focus on stratifying patients according to cancer type, cancer stage, patient well-being and the presence of any comorbid conditions (44). Patients may also be stratified according to the presence of specific biomarkers, medical history or previous adverse events that may impact treatment outcomes (45).

8. Future directions of the R848 and R837 immunotherapy strategy

Drug delivery systems, such as nanoparticles or liposomes, may be optimized to enhance the targeted delivery of R848 and R837, and to control off-target effects. Administering a sustainable release system of drugs may improve therapeutic outcomes via the reduction in the frequency of treatments and improved convenience for patients. In addition, these factors may enhance compliance within immunotherapeutic practice. Developing systems that are FDA-approved will also lead to increased uptake in clinical practice. At present, systems that use FDA-approved lipids to deliver mRNA to dendritic cells are ongoing in clinical trials (46,47). Immunotherapies specific to patient profiles may be managed through biomarker-driven approaches. These approaches include genetic profiling and immune system analysis. In addition, personalized cancer vaccines may be established via neoantigen identification advancements. Numerous immunotherapeutic approaches, such as ICIs, agonistic antibodies, cancer vaccines, lymphocyte-activating cytokines, CAR T-cells and bispecific antibodies exhibit potential in the treatment of cancer (48). R848 and R837 may also exhibit potential as an immunotherapy, with precise specifications and a targeted approach. Moreover, the combination of R848 and R837 with chemotherapy, radiation therapy, nanoparticle-mediated immunoadjuvant therapy or phototherapy may exhibit potential in the treatment of cancer (6,49).

In the preliminary stage of malignancy, the TME is more susceptible to treatment, and cancer cells may not develop immunosuppressive mechanisms. Thus, introducing agonists in the early stages of disease may lead to earlier activation of immune cells, such as dendritic cells, and the initiation of an adaptive immune response. Notably, tumors may be recognized by immune cells during remission, demonstrating the potential of TLR agonists in the early stages of disease (50,51).

9. Conclusions

Numerous previous studies have focused on TLR7/8 signaling in immune cells and progenitor cells, and demonstrated that agonists exhibit high levels of efficacy in tumor cells, in which TLR7/8 signaling either promotes or inhibits tumor growth.

R848 and R837 are considered innate immunity activators that target tumor growth and metastases, and these exhibit potential as a novel therapeutic option for the treatment of cancer. Clinical trials focused on the safety and efficacy of these molecules are ongoing. Notably, Imiquimod and Resiquimod exhibit increased levels of anti-tumor activity when used in combination therapies; thus, R848 and R837 may exhibit improved outcomes when used in combination. Therapeutic drug delivery using nanoparticles may be limited due to the off-target effects of R848 and R837, and improvements in targeted delivery and control are required. In conclusion, further investigations into drug delivery, personalized approaches and tumor resistance are required for the development of R848 and R837 as an immunotherapy.

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Authors' contributions

KB was responsible for study design and writing the manuscript. MA was responsible for editing the manuscript. MW and AM was responsible for final revision of the manuscript. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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