WILEY

Janssen-Sponsored Satellite Symposium at the 30th EADV Virtual Congress 2021

The art of joint forces: crafting psoriatic arthritis care for dermatologists

This virtual satellite symposium will focus on the necessity for practicing dermatologists to understand the burden of psoriatic arthritis in patients with psoriasis. It will emphasize how important it is that dermatologists detect early signals of psoriatic arthritis in patients with psoriasis and also understand why targeting IL-23 directly can be effective in treating and potentially also preventing the development of psoriatic arthritis for their psoriasis patients

Janssen Immunology

PHARMACEUTICAL COMPANIES OF Johnson & Johnson

CP-255722

Received: 13 August 2021 | Revised: 1 October 2021 | Accepted: 17 October 2021

DOI: 10.1111/exd.14479

REVIEW ARTICLE

Experimental Dermatology WILEY

Methodological shortcomings in the reports of the imiquimod psoriatic model

M. Pilar Vinardell

Department of Biochemistry and Physiology, Faculty of Pharmacy and Food Sciences, Universitat de Barcelona, Barcelona, Spain

Correspondence
Maria P. Vinardell, Dep Biochemistry and Physiology, Faculty of Pharmacy and Food Sciences, Universitat de Barcelona, Av. Joan XXIII 27-31, 08028 Barcelona (Spain).
Email: mpvinardellmh@ub.edu

Abstract

Psoriasis is a chronic inflammatory skin disease affecting about 2–3% of the worldwide population. More of the knowledge of psoriasis is due to the in vitro and in vivo models tried to reproduce the disease and to know the mechanisms of pathogenesis, as well as to develop new therapies. One of the more simple, cheap and more used models is the imiquimod model based on the application of imiquimod in the depilated skin of mice. Several studies describing the methodology employed to develop an animal disease model does not present all the details about the model more especially related to the use of one sex or another and other methodological aspects that are relevant for researchers and to consider the accuracy of the study. In this review, we have selected 100 papers published in the last five years using the imiquimod psoriatic model recording different data such as animal, strain, sex, dose of imiquimod, area of administration, housing information, anaesthesia/euthanasia information, number of animals per group and control details among others. Our results revealed several methodological shortcomings in the models of imiquimod to study psoriasis namely sex bias and discrepancies in dose applied or time of imiquimod application among others. As long as these discrepancies exist in animal methodologies, there will be a poor translation from animal studies to clinical applications in dermatology and in other areas.

KEYWORDS
methodology, mice, preclinical research, skin disease, translation

1 | INTRODUCTION

1.1 | Psoriasis

Psoriasis is a chronic inflammatory skin disease affecting about 2–3% of the worldwide population with lower prevalence in Asian and some African populations and up to 11% in Caucasian and Scandinavian populations.[1] Psoriasis is a multifactorial disease with different factors such as genetic factors, alterations in the immune system, and environmental conditions among others and characterized by infiltration of immune cells, epidermal hyperproliferation, and abnormal keratinocyte differentiation.[2] There are different types of psoriatic manifestations, the most common form corresponds to plaques, typically has raised red or white scaly skin lesions with a thickened epidermis and covering large areas of the skin.[3] The presence of vascular engorgement and alteration on epidermal cell cycles are characteristics of patients with psoriasis. Such changes may be related to the various inflammatory cytokines released in

the inflammatory process, such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and interleukin-17 (IL-17). The alteration on the differentiation of psoriatic keratinocytes presents an upregulation of the early differentiation markers such as involucrin, keratin 6, keratin 16 among others and downregulation of the late keratinocyte differentiation markers such as filaggrin, loricrin, and caspase-14.[4] More of the knowledge of psoriasis is due to the in vitro and in vivo models tried to reproduce the disease and to know the mechanisms of pathogenesis, as well to develop new therapies.

1.2 | Characteristics of the psoriasis animal models

There are different reviews analysing the currently available murine models.[5–7] Table 1 shows the most useful animal models of psoriasis with their respective advantages and disadvantages. Among the different *in vivo* models, ones are spontaneous mutant models developed many years ago with poor application for psoriasis. Genetically engineered mouse models of psoriasis as the Stat3C transgenic animal model based on epithelial growth factors genes affecting keratinocytes and promoting epidermal growth and differentiation, inducing hyperplasia of the keratinocytes o acanthosis, but not including the immune component of the disease. Other models such as K14-VEGF transgenic mouse model develops a psoriasiform inflammatory skin with infiltration of lymphocytes or with hyperplastic and inflamed dermal blood vessels. All these models are based in only one gene and considering the complexity of this disease they are not good models because psoriasis involves more genes.[8]

Xenotransplantation models have been developed because psoriasis is a human disease that usually does not occur in animals and then it is necessary to humanized immunodeficient mouse with skin from patients. But the characteristics of the mouse transplanted skin are different to the patient's skin.[9] These xenotransplantation models are useful to study the role of T cells on the pathogenesis of psoriasis and for drug discovery.[6]

There are models based on cytokine injections such as the intradermal injection of IL-23 that stimulates the production of other cytokines such as IL-19 and IL-24, which affects keratinocyte differentiation and proliferation inducing some changes similar to psoriasis with acanthosis, parakeratosis, and dermal inflammatory infiltration of immune cells.[10] The injection of IL-21 into the mouse skin induces epidermal hyperplasia, parakeratosis, and the accumulation of T cells in the skin.[11]

Nevertheless, the number of models of psoriasis developed in the last years, these models do not reproduce the human psoriatic phenotype due to the differences in architecture of mouse and human skin.

The continuous topical application of Aldara® cream with 5% imiquimod (IMQ) induced psoriatic lesions on patients using it for the treatment of human papillomavirus. This finding stimulates the use of Aldara to induce the disease in mice.[12] Balb/c mice receiving Aldara® cream at a dose of 62.5 mg daily for 5–7 days on the skin of the back presented skin lesions similar to the psoriatic lesions. IMQ is a Toll-like receptor 7 ligand and a potent immune activator for macrophages, monocytes, and DCs. It contributes to strong activation of the immune system. Imiquimod induces in mice the epidermal expression of different interleukins such as IL-17A, IL-17F, and IL-23 and histopathological alterations similar to humans.[13]

Since 2009, the IMQ-induced mouse model has become the most used mouse model in preclinical psoriasis studies.[14] This model has several advantages, such it is cheap and very easy to use by the application of a commercially available cream on the depilated back or ears of mice. The more used strain are BALB/c and C57Bl/6 mice. But the model has also some disadvantages such as it is an acute skin inflammation in mice and not a chronic one as in human, the inflammatory and immune responses are not limited to the skin. Moreover, mice scratch the area of application inducing excoriation of the epidermis, the application for periods of two weeks can cause death and loos of body weight in shorter periods. The model does not present in mice other alterations observed in humans such as arthritis and cardiovascular disease.[15] Another disadvantage of this model is dependence on the mice strain, being C57Bl/6 mice, the best providing a better background than other strains for modelling psoriasis disease mechanisms.[16] Moreover, imiquimod is also used to develop other skin diseases such as lupus erythemathosus.[17]

In conclusion, in spite of its limitations, the imiquimod induced psoriasis-like model in mice represents an adequate model to study psoriasis and its treatment. In the last five years more than the half of the animal models of psoriasis correspond to the imiquimod model. For this reason, we consider this model for our review.

TABLE 1 Advantages and disadvantages of the different psoriatic animal models

Psoriatic animal model	Advantages	Disadvantages
Spontaneous models	Keratinocyte hyperproliferation	Multiorgan inflammation Poor responses to anti-psoriatic drugs
Genetically engineered mouse	Gives information about the genes involved in psoriasis	Usually involves only one gene does not represent the full phenotype of psoriasis
Xenotransplantation model	Human skin and immune cells transplanted Useful for drug discovery	Limited to early stages of psoriasis
Cytokine injection	Easy methodology	Induce other skin disease such as atopic dermatitis
Imiquimod model	Cheap method Very easy to use	Do not mimic the chronic inflammation of psoriasis Comorbidities of psoriasis are not presented Systemic inflammation

Several studies describing the methodology employed to develop an animal disease model do not present all the details about the model more especially related to the use of one sex or another and other methodological aspects that are relevant for researchers and to consider the accuracy of the study. In more cases, there was poor information which difficult the reproduction of the methods or findings. The objective of the present review is to analyse the shortcoming in the methodology described in different research papers using the psoriatic Imiquimod model.

2 | LITERATURE SEARCH

A careful literature search was performed to find publications using the imiquimod model of psoriasis. An online search was conducted on PubMed for studies published in English in the last 5 years using the following search term: animal model and psoriasis and imiquimod. First, we chose the open access articles and completed up to a hundred with articles that did not have free access.

The inclusion criteria were: (a) experimental psoriasis induced in rodents (ie rats or mice), (b) imiquimod and, (c) studies published in English. The exclusion criteria were: (a) not an original paper (eg review, letter, opinion, etc.), (b) studies in humans, and (c) other models (Figure 1).

From the 100 publications selected we recorded the following data: animal, strain, sex, dose of imiquimod, area of administration, housing information, anaesthesia/euthanasia information, number of animals per group and control details. A detailed study of these data has been done calculating the percentage of articles describing the different items.

3 | RESULTS AND DISCUSSION

From the 100 publications selected the majority used mouse and only 2 used rats as experimental animal.

The mice strains more used in the imiquimod model are BALBc the first strain used in the development of the model and C57BL/6J. However, authors have also used knockout mice to study the effect of determined gens in the pathology of psoriasis.

The more significant results are present in Figure 2 (subsections a–d). In relation to the sex of animals 38% of the experiments were done in males, 32% in females and 9% in both sexes (Figure 2A). Curiously 21% of the studies did not inform about the sex of the animals used and only says mice with some details about the weight of the animals.[18,19]

The information about the number of animals in each group not is always described in material and methods and in some cases, it is necessary to extract the information from the tables and/or the figures presented in results. Similarly occurs with some important information such as the use of controls. 12% of the publications did not give information about the use of controls. Some papers used Vaseline, excipient of vehicle as control. In other cases, controls were untreated animals and, in some papers, authors indicated that they used controls but no details about them were included.

The area of application of the imiquimod is usually the back or the ears. 64% of the studies applied the imiquimod only on the back after shaving, 10% only on the ears and 26% on both areas (Figure 2B). There is also one study which did not describe the area of application. There are also discrepancies in the dose of imiquimod applied and the duration of the treatment. The majority of the studies use 62.5 mg of imiquimod as was described in the original paper,[12] but there are also studies with less doses such as 45 or 50 mg (Figure 2C). 30% applied imiquimod for 7 days, 25% for 6 days, and 20% 5 days. The rest of the studies vary the time of application from the less 3 days to a longer period of 16 days (Figure 2D). That shows the great variability in the protocol used by different researchers. These differences in dose and in time of application difficult comparation between studies.

There was poor information about the use of anaesthesia and euthanasia. In this sense, only 5 papers inform about the use of different anaesthetics such as isoflurane, sevoflurane, ketamine and zylazine and sodium pentobarbital. 16 papers explained the method of euthanasia and it was consistent on CO_2, cervical dislocation, cervical dislocation previous anaesthesia and excess of different anaesthetics. One article says that they have humanely euthanized the animals with no more information.

Sixty-four per cent of the studies did not give information about the housing conditions of the animals and in some cases the information is limited to poor sentences such as experiments were conducted in standard laboratory conditions of temperature and humidity.

Only four of the 100 studies analysed indicated that they followed the ARRIVE guideline.[20-23] However, irrespective of the indication that they followed the ARRIVE guideline, one did not give information about the sex of the animals and another one has no data about the housing conditions of the animals. By the other hand there are studies very well detailed but no indication about the following of the guideline.

The ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments) present a checklist of the basic information that should be included in publications describing animal research (https://arriveguidelines.org/). The guidelines ensure that studies are reported in enough detail. Scientific publications reporting animal research often lack important information, limiting their utility. Reporting animal research in adherence with the ARRIVE guidelines 2.0 ensures transparency. This enables readers and reviewers to examine the research adequately, evaluate its methodological rigour, and reproduce the methods or findings. The initiative for the ARRIVE guideline was from the conclusion of a previous review of more than 250 articles from different journals in which authors found poor information about the number of animals used in the methodology or in the results.[24] The first guideline was published in 2010[25] and from then there have been different updates, the last in 2020.[26]

To avoid animal suffering in experiments before them are conducted the protocol should be reviewed by animal ethics committees

at institutional or national levels and the principle of the 3Rs (replacement, reduction and refinement) should be followed. These committees give an authorization number for each protocol. In the present review, near all the articles have sentences related to the approval by their respective Ethical Committees but only 33% of the articles included the number of the authorization from the Ethical Committee.

Our results revealed several methodological shortcomings in the models of imiquimod to study psoriasis. Similar conclusion has been detected in other studies in other areas of animal research.[27–29] Poor methodological quality may weaken study validity and may have contributed to the poor translation of animal research to the clinical setting.[30]

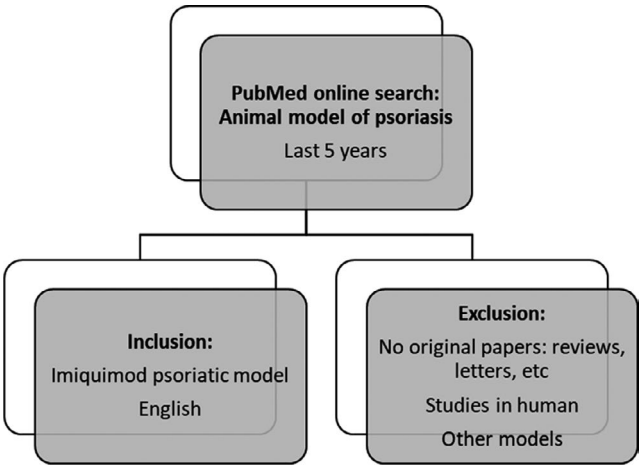

FIGURE 1 Diagram of articles selection

Scientific and ethical reasons mandate that animal experiments be appropriately designed, properly executed, and transparently reported. All essential methodological and ethical information must be included in scientific publications to improve quality of research, public perception of animal research, and safety of humans in translational research.

In conclusion, our results highlight the need for further efforts to improve methodological and ethical quality of animal research publications. Researchers should improve experimental design it properly to guarantee the effectivity of the research and no performing animals' experimentation without enough accuracy and this begin by reporting all the methodology with enough detail which allows other researchers to reproduce the same methodology and specially for comparative purposes. As long as these discrepancies exist in animal methodologies, there will be a poor translation form animal studies to clinical applications in dermatology and in other areas. Then the efforts of all those involved, researchers, editors, reviewers, etc. is needed to ensure that the information presented in the studies involving experimental animals is accurate and contrasted to achieve a correct clinical translation.

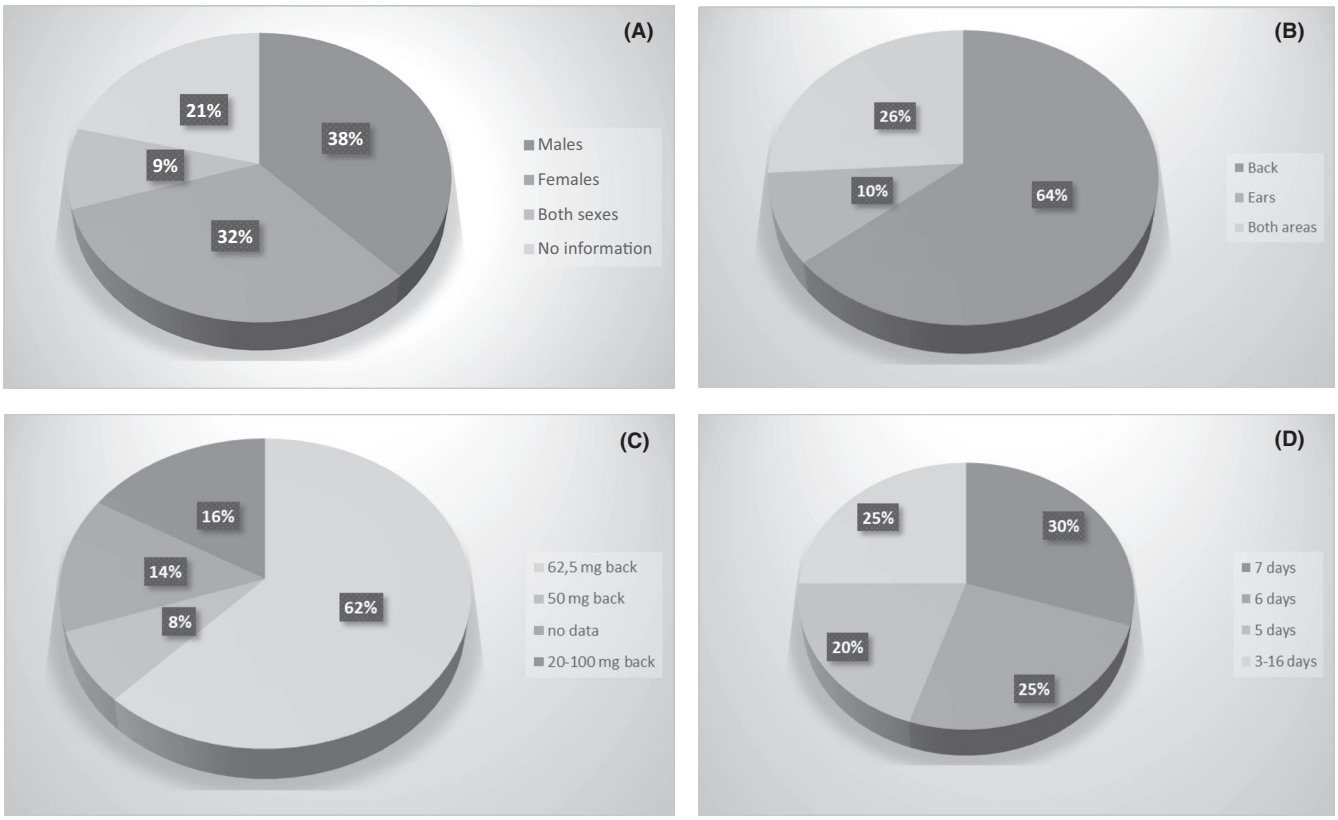

FIGURE 2 Information reported in the studies analysed: Sex distribution (a); Imiquimod's application areas (b); doses of imiquimod applied to the mice (c); days of application of imiquimod (d)

ACKNOWLEDGEMENT

The author is thankful to "Serveis Lingüístics" from Universitat de Barcelona for language support and English correction.

CONFLICT OF INTEREST

There is no conflict of interest.

AUTHOR CONTRIBUTION

Maria P. Vinardell designed the research study performed the research analysed the data and wrote the paper.

ORCID

M. Pilar Vinardell https://orcid.org/0000-0003-4533-5114

REFERENCES

1. Parisi R, Symmons DP, Griffiths CE, Ashcroft DM. Global epidemiology of psoriasis: A systematic review of incidence and prevalence. *J Investig Dermatol*. 2013;133:377-385.
2. Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. *Annu Rev Immunol*. 2014;32:227-255.
3. Rendon A, Schäkel K. Psoriasis pathogenesis and treatment. *Int J Mol Sci*. 2019;20(6):1475.
4. Bocheńska K, Smolińska E, Moskot M, Jakóbkiewicz-Banecka J, Gabig-Cimińska M. Models in the research process of psoriasis. *Int J Mol Sci*. 2017;18(12):2514.
5. Guerrero-Aspizua S, Carretero M, Conti CJ, Del Río M. The importance of immunity in the development of reliable animal models for psoriasis and atopic dermatitis. *Immunol Cell Biol*. 2020;98(8):626-638.
6. Chuang SY, Lin CH, Sung CT, Fang JY. Murine models of psoriasis and their usefulness for drug discovery. *Expert Opin Drug Discov*. 2018;13(6):551-562.
7. Schön MP, Manzke V, Erpenbeck L. Animal models of psoriasis-highlights and drawbacks. *J Allergy Clin Immunol*. 2021;147(2):439-455.
8. Gao J, Sun L, Zhang X. The genetic progress of psoriasis in the Han Chinese population. *J Invest Dermatol Symp Proc*. 2015;17(1):46-47.
9. Gudjonsson JE, Johnston A, Dyson M, Valdimarsson H, Elder JT. Mouse models of psoriasis. *J Invest Dermatol*. 2007;127(6):1292-1308.
10. Chan JR, Blumenschein W, Murphy E, et al. IL-23 stimulates epidermal hyperplasia via TNF and IL-20R2-dependent mechanisms with implications for psoriasis pathogenesis. *J Exp Med*. 2006;203(12):2577-2587.
11. Zheng Y, Danilenko DM, Valdez P, et al. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature*. 2007;445(7128):648-651.
12. van der Fits L, Mourits S, Voerman JS, et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J Immunol*. 2009;182:5836-5845.
13. Terhorst D, Chelbi R, Wohn C, et al. Dynamics and transcriptomics of skin dendritic cells and macrophages in an imiquimod-induced, biphasic mouse model of psoriasis. *J Immunol*. 2015;195(10):4953-4961.
14. Lin YK, Yang SH, Chen CC, Kao HC, Fang JY. Using imiquimod-induced psoriasis-like skin as a model to measure the skin penetration of antipsoriatic drugs. *PLoS One*. 2015;10(9):e0137890.
15. Hawkes JE, Gudjonsson JE, Ward NL. The snowballing literature on imiquimod-induced skin inflammation in mice: A critical appraisal. *J Invest Dermatol*. 2017;137(3):546-549.
16. Swindell WR, Michaels KA, Sutter AJ, et al. Imiquimod has strain-dependent effects in mice and does not uniquely model human psoriasis. *Genome Med*. 2017;9(1):24.
17. Yokogawa M, Takaishi M, Nakajima K, et al. Epicutaneous application of toll-like receptor 7 agonists leads to systemic autoimmunity in wild-type mice: a new model of systemic Lupus erythematosus. *Arthritis Rheumatol*. 2014;66(3):694-706.
18. Kodji X, Arkless KL, Kee Z, et al. Sensory nerves mediate spontaneous behaviors in addition to inflammation in a murine model of psoriasis. *FASEB J*. 2019;33(2):1578-1594.
19. Luan C, Chen X, Zhu Y, et al. Potentiation of psoriasis-like inflammation by PCSK9. *J Invest Dermatol*. 2019;139(4):859-867.
20. Ju N, Shimamura M, Hayashi H, et al. Preventative effects of the partial RANKL peptide MHP1-AcN in a mouse model of imiquimod-induced psoriasis. *Sci Rep*. 2019;9(1):15434.
21. Duan X, Liu X, Liu N, et al. Inhibition of keratinocyte necroptosis mediated by RIPK1/RIPK3/MLKL provides a protective effect against psoriatic inflammation. *Cell Death Dis*. 2020;11(2):134.
22. Irrera N, Bitto A, Vaccaro M, et al. PDRN, a bioactive natural compound, ameliorates imiquimod-induced psoriasis through NF-κB pathway inhibition and Wnt/β-catenin signaling modulation. *Int J Mol Sci*. 2020;21(4):1215.
23. Wang X, Liu X, Liu N, Chen H. Prediction of crucial epigenetically-associated, differentially expressed genes by integrated bioinformatics analysis and the identification of S100A9 as a novel biomarker in psoriasis. *Int J Mol Med*. 2020;45(1):93-102.
24. Kilkenny C, Parsons N, Kadyszewski E, et al. Survey of the quality of experimental design, statistical analysis and reporting of research using animals. *PLoS One*. 2009;4:e7824.
25. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol*. 2020;8(6):e1000412.
26. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biol*. 2020;18(7):e3000410.
27. Yoon SJ, Yoon DY, Cho YK, et al. Characteristics and quality of published animal research in the field of radiology. *Acta Radiol*. 2017;58(6):685-691.
28. Tihanyi DK, Szijarto A, Fülöp A, et al. Systematic review on characteristics and reporting quality of animal studies in liver regeneration triggered by portal vein occlusion and associating liver partition and portal vein ligation for staged hepatectomy: adherence to the ARRIVE Guidelines. *J Surg Res*. 2019;235:578-590.
29. Faggion CM Jr, Aranda L, Diaz KT, Shih MC, Tu YK, Alarcón MA. The quality of reporting of measures of precision in animal experiments in implant dentistry: a methodological study. *Int J Oral Maxillofac Implants*. 2016;31(6):1312-1319.
30. van der Worp HB, Howells DW, Sena ES, et al. Can animal models of disease reliably inform human studies? *PLoS Medicine* 2010;7(3):e1000245.

How to cite this article: Vinardell MP. Methodological shortcomings in the reports of the imiquimod psoriatic model. *Exp Dermatol*. 2021;00:1–5. doi:10.1111/exd.14479