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Conflict of interest: NA

Novel haematopoietic prostaglandin D2 synthase inhibitor, CLS122, alleviated clinically relevant symptoms in an atopic dermatitis-like mouse model

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Learning Objectives: To evaluate the therapeutic effect of novel haematopoietic prostaglandin D2 synthase (HPGDS) inhibitor, CLS122, within inflammatory *in vitro* assays and the preclinical AD model, 2,4-dinitrochlorbenzene (DNCB) challenged mice.

Takeaway Message: Targeted reduction of prostaglandin D2 by upstream HPGDS inhibition shows reduced inflammatory gene expression and offers significant symptom alleviation in a DNCB-challenged mouse model.

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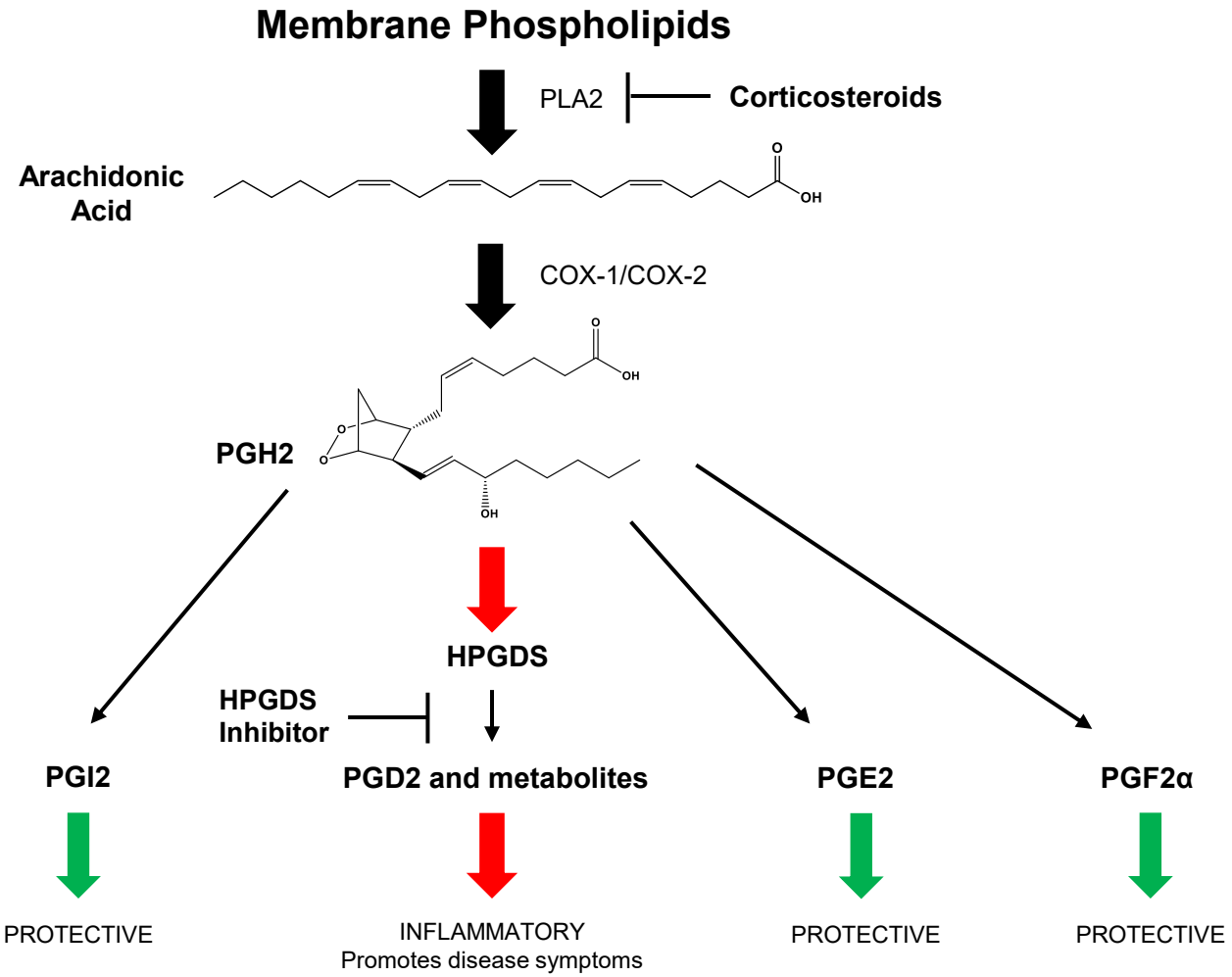
Background

- Inflammation is a key driver of atopic dermatitis (AD), characterised by the infiltration of immune cells like eosinophils and mast cells that produce prostaglandin synthase (HPGDS) and its product, PGD2.

- Corticosteroids, though standard of care, come with side effects and block downstream beneficial prostaglandins (PGs).

- HPGDS inhibition is a clinically validated pathway that offers precision targeting, blocking inflammatory PGD2 while preserving pro-resolution mediators.
  - TAS-205 (Taiho Pharma) is an HPGDS inhibitor currently in Phase 3 clinical trials for another indication

TAIHO PHARMA



Methods

In vitro potency determination

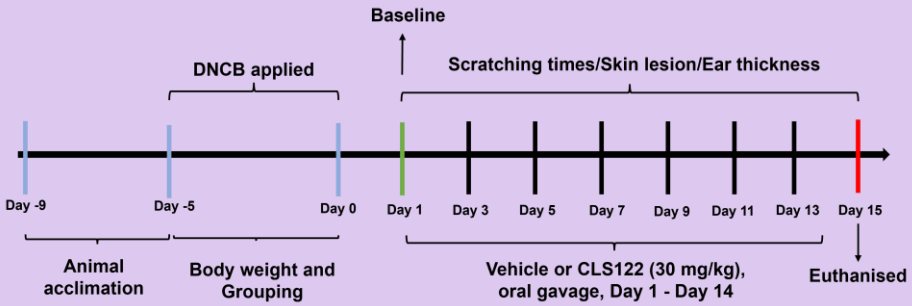
Purified enzyme (fluorescence polarisation, Cayman Chemical) and cell-based (LPS stimulated RAW 264.7) potency was determined against clinically relevant HPGDS inhibitor, TAS-205 (Taiho Pharmaceutical).

Inflammatory gene expression

qPCR evaluated reduced proinflammatory gene expression in LPS-stimulated RAW 264.7 macrophages using SYBR green reporter.

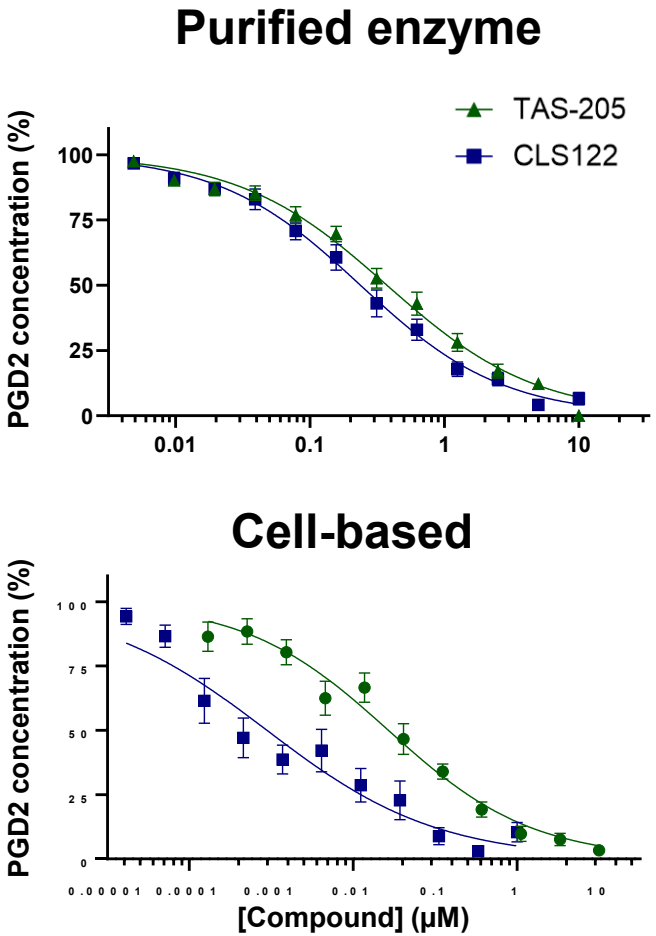
In vivo symptom alleviation

CD-1 female mice (sham, vehicle, CLS122 *n*=8/group) DNCB dissolved in acetone-olive oil (4:1); 1% DNCB (100 uL) topically applied to shaved dorsal skin, neck, and ears for CLS122 and vehicle groups.



CLS122 demonstrated increased potency against TAS-205

A

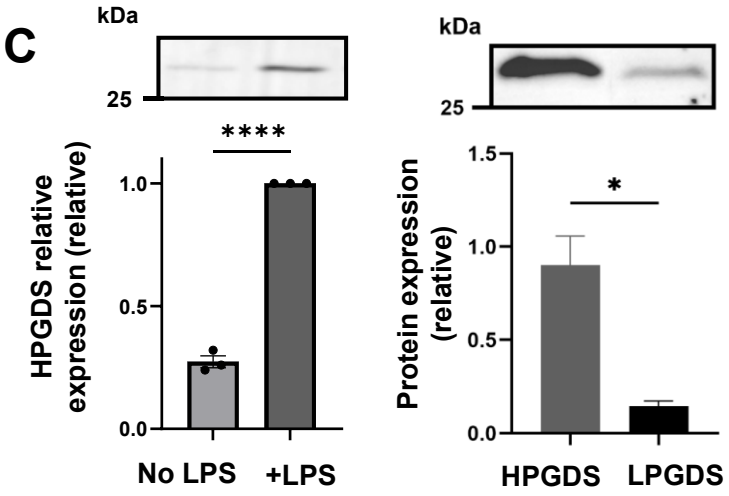


B

HPGDS inhibitor	PE IC50 (μM) ± SEM	Cell (nM) ± SEM
CLS122	0.26 ± 0.06	2.46 ± 3.60
TAS-205	0.41 ± 0.09	28.34 ± 4.71

11-fold greater potency

C



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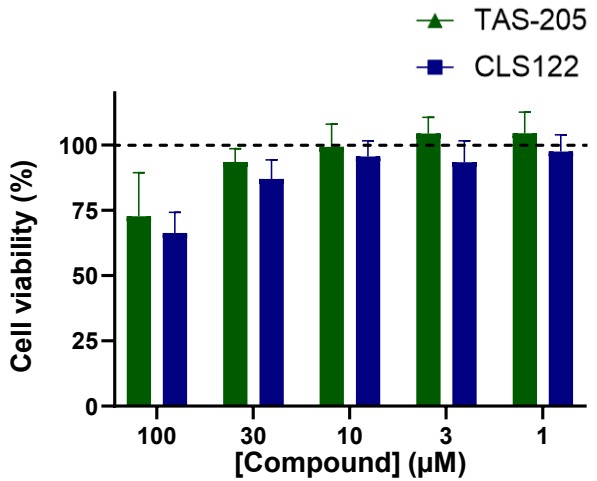


Figure 1: (**A**) Purified enzyme (fluorescence polarisation) and cell-based (ELISA; LPS-stimulated RAW 264.7 cells) potency assays. (**B**) IC50 values for purified enzyme and cell potency assays. (**C**) Quantification of HPGDS in LPS and no LPS RAW 264.7 cells, as well as LPGDS vs HPGDS relative protein expression in LPS-stimulated RAW 264.7 cells via Western Blot. (**D**) cell viability (%) after treatment with varying concentrations of TAS-205 and CLS122. Assays were completed at a minimum biological triplicates (*n*=3). Data shown as mean ± SEM.

Macrophages pre-treated with CLS122 and stimulated with LPS showed reduction in pro-inflammatory gene expression

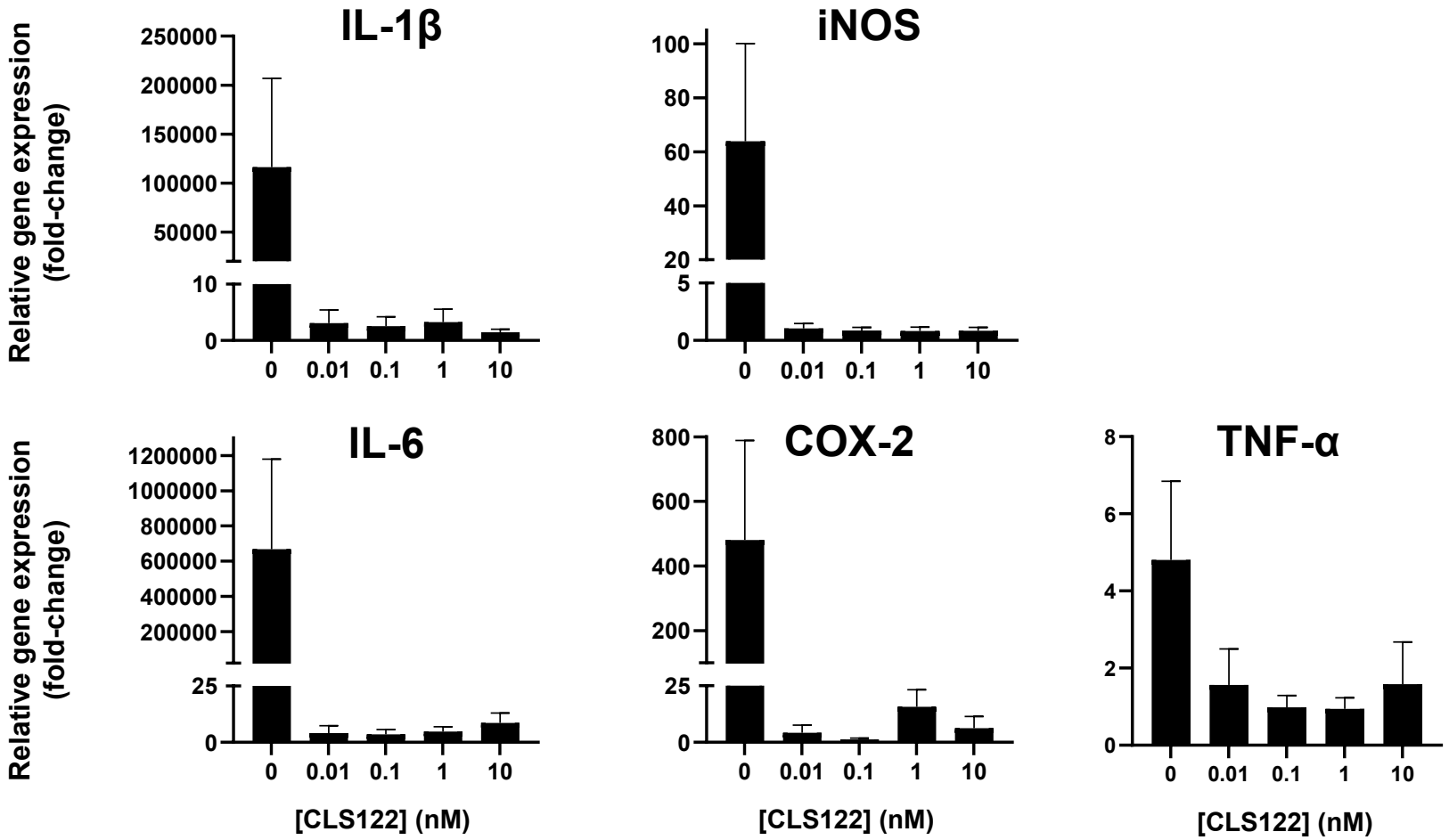
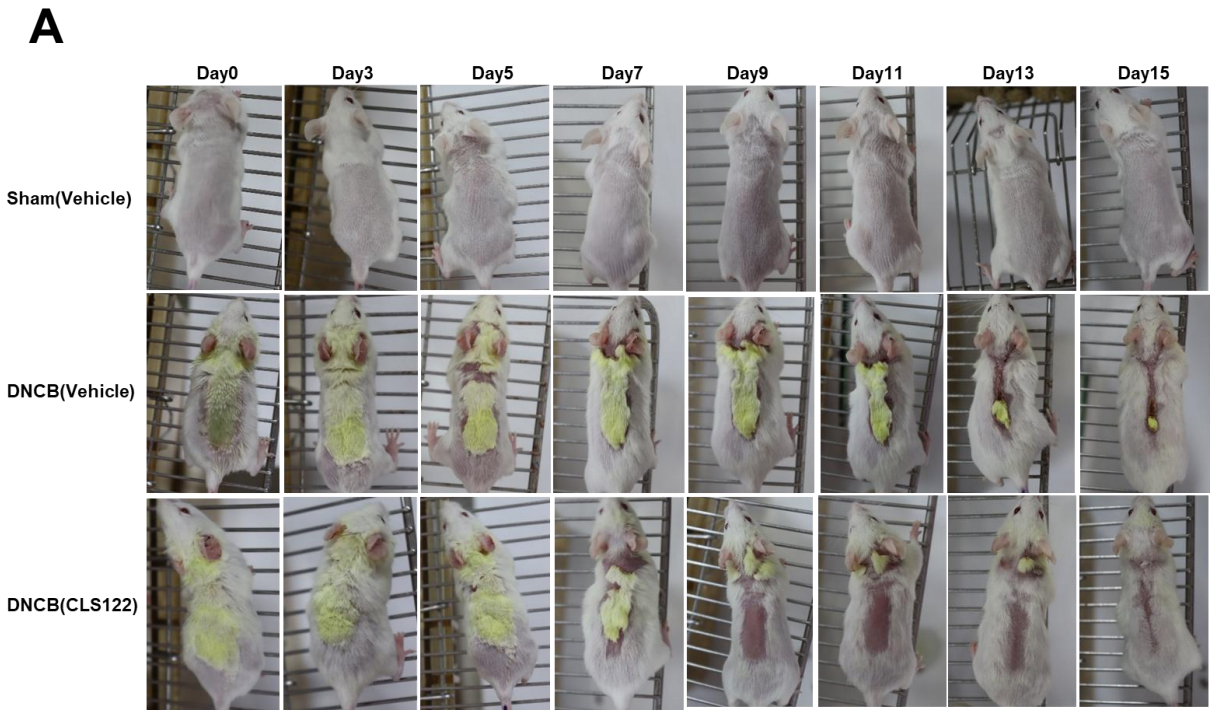


Figure 2: Relative gene expression (fold-change) of TNF-α, iNOS, IL-1β, IL-6, COX-2, and HPGDS in response to CLS122 (10 – 0.01 nM) treated RAW 264.7 macrophages. Quantification via qPCR utilising SYBR Green Reporter. Assays were completed as biological triplicates (*n*=3) with technical duplicates (across two plates). Data shown as mean ± SEM.

DNCB-challenged mice treated with HPGDS inhibitor CLS122 demonstrated decreased skin lesion severity



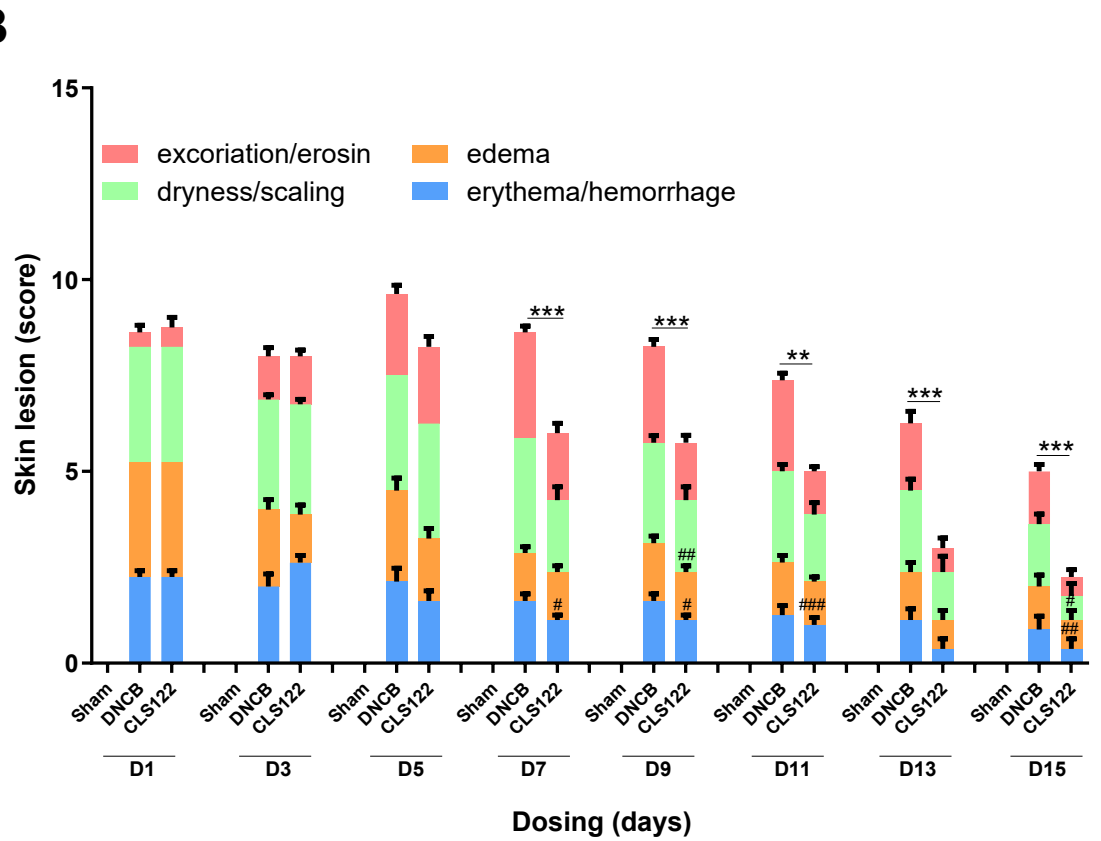


Figure 3: **(A)** CLS122 treatment shows visual reduction of scabbing (yellow patches), erosion (skin lesions), edema (swelling), and erythema (redness). **(B)** Severity scores ranging from 0 (none) to 3 (severe) (n=8/group). Statistical * comparison of total score; statistical # comparison of each symptom. Two-way ANOVA Tukey's multiple comparisons test. p#<0.05; p##<0.01; p###<0.001; p**<0.01; p***<0.001. Data shown as mean ± SEM.

Scratching behaviour was decreased in DNCB-challenged mice treated with HPGDS inhibitor CLS122

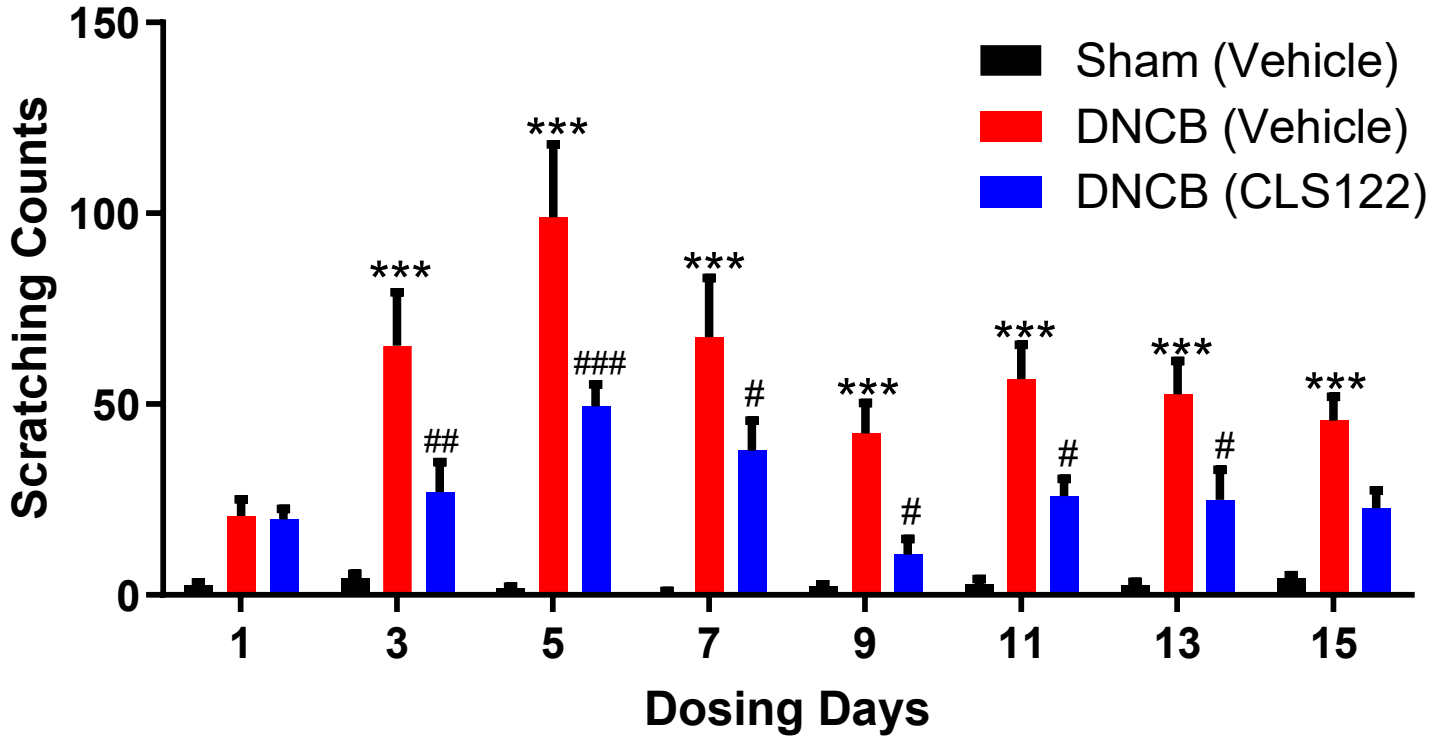


Figure 4: Effect of CLS122 on the development of DNCB-induced AD-like scratching counts in CD-1 female mice. Data shown as mean ± SEM. * DNCB (Vehicle) compares with Sham (Vehicle); # DNCB (CLS122) compares with DNCB (Vehicle). Two-way ANOVA followed by Tukey's multiple comparisons test. *p*#<0.05; *p*##<0.01; *p*###<0.001; *p***<0.01; *p****<0.001.

Ear thickness and weight was reduced in DNCB-challenged mice treated with HPGDS inhibitor CLS122

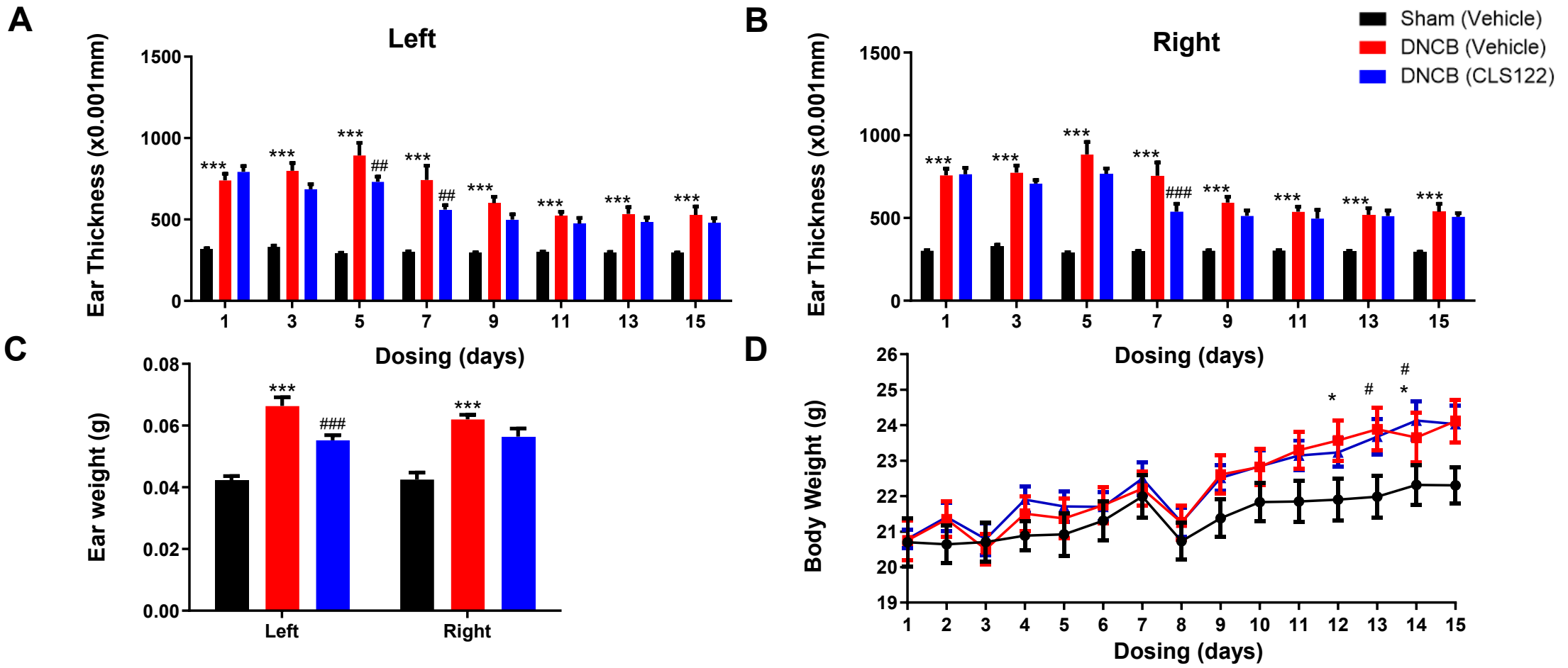
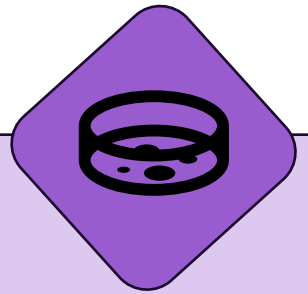


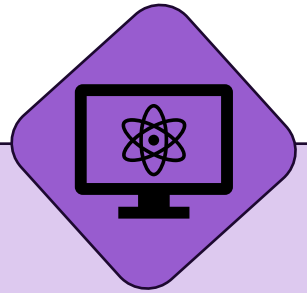
Figure 5: CLS122 on the development of DNCB-induced **(A)** left and **(B)** right ear thickening and **(C)** ear weight. **(D)** CLS122 treatment was well-tolerated, and no body weight change observed. * DNCB (Vehicle) compares with Sham (Vehicle); # DNCB (CLS122) compares with DNCB (Vehicle). Two-way ANOVA followed by Tukey's multiple comparisons test. *p*#<0.05; *p*##<0.01; *p*###<0.001; *p**<0.05; *p****<0.001. Data shown as mean ± SEM.

Conclusions: PGD2 is a potential target for novel atopic dermatitis therapeutics



In vitro potency determination

CLS122 showed nanomolar potency in an HPGDS expressing cell line, 11-fold more potent than clinical trial compound TAS-205



Inflammatory gene expression

Macrophages pre-treated with CLS122 displayed considerably reduced pro-inflammatory gene expression (IL-1β, iNOS, TNF-α, IL-6, and COX-2).

In vivo symptom alleviation

DNCB-challenged mice treated with CLS122 demonstrated a significant alleviation in AD-like symptoms:
Skin lesion severity score
Scratching count
Ear thickness and weight

Body weight did not differ from vehicle, suggesting the HPGDS inhibitor was well tolerated.

Overall, targeting HPGDS and PGD2 for AD treatment offers significant advantage by protecting skin barrier function and its non-invasive route of administration, increasing patient adherence.

Future directions – testing tool HPGDS inhibitor CLS122 in AD

Confirm dose-dependent efficacy of CLS122 in similar DNCB model (PO and topical)

Comparison of efficacy to current standard of care (eg steroid)

Histology – epidermal barrier function and immune cell infiltration

Disease severity efficacy – evaluation in different AD murine models

Mechanism studies – inflammatory mediation

Thank you!

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Australian Government
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