IRL201104, A Novel Immunomodulatory Peptide, Shows Efficacy In An Allergen Driven Model Of Atopic Dermatitis

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REVOLO BIOTHERAPEUTICS

Rationale

- Atopic dermatitis (AD) is a chronic inflammatory skin condition with a complex underlying pathology (1) Despite recent therapeutic advances, new treatments are still needed (2).
- IRL201104 is a novel clinical stage immunomodulatory peptide in development for allergic conditions that has shown promising efficacy in a Phase 2a study of Eosinophilic Esophagitis and a range of preclinical models of allergic inflammation (3-7).
- Our aim was to explore its therapeutic potential in an allergen driven model of atopic dermatitis.

Methods

Model protocol

- Mice were actively sensitised to ovalbumin (OVA; 15 µg, s.c.) and 25 µL of Imject Alum on days 1 and 7.
- On day 14, animals were anaesthetised with isoflurane to allow for the dorsal skin to be shaved using clippers and hair removal cream followed by tape stripping which was conducted 6 consecutive times.
- After shaving, the mice were topically challenged with OVA patches prepared with 1 cm^2 sterile gauze moistened with OVA (300µg) in phosphate buffered saline and attached to the dorsal skin with a transparent dressing for 7 consecutive days (day 14-20). Patches were changed daily.
- Patches were then removed and animals left to rest for 7 days until day 28 when a new 7 days challenge phase took place (day 28-34).
- Animals were dosed either vehicle, IRL201104 (80ug/kg or 2mg/kg iv) or dexamethasone (3mg/kg ip) at day 18, 19, 20, 21, 23, 25, 27, 32, 33, and 34.

Serum Collection

- 24hrs after the last challenge, on day 35, a terminal blood sample was collected via cardiac puncture and placed into a serum tube. Samples were kept at room temperature for 45 minutes to allow coagulation, before being centrifuged (2000g, 15 min at 4°C). Supernatant was separated, aliquoted and stored at -80°C for analysis.

Assessment of Skin Pathology

- Immediately after blood collection the dermal area exposed to the OVA challenge was visually assessed for pathological features associated with atopic dermatitis. These included: Erythema/haemorrhage, oozing/crust, erosion/excoriation and lichenification. Each pathology was evaluated using the following scoring system:

Score	Symptoms
0	None
1	Very Slight
2	Well defined
3	Moderate

Skin Thickness Measurements

- After the pathological assessment, the dorsal skin was carefully excised. Randomly, 3 positions on the dorsal skin were measured doubled over by using digital calliper and the average thickness (µm) calculated for each mouse.

OVA specific IgE level measurement

- OVA specific IgE level in serum was determined using an ELISA kit (AssayGenie) as per the manufacturer's instructions. Results were expressed as concentration in pg/mL.

Cytokine/chemokine measurements

- An 8-Plex cytokine/chemokine panel (IL-4, IL-5, IL-13, IL-1β, TNF-α, IFN-γ, IL-17, CCL-22) was run in the serum samples using a magnetic multiplex assay (Biotechne) as per the manufacturer's instructions. Levels were measured using a Magpix system (Luminex Corp). Results were expressed as concentration in pg/mL. CCL-17 and IL-31 concentrations were measured using ELISA kit (Biotechne and Invitrogen respectively) as per the manufacturer's instructions. Optical density was measured at 450 nM using a microplate reader (SpectraMax 340PC). Results were expressed as concentration in pg/mL.

Statistical analysis

- Data are shown as mean ± S.E.M. (standard error of the mean). Inter-group deviations were statistically analysed by a one-way analysis of variance (ANOVA) followed by a Dunnett's test. $p < 0.05$ was considered statistically significant.

Results (I)

Effect of treatment on skin thickness

At day 35, exposure of the sensitised skin to allergen challenge led to a significant increase in skin thickness. IRL201104 significantly and dose dependently reduced skin thickness similarly to positive control dexamethasone (Fig1).

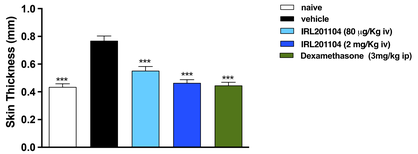

Figure 1 – Effect of treatment on OVA-induced increase in skin thickness. *Data are expressed as average thickness in millimetres (mm) ± SEM. Groups were compared to OVA/vehicle group using a one-way ANOVA, followed by a Dunnett's test; ***P<0.001; n=8.*

Effect of treatment on skin pathology

At day 35, exposure of sensitised skin to allergen challenge led to the development of significant skin pathology (Fig2) characterised by increased erythema/haemorrhage, increased oozing/crust and increased excoriation/erosion. No lichenification was observed. IRL201104 treatment significantly reduced erythema/haemorrhage and erosion/scoriation score while showing a strong trend to reduce oozing/crust score similarly to positive control dexamethasone (Fig3A, B, C).

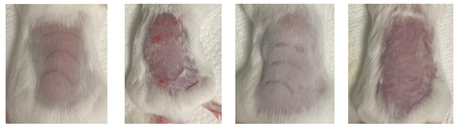

Figure 2 – Representative images of skin pathology. *Images of dorsal skin area at day 35, showing a representative (A) naïve; (B) OVA/vehicle; (C) OVA/IRL201104 (2mg/kg i.v.); (D) Dexamethasone (3mg/kg i.p).*

Results (II)

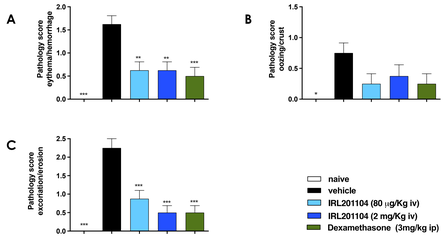

Figure 3 – Effect of treatment on OVA-induced skin pathology. *Effect on (A)* erythema/haemorrhage score, *(B)* oozing/crust score *and (C) excoriation/erosion score. Data are expressed as mean score ± SEM. Groups were compared to OVA/vehicle group using a one-way ANOVA, followed by a Dunnett's test; *P<0.05, **P<0.01, ***P<0.001; n=8.*

Effect of treatment on OVA specific IgE levels

OVA challenge elicited an increase in OVA specific IgE. Both positive control dexamethasone and IRL201104 treatment (at both doses) significantly reduced OVA specific IgE levels in serum at day 35 (Fig4).

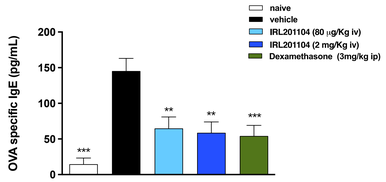

Figure 4 – Effect of treatment on OVA specific IgE serum levels. *Data are expressed as pg/mL of serum, mean ± SEM. Groups were compared to OVA/vehicle group using a one-way ANOVA, followed by a Dunnett's test; **P<0.01, ***P<0.001; n=8.*

Results (III)

Effect of treatment on cytokine/chemokine release in serum

At day 35, exposure to OVA challenge elicited the release of T2 cytokines IL-4, IL-5, IL-13 and IL-31; T1 cytokines TNF-α, IL-1β, and IFN-γ; IL-17 and the key AD biomarkers CCL-17 and CCL-22. Apart from IL-17, and similar to dexamethasone, IRL201104 treatment led to a significant dose dependent reduction of cytokines/chemokines in serum at day 35 (Table 1).

Day 35	Serum concentration (pg/ml)	Naive	OVA/vehicle	OVA/1104 80µg/Kg i.v.	OVA /1104 2mg/kg i.v.	OVA/Dexamethasone 3mg/kg i.p.
	IL-4	18.5±11***	192±25	83±17***	61±15***	43±14***
	IL-5	17±10***	213±22	91±22***	64±17***	62±16***
	IL-13	18±11***	145±22	59±20**	45±14***	39±13***
	IL-31	19±11***	229±23	90±11***	96±13***	87±20***
	TNF-a	16±9***	126±14	53±14***	47±13***	34±11***
	IL-1b	9±9***	83±10	34±13**	21±10***	11±7***
	IFN-g	18±10***	111±16	42±15**	37±15**	28±13***
	IL-17	15±9	74±9	41±20	28±14	24±12
	CCL-17	19±11***	227.5±27	96±19***	72±17***	60±16***
	CCL-22	18±10***	175±25	70±17***	45±15***	39±13***

Table 1– Effect of IRL201104 on cytokines/chemokines in serum after OVA challenge. *Effect on IL-4, IL-5, IL-13, IL-31, TNF-α, IL-1β, IFN-γ, IL-17, CCL-17 and CCL-22. Data are expressed as picograms per mL of serum, mean ±SEM. Groups were compared to the OVA/vehicle group using a one-way ANOVA, followed by a Dunnett's test; **P<0.01,***P<0.001; n=8.*

Discussion

- **Administration of IRL201104 significantly reduced skin thickness and skin pathological score comparable to positive control dexamethasone.**
- **Furthermore, IRL201104 treatment significantly reduced serum levels of allergen specific IgE, T2 cytokines (IL-4, IL-5 and IL-13), T1 cytokines (TNF-α, IL-1β, and IFN-γ), the pruritogenic cytokine IL-31 and AD biomarkers CCL-17 and CCL-22.**
- **This study shows the potential of IRL201104, a clinical stage immunomodulator, as a novel treatment for atopic dermatitis.**

REFERENCES

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