IRL201104, A Novel Immunomodulatory Peptide, Drives An Increase In T And B Regulatory Cell Phenotypes And Shows Long Lasting Efficacy Through Different Routes Of Administration In A Mouse Model Of Allergic Inflammation

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REVOLO BIOTHERAPEUTICS

Rationale

- IRL201104, a novel clinical stage immunomodulatory peptide, has a short half-life (~10-15min) but long-lasting effects in allergic inflammation models (1-3,7)
- Human ex vivo and clinical data highlight IRL201104 ability to drive a tolerogenic response involving T and B regulatory cells (4-6)
- Our aim was to test two new potential clinical subcutaneous IRL201104 formulations vs intravenous in an ovalbumin (OVA) re-challenge model of allergic inflammation and simultaneously explore IRL201104 capacity to drive a similar tolerogenic response as seen in human studies.

Methods

Clinical formulations

- Two subcutaneous formulations of IRL201104, in development for clinical applications, were tested in this study vs our preclinical Intravenous formulation (IV): prototype I (SC1) and prototype II (SC2).

Model protocol

- Female Balb/C mice (~25g) were sensitised with ovalbumin (15 µg, s.c.) and Imject Alum as an adjuvant on days 1 and 7. In order to elicit a local inflammatory response in the lungs, mice were challenged on day 15, 16 and 17 with an aerosol of either 1% w/v ovalbumin in phosphate buffered saline (PBS) or just PBS, generated with an ultrasonic nebuliser (Aerogen) for 20 min.
- IRL201104 or vehicle were administered subcutaneously (s.c.; 80µg/Kg) or intravenously (i.v.; 80 µg/Kg) 15 minutes prior to the OVA or PBS challenges on days 15, 16 and 17. 24 hours after the final challenge to OVA or PBS (day 18), samples were collected.
- In a separate cohort of animals, a second series of challenges on days 27, 28 and 29 with an aerosol of 1% w/v ovalbumin or PBS were carried out without further drug treatment. Samples were collected from these animals 24 hours after the final challenge (day 30).

BALF and blood/serum collection and differential cell counts

- 24 hours after the last challenge (day 18 or 30) two blood samples were taken by venepuncture (via the lateral tail vein). A fresh sample was processed for blood immunophenotyping and a second blood sample was processed to serum and stored at -80°C for OVA specific IgE analysis.
- Animals were then overdosed with pentobarbitone and bronchoalveolar lavage was carried out using phosphate buffered saline. The isolated BALF was then centrifuged at 1500 rpm for 10mins at 4°C and the supernatant was aliquoted and stored at -80°C for cytokine analysis.
- BALF cells were analysed for total and differential cell numbers using a XT-2000iV analyser (Sysmex). Results were expressed as cells/mL.

OVA specific IgE level measurement

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

Cytokine/chemokine measurements

- A 12-Plex cytokine/chemokine panel (IL-4, IL-5, IL-10, IL-12p70, IL-13, IL-17, KC, Eotaxin, G-CSF, GM-CSF, MCP-1 and RANTES) was run in BALF supernatant using a magnetic multiplex assay as per the manufacturer's instructions. Results were expressed as concentration in pg/mL.

Blood immunophenotyping

- To increase the number of cells available, blood from individuals in each group were pooled in pairs. Red blood cells (RBC) were removed from fresh blood sample using RBC lysis buffer, then cells were treated with Fc block. Following this treatment, cells were stained with a fixable viability dye and labelled with relevant indicated antibodies. For intracellular cytokine detection, cells were fixed and permeabilised prior to labelling with antibodies. Sample data was acquired on the Novocyte 3000, with compensation defined and applied using FMO controls.

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 OVA induced cell infiltration

24 hours post-challenge at day 18, exposure to OVA aerosol triggered inflammatory infiltration in the lung as measured by BALF differential cell counts. IRL201104 significantly reduced OVA-induced lung infiltration of eosinophils, neutrophils, lymphocytes and macrophages (Fig1A,B,C,D) through both routes of administration. The effect of IRL201104 was maintained upon OVA re-challenge (day 30), 13 days after the last dose of the compound (Fig1E,F,G,H).

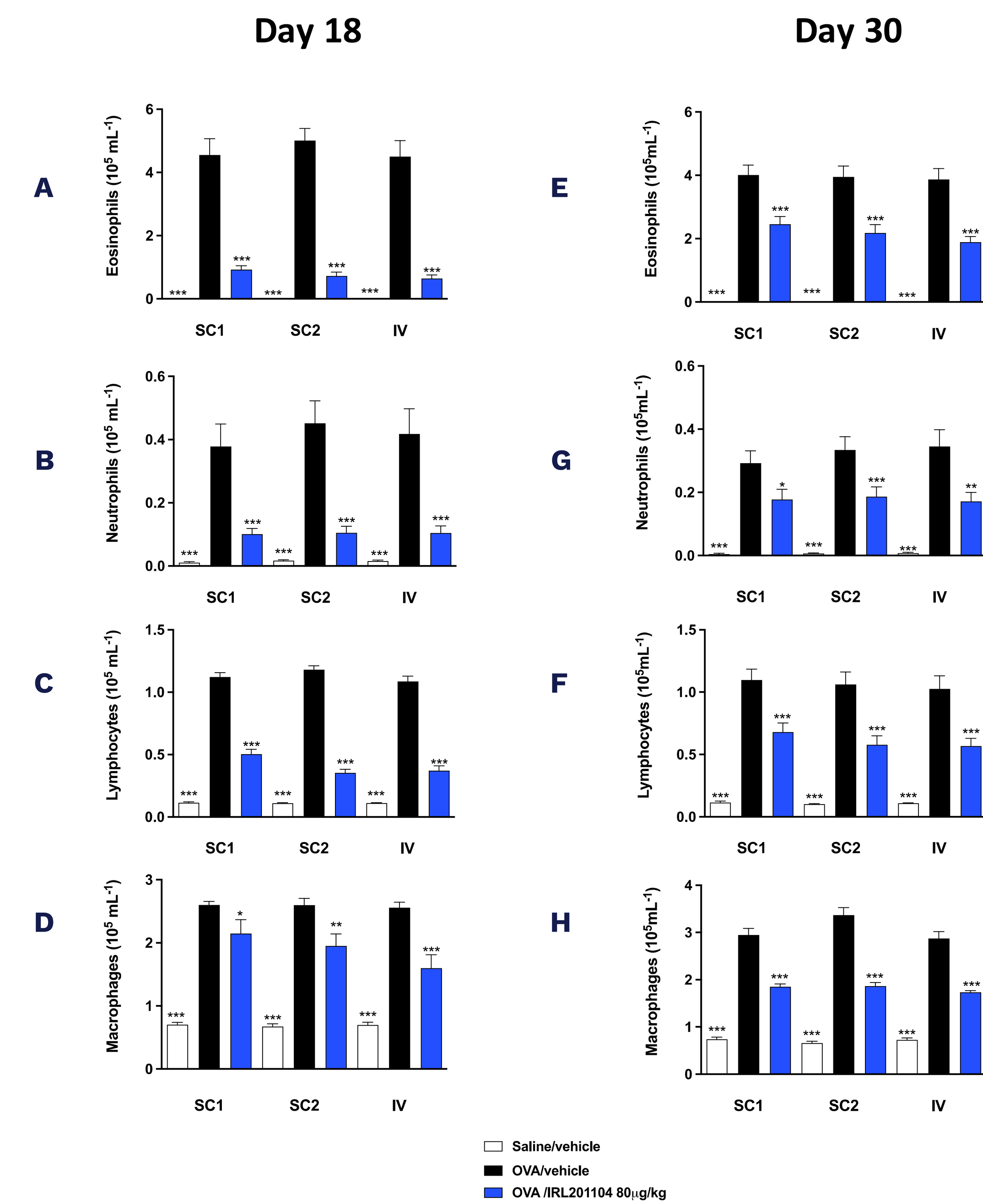

Figure 1 – Effect of treatment on OVA-induced cellular infiltration. *Effect of IRL201104 on (A,E) eosinophils, (B,G) neutrophils, (C,F) lymphocytes and (D,H) macrophages lung infiltrate. Data are expressed as cells per mL of BALF, mean ± SEM. Groups were compared to OVA/vehicle group using a one-way ANOVA, followed by a Dunnett's test; *P<0.05, **P<0.01, ***P<0.001; n=8.*

Results (II)

Effect of treatment on OVA specific IgE levels

OVA challenge elicited an increase in OVA specific IgE (Fig2). IRL201104 treatment through both routes of administration significantly reduced OVA specific IgE in serum at day 18 (Fig2A) and the effect was maintained upon OVA re-challenge (day 30), 13 days after the last dose of the compound (Fig2B)

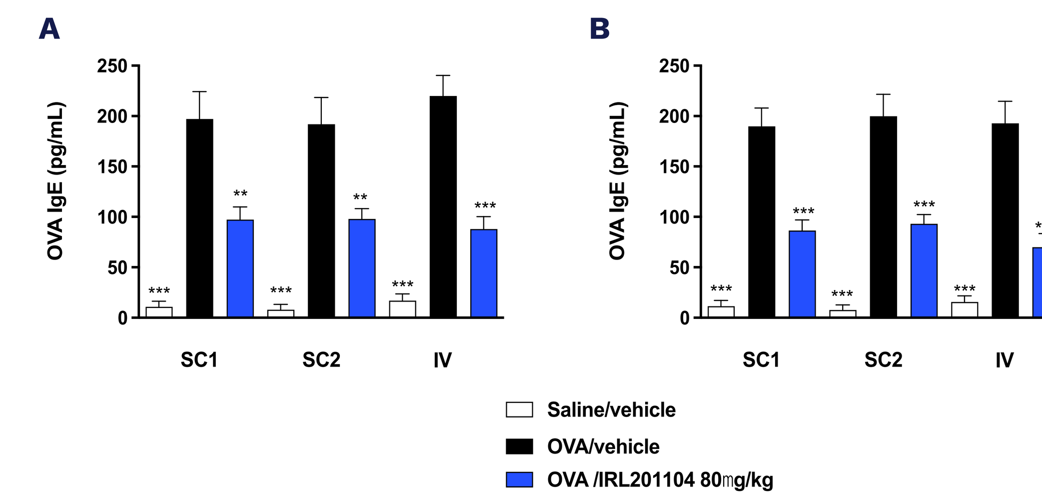

Figure 2 – Effect of 1104 treatment on serum OVA specific IgE. *Effect at day 18 (A) and day 30 (B) on OVA specific IgE serum levels. Data are expressed as pg/mL of serum, mean ± SEM. Groups were compared to OVA/vehicle group using a one-way ANOVA, followed by a Dunnett's test; **P<0.01, ***P<0.001; n=8.*

Effect of treatment on tolerogenic response induction

IRL201104 treatment through both routes of administration seemed to drive an increase the percentage of T regulatory (CD3+FOXP3+IL10+; Fig3A) and B regulatory (CD19+IL10+; Fig2A) lymphocytes in the blood at day 30, 13 days after the last dose of the compound consistent with what has been previously observed in human clinical and ex vivo data.

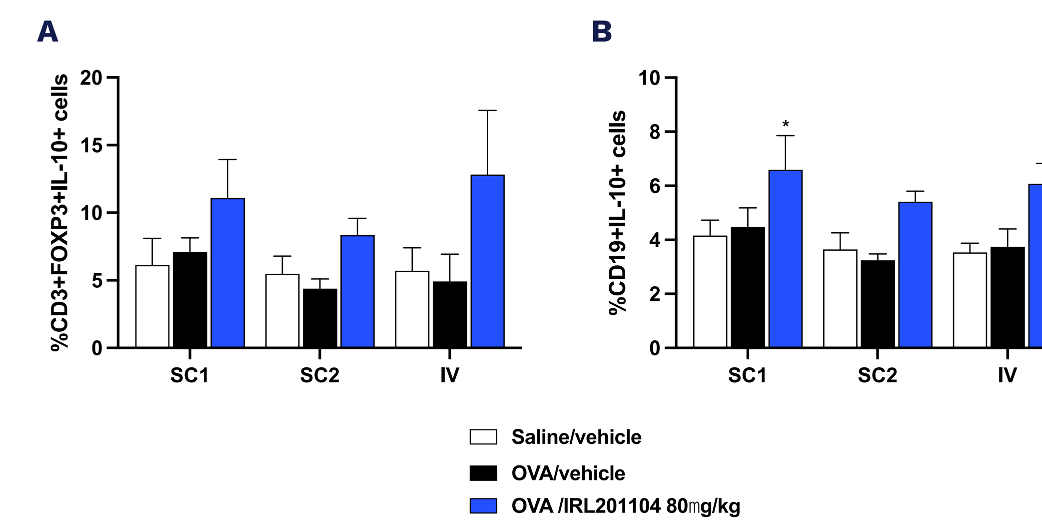

Figure 3 – Effect of treatment on tolerogenic T and B lymphocyte populations. *Effect of IRL201104 on % of (A) CD3+FOXP3+IL10+ lymphocytes and (B) CD19+IL10+ in blood. Data are expressed as % of either CD3+ or CD19+ cells mean ± SEM. Groups were compared to OVA/vehicle group using a one-way ANOVA, followed by a Dunnett's test; *P<0.05; n=4.*

Results (III)

Effect of treatment on cytokine/chemokine release in BALF

24 hours post-challenge at day 18, exposure to OVA aerosol elicited the release of IL-4, IL-5, IL-13, IL-10, IL-12(p70), IL-17, Eotaxin, GM-CSF, G-CSF, MCP-1, RANTES and Periostin in BALF supernatant. IRL201104 significantly reduced OVA-induced cytokines/chemokine release through both routes of administration. This effect of IRL201104 was maintained through both routes of administration upon OVA re-challenge (day 30), 13 days after the last dose of the compound (Table 1).

Day 18	BALF concentration (pg/ml)	Saline/vehicle SC1	OVA/vehicle SC1	OVA/1104 80µg/Kg SC1	Saline/vehicle SC2	OVA/vehicle SC2	OVA/1104 80µg/Kg SC2	Saline/vehicle IV	OVA/vehicle IV	OVA/1104 80µg/Kg IV
	IL-4	19±7***	267±25	102±14***	13±6***	279±25	90±12***	20±8***	263±23	51±16***
	IL-5	12.5±6***	344±30	103.5±17***	12.5±6***	366±36	115±16***	21±8***	336±29	67±21***
	IL-13	20±8***	202±37	73±18**	15±8***	221.5±26***	70±12	15±7***	194±33	49±16***
	IL-10	13±6***	208±27	108±14***	15±7***	227±23	95±12***	19±7***	199±22	71±16***
	IL-12p70	19±7***	122±14	74±11**	8±5***	127±12	65±8***	13±6***	115±13	50±12***
	IL-17	12.5±6***	154±19	81±9**	8±5***	175±18	76±9***	17±8***	152±19	52±13***
	Eotaxin	21±8***	157±17	73±8***	17±8***	176±16	69±9***	19±7***	143±20	44±11***
	GM-CSF	13±6***	113±14	60±9**	13±6***	122±16	46±9***	19±7***	104±19	30.5±13**
	G-CSF	13±6	31±10	19±7.5	8±5	39±12	18±9	17±8	34±13	24±9
	MCP-1	13±6***	114±22	51±9**	12±6***	114±22	48±10**	12±6***	121±20	44±12**
	RANTES	22±9***	110±10	62±9**	14±7***	116±15	73±7*	18±7***	103±13	40±17**
	Periostin	13±6***	149±23	74±10**	9±6***	162±18	71±7***	20±8***	163±17	55±6***
Day 30	**BALF concentration (pg/ml)**	**Saline/vehicle SC1**	**OVA/vehicle SC1**	**OVA/1104 80µg/Kg SC1**	**Saline/vehicle SC2**	**OVA/vehicle SC2**	**OVA/1104 80µg/Kg SC2**	**Saline/vehicle SC2**	**OVA/vehicle IV**	**OVA/1104 80µg/Kg IV**
	IL-4	21±8***	245±23	125.5±13.5***	14±7***	260±21	115±15.5***	23±9***	252±24	101±18***
	IL-5	22±9***	326±23	151.5±18***	17±8***	325±30	136±16***	19±8***	312±31	114±23***
	IL-13	22±8***	199±29	112±17*	17±9***	216±23	98±17***	19±7***	187±26	86±20**
	IL-10	15±8***	184±21	117±14**	14±7***	181±16	106±12***	24±9***	200±18	107±16**
	IL-12p70	17±9***	127±10	94±9*	15.5±8***	140±16	92±8*	18±7***	125±11	88±10*
	IL-17	18±7***	119±17	77±10*	14±7***	123.5±13	75±9**	20±8***	113.5±13	43±15**
	Eotaxin	20±8***	152±12	82±11***	16±8***	134.5±13	81±10**	13±6***	144±19	65±12***
	GM-CSF	15±7***	99±16	60±6*	8±5***	110±17	60±7**	14±7***	109.5±12	47±15**
	G-CSF	19±9	33±13	22±11	12±6	33±16	20±10	11±6	36±14	18±7
	MCP-1	14±7***	101±18	77±11	14±7***	104±15	57±12*	19±7***	123±14	63±16**
	RANTES	19±7***	113±12	85±9	9±6***	120±13	93±8	17±7***	102.5±12	78±9
	Periostin	15±7***	177±18	109±17**	10±6***	182±15	114±13**	18±7***	161±17	86±10

Table 1– Effect of IRL201104 (1104) on cytokines/chemokines in BALF after OVA challenge. *Effect on IL-4, IL-5, IL-13, IL-10, IL-12(p70), IL-17, Eotaxin, GM-CSF, G-CSF, MCP-1, RANTES and Periostin. Data are expressed as picograms per mL of BALF, mean ±SEM. Groups were compared to their respective OVA/vehicle groups using a one-way ANOVA, followed by a Dunnett's test; *P<0.05, **P<0.01,***P<0.001; n=8.*

Discussion

- **Administration of the same dose of IRL201104 using our two new clinical subcutaneous formulations showed similar efficacy to the intravenous route on OVA induced lung infiltration, serum OVA specific IgE and BALF cytokines/chemokines, supporting transition to this route of administration for future clinical trials.**
- **Despite the short half life, IRL201104 shows a long pharmacodynamic effect through both routes of administration which is consistent with the induction of tolerogenic T and B regulatory cell phenotypes.**

REFERENCES

1. Page C et al. Am J Respir Crit Care Med 2019;199:A2861. **2.** Riffo-Vasquez Y et al, Clin Exp Allergy. 2020 Apr;50(4):508-519. **3.** De Alba J et al. Am J Respir Crit Care Med 2022; 205:A3743. **4.** Dellon et al. The American Journal of Gastroenterology 2023; 118(10S):p S335-S336. | DOI: 10.14309/01.ajg.0000951480.82456.a1. **5.** Keane S et al. Allergy 2023; 78:p 181. **6.** Hikmawati et al. Allergy 2023; 78: p 181-182. **7.** De Alba J et al. Am J Respir Crit Care Med 2024;209:A6982.

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