IOMX-0235, a CCR9+ Cell Depleting Antibody for the Treatment of IBD with Best-in-Class Potential

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Deliver Safe & Effective Precision Therapies to Every IBD Patient

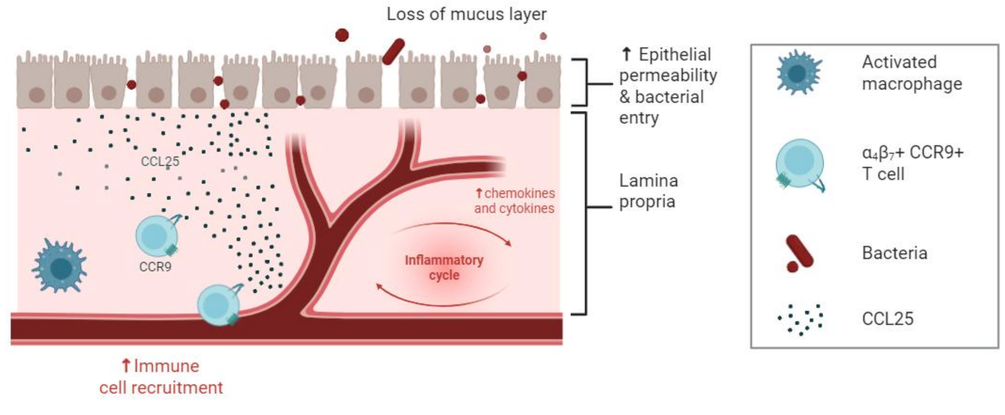
Introduction

Inflammatory Bowel Disease (IBD) is a chronic condition of the intestine characterized by leukocytic infiltration and pro-inflammatory cytokine production[1]. CCR9 is a gut homing receptor expressed on specific subsets of leukocytes, which mediates the migration of leukocytes into the gut towards high levels of its ligand CCL25 selectively upregulated in the inflamed intestine[2]. Despite proven efficacy of CCR9 or CCL25 knockout and CCL25-dependent signaling inhibition in preclinical models of colitis, blockade of CCR9 function failed to demonstrate clinical benefit in human trials[3,4,5,6].

Nevertheless, the removal of CCR9-positive cells using extracorporeal leukapheresis led to clinical responses in patients suffering from ulcerative colitis underscoring CCR9 as a promising target for selective depletion of inflammation driving immune cells in IBD[7].

IOMX-0235 is an optimized anti-CCR9 specific antibody designed for enhanced killing activity. It represents a novel opportunity for the treatment of IBD by selectively targeting CCR9+ pro-inflammatory immune cells. IOMX-0235 leverages a novel mechanism of action of robust cell depletion *in vitro* as well as in *in vivo* models without interference with ligand binding providing best-in class potential. IOMX-0235 is ready to enter IND-enabling activities.

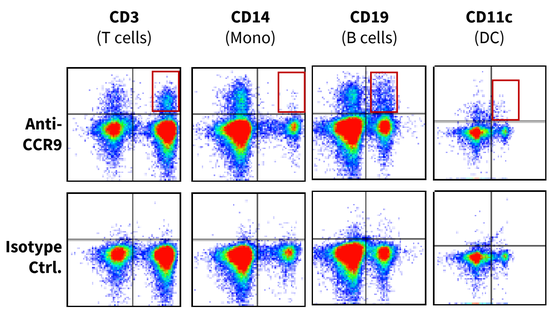
IOMX-0235 represents a promising therapeutic approach for IBD, with the potential to provide meaningful clinical benefits by depleting CCR9+ immune cells from patients. Its unique mode of action and readiness for clinical development make it a valuable candidate in the IBD field.



CCR9 Expression – Restricted Expression on Healthy Peripheral Blood Cells

- CCR9 is expressed on low proportions of T, B & dendritic cells, approx. 5% of hu T cells in circulation are CCR9⁺ in healthy donors[8,9,10]
- CCR9 is not enriched in any specific T-cell subset
- Thrombocytes, monocytes, granulocytes and erythrocytes are negative for CCR9
- The CCR9 protein expression pattern demonstrated by FACS is confirmed by published RNA expression data of immune cell subsets
- Elimination of CCR9⁺ cells has positive effect on inflammatory symptoms in UC patients by reducing the Mayo score & rectal bleeding[7]

A) CCR9 expression in Immune Cell Subsets



B) CCR9 protein and RNA expression levels

Cell Type	Marker	% CCR9⁺	RNA (pTPM)[2]
T cells	CD3+	3-8%	*n.d.*
CD4+ T cells	CD3+ CD4+	ca. 4%	4,0+
CD8+ T cells	CD3+ CD8+	ca. 3%	3,3+
Treg	CD4+ CD25+ CD127-	11-26%	9,4
B-cells	CD19+	4-15%	40,8+
Dendritic cells	CD11c+ (CD14-)	2-6%	0,8**
NK cells	CD56+	<2%	None
NK/T cells	CD3+ CD56+	<1%	*n.d.*
Monocytes	CD14+	<1%	None
Granulocytes	CD66b+	None	*n.d.*
Erythrocytes	CD235+	None	*n.d.*
Thrombocytes	CD41+	None	*n.a.*

FIGURE 1

CCR9 expression in primary human immune cell subtypes of PBMCs of healthy donors was assessed by flow cytometry **(A)** and compared to RNA levels in corresponding subsets **(B)**. RNA expression data were obtained from human Protein Atlas[11].

Discovery & Development of IOMX-0235

- Immunization of rabbits with human CCR9 over-expressing cells
- Single B-cell screenings on target-expressing cells
- Sequence optimization and humanization
- Fc-engineering to improve binding to FcgRIIA and FcgRIIIA and ADCC/ADCP activity

A) Discovery & Development of IOMX-0235

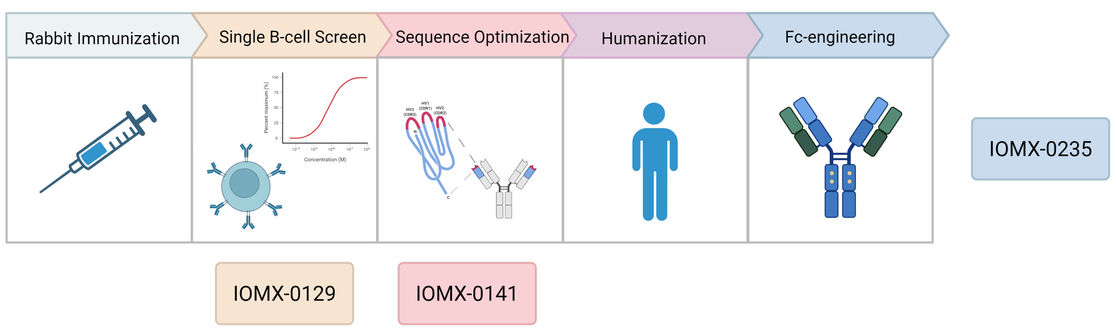


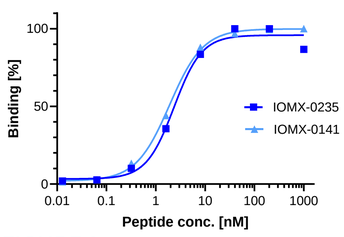
FIGURE 2

A) CCR9-binding antibodies were identified by sequencing B cells from immunized rabbits. Candidate antibodies, including the parental clone IOMX-0129, were sequence-optimized and humanized. Final candidates were evaluated in an Fc-enhanced format using biochemical assays, *in vitro* functional studies, and *in vivo* mouse models.

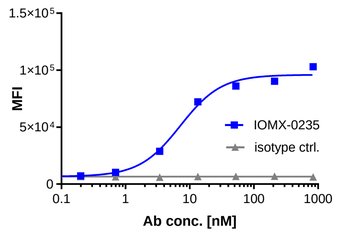
Biochemical Characterization - IOMX-0235 Binds CCR9 With High Affinity

- IOMX-0235 retains the binding profile of its parental clone IOMX-0141 following humanization.
- IOMX-0235 is fully cross-reactive to cynomolgus CCR9 and binds human and cynomolgus monkey CCR9 peptides with comparable affinity.
- CCR9⁺ cell binding on human CCR9⁺ MOLT-4 cell line was confirmed by flow cytometry analysis.
- BLI analysis with human CCR9 peptide demonstrates picomolar apparent binding affinity.

A) Binding of IOMX-0235 to human and cyno CCR9



B) Binding of IOMX-0235 on MOLT-4 cells



C) Affinity of IOMX-0235 to human CCR9

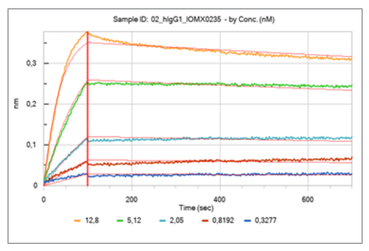
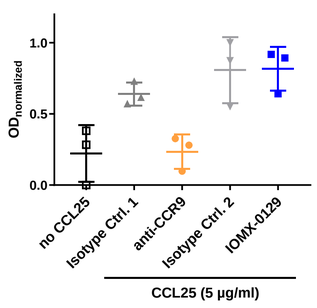


FIGURE 3

A) Binding of IOMX-0235 (hIgG1 isotype) to human and cynomolgus monkey CCR9 peptide by ELISA. Antibody was coated at 2 µg/ml and peptide titrated across the indicated concentration range. Data normalized to maximum binding. **B)** Binding of IOMX-0235 (hIgG1 isotype) to MOLT-4 T-cells, endogenously expressing CCR9, by flow cytometry. **C)** Apparent affinity of IOMX-0235 to recombinant CCR9 peptide using BLI.

Functional Characterization - IOMX-0235 Binding is Not Competing with CCL25 Binding

CCL25 – CCR9 Chemotaxis is not affected



- Binding to CCR9 does not impair CCL25-mediated chemotaxis of CCR9⁺ cells, as shown for IOMX-0235 parental mAb IOMX-0129.
- Binding of IOMX-0235 to CCR9 does not compete with binding of ligand CCL25.
- This allows CCR9+ cell depletion by IOMX-0235, without the necessity to compete with the high affinity binding of CCL25, offering a potential advantage over ligand blocking antibodies.

FIGURE 4

Chemotaxis of CCR9⁺ MOLT-4 cells towards a CCL25 stimulus in the presence of IOMX-0129 assessed in a transmigration assay. Cells were allowed to migrate towards 2.5 µg/µl CCL25 for 3 days in presence of 0.5 µM of antibody.

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References

[1] Wendt et al. Clinical and Experimental Gastroenterology 2015; [2] Wendland et al. PNAS 2007; [3] Wurbel et al. J Immunol 2007; [4] Wurbel et al. PLoS ONE 2011; [5] Bekker et al. Mediators of Inflammation 2015; [6] Feagan et al. Aliment Pharmacol Ther 2015; [7] Eberhardson et al. Journal of Crohn's and Colitis 2017; [8] Papadakis et al. J Immunol 2000; [9] Qiuping et al. Cancer Res. 2003; [10] Olaussen et al. Gastroenterology 2007; [11] https://www.proteinatlas.org/ENSG00000173585-CCR9/blood ,

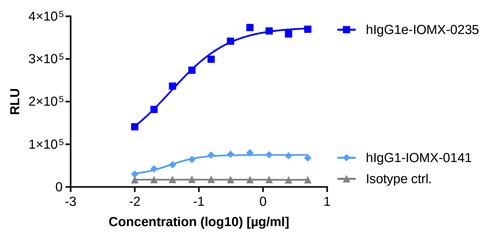
Abbreviations

CCR9: CC motif chemokine receptor 9; CCL25: chemokine (CC motif) ligand 25; cyno: cynomolgus monkey; BLI: biolayer interferometry; KD: dissociation constant; FACS: Fluorescence-activated cell sorter; IBD: inflammatory bowl disease; Fc: fragment crystallizable; FcgR: Fc gamma receptor; Fig.: figure; UC: Ulcerative Colitis (UC); CD: Crohn's Disease CD; NK: natural killer; CD: cluster of differentiation; IND: investigational new drug; ADCC: antibody dependent cellular cytotoxicity, ADCP: antibody-dependent cellular phagocytosis

Functionality - IOMX-0235 Induces Potent *In Vitro* ADCC Activity on CCR9⁺ Cells

- Potent binding of IOMX-0235 to CCR9 combined with Fc enhancement leads to strong activation of FcγRIIIa signaling in a reporter assay.
- Fc enhancement of IOMX-0235 enables strong FcγRIIIA activation driving efficient NK-cell mediated lysis of CCR9 transfected MO3.13 cells.
- NK cell mediated ADCC is triggered at sub-nanomolar concentrations of IOMX-0235.

A) Reporter assay confirms enhanced FcγRIIIA signaling



B) NK cell mediated ADCC activity

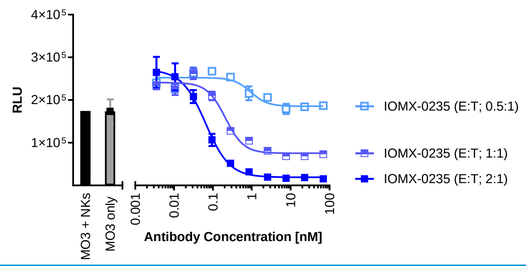


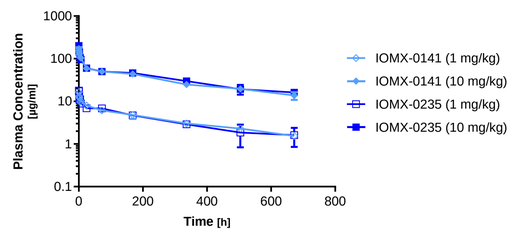
FIGURE 5

A) Reporter Assay: CCR9 expressing MOLT-4 cell line was co-cultured with Jurkat reporter cells expressing FcγRIIIa and NFAT-driven luciferase. Fc receptor clustering on Jurkat cells triggered NFAT-mediated luciferase activity, simulating ADCC mechanisms **B)** ADCC assay conducted with primary human NK cells, showing the titration of IOMX-0235 on CCR9 over-expressing MO3.13 cells using different E:T ratios.

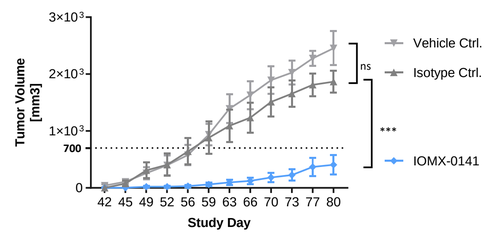
Safety & Efficacy – Efficient CCR9+ T Cell Depletion in Mouse Models

- IOMX-0235 demonstrates a linear, dose-dependent pharmacokinetic profile in rodents (Fig. 5A).
- Depletion of CCR9⁺ T cells leads to significantly delayed tumor onset and reduced tumor take rate, with 5 of 12 animals remaining tumor-free in a prophylactic setting (Fig. 5B).
- In a therapeutic subcutaneous tumor model using MOLT-4-tumor bearing animals, IOMX-0141 treatment resulted in a 65% tumor growth reduction 28 days after therapy start.
- Tumor growth inhibition was associated with increased infiltration of ADCC/ADCP-competent immune cells, F4/80⁺ macrophages and CD335⁺ NK cells, compared to isotype control confirming the CCR9⁺ cell depletion through ADCC/ADCP activity.

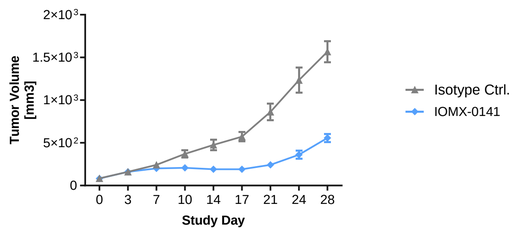
A) Pharmacokinetic Profile in Rodents



B) CCR9⁺ cell depletion in a prophylactic MOLT-4 tumor model



C) CCR9⁺ cell depletion in a therapeutic MOLT-4 tumor model



D) Enhanced infiltration of ADCC/ADCP-competent cells

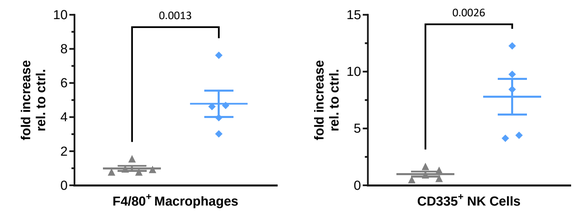


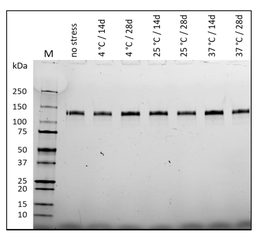
FIGURE 6

Pharmacokinetics analysis for single intravenous administration of 1 or 10 mg/kg of IOMX-0141 and IOMX-0235 (hIgG1) in C57Bl6 animals. EDTA plasma samples from three animals per time point were analyzed by ELISA **(A)**. Human acute T lymphoblastic leukaemia cell line MOLT4 was implanted subcutaneously into in immunodeficient Rag2-/- mice. Animals were treated directly after implantation (**B**) or after randomization at 80mm³ **(C)** twice weekly with 10 mg/kg IOMX-0141, vehicle, or mIgG2a-isotype control antibody. Statistical analysis by unpaired two-sided Student T-Test. Mean±SEM depicted. **D)** Tumor infiltrating macrophages and NK cells were analyzed by flow cytometry. F4/80+ macrophages and CD335+ NK cells are shown as mean ± SEM relative to the isotype ctrl. Statistical analysis by unpaired two-sided Student T-Test.

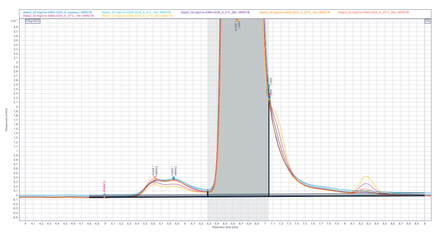
Developability – IOMX-0235 with favorable developability profile ready for CMC activities

- *In silico* sequence analysis: IOMX-0235 lacks sequence hotspots for deamidation or isomerization in any of the CDRs.
- Upon stress incubation at elevated temperatures IOMX-0235 exhibits excellent stability.
- No fragmentation apparent on SDS-PAGE analysis after storage for 28 days at elevated temperatures (Fig. 6A).
- IOMX-0235 is stable for up to 28 days, with only ca. 1% of high molecular weight (HMW) species formed in size exclusion chromatography (Fig. 6B).
- Loss in relative activity is <10% after 28 days at 37°C (Fig 6C.).
- Research cell bank established with productivity prior to optimization up to 4.5 g/L.

A) Non-reducing SDS-PAGE analysis



B) No HMW formation



C) Relative Active Concentration

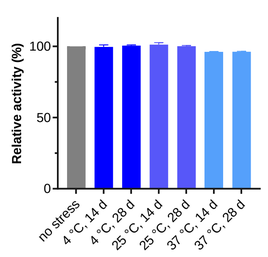


FIGURE 7

Developability asessment of IOMX0235 in final Fc- enhanced format. Lead molecules were formulated in Histidine-based buffer and incubated at elevated temperatures for up to 28 days. Samples were analyzed for aggregation, fragmentation, degradation propensity (SDS-PAGE, HP-SEC) and for functional integrity (relative active concentration, Octet).

Conclusion

- CCR9 is a highly validated target in IBD.
- CCR9 shows restricted expression on healthy peripheral blood cells with only low proportions of T-cells, B-cells, and dendritic cells being CCR9 positive.
- Proof-of-principle for depleting CCR9⁺ immune cells achieving a clinical effect in IBD patients.
- IOMX-0235 is a humanized, optimized and ADCC enhanced CCR9 specific antibody with high binding affinity.
- Anti-CCR9 antibody IOMX-0235 efficiently depletes CCR9⁺ cells, both *in vitro* and *in vivo.*
- IOMX-0235 is fully cynomolgus monkey cross-reactive to facilitate *in vivo* efficacy and toxicology testing.
- IOMX-0235 is ready to enter IND-enabling activities.