Insights into Technical Development of LILRB1 and LILRB2 Cross-Specific Antibody IOMX-0675 on its Way to the Clinic

Stefan Kaden, Jonas Schilz, Stephanie Seifert, Madleen Schübel, Bettina Langer, Michal Swiat, Christina A. Hartl, Ilona-Petra Maser, Maximilian Aigner and Simone Friedrich

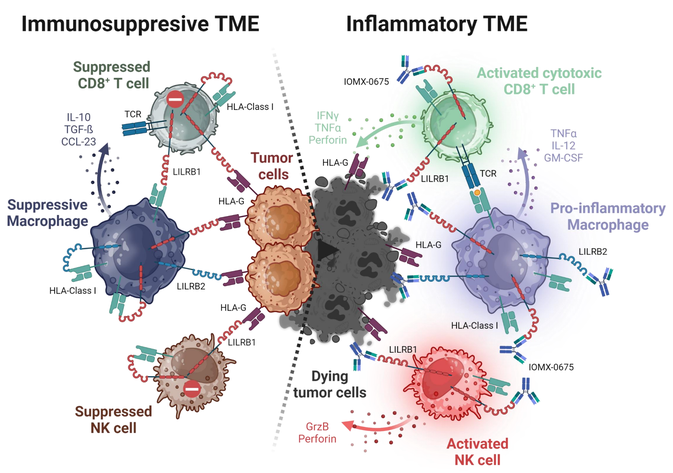
iOmx Therapeutics AG, Martinsried/Munich, Germany

Introduction

Aiming at reprogramming immunosuppressive myeloid cells and restoration of cytotoxic T cell activity in the tumor microenvironment, we initiated discovery efforts focusing on cross-specific antibodies selectively binding to the inhibitory leukocyte immunoglobulin-like receptors (LILR) LILRB1 (ILT2) and LILRB2 (ILT4), while sparing the closely related immuno-activating LILR family members LILRA1 and LILRA3.

We identified the fully human, Fc-silenced, cross-specific antibody IOMX-0675 with highly differentiated binding profile that demonstrates remarkable superior potency in various biochemical and *in vitro* functional assays compared to clinical competitors targeting the same pathway. IOMX-0675 effectively blocks LILRB1 and LILRB2 from binding to their natural, mutually shared ligands which ones are classical and non-classical MHC-I molecules, e.g. HLA-G. In immune cell co-cultures, IOMX-0675 reprograms immunosuppressive macrophages in a dose-dependent manner, restoring lymphoid immune cell function. Our dual-targeting antibody also boosts macrophage phagocytosis and pro-inflammatory cytokine secretion from stimulated PBMCs in whole blood. Finally, IOMX-0675 showed potent anti-tumor effects and modulated tumor-associated macrophages in an aggressive CD34⁺ stem cell-engrafted humanized mouse melanoma model, echoing *in vitro* results.

In parallel to single out an antibody candidate with outstanding biological function, we performed a thorough developability assessment to assess the feasibility of the technical development of potential candidates. We herein illustrate our strategy to funnel down candidates by challenging their manufacturability and stability as well as briefly touching on major safety and efficacy aspects, leading to the selection of IOMX-0675 and paving its way to the clinic.



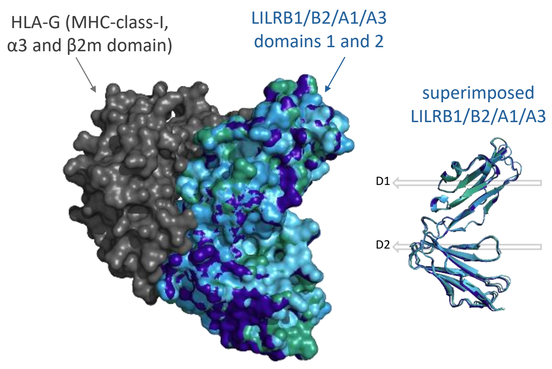
Conclusion

- IOMX-0675 was identified by driving *de novo* phage display selections, focused affinity maturation and effective functional screening.
- IOMX-0675 is a fully human, Fc-silenced, cross-specific IgG1 antibody binding with high affinity to the immunosuppressive receptors LILRB1 and LILRB2, while sparing the closely related immuno-activating LILR family members LILRA1 and LILRA3.
- IOMX-0675 offers highly preferrable prerequisites for technical development as proven in our manufacturability and stability assessment including manufacturing process oriented small-scale productions and quality assessment after physical and mechanical stress conditions.
- IOMX-0675 demonstrated best-in class potential in our efficacy and safety assessment including *in silico*, *in vitro* and *in vivo* assays.
- Clinical Trial Application (CTA) for IOMX-0675 was filed and is currently under review at the authorities.

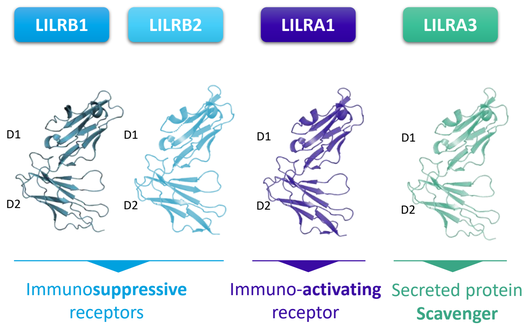
Selectively Tuning a Network of Immune-Modulatory Receptors: Challenge Accepted

- Leukocyte immunoglobulin-like receptors (LILR) share classical and non-classical MHC-I molecules (e.g. HLA-G) (Fig. 1A)
- High structural and sequence homology between immunosuppressive LILRB1/2 receptors and immuno-activating LILRA1/3 receptors (Fig. 1B)
- Challenge: identify candidates selectively binding inhibitory receptors LILRB1 and LILRB2 while sparing immuno-activating LILRA1 and LILRA3
- *De novo* phage display selections, affinity maturation, functional screening and developability assessment led to the identification of six lead molecules including IOMX-0675 (Fig. 1C)
- IOMX-0675 demonstrates an exceptionally differentiated binding profile with high affinity to immunosuppressive LILRB1 and LILRB2 receptors (Fig. 1D)
- IOMX-0675 provides superior blockade of the HLA-G – LILRB1/2 ligand–receptor interaction compared to a competitor antibody (Fig. 1E)

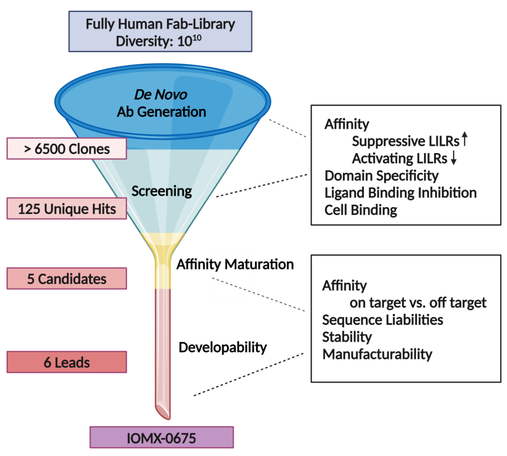
A) Interaction between LILRs and its ligand



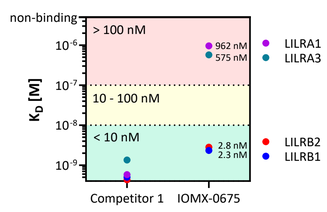
B) High structural homology between LILRB- and LILRA-family members



C) Technical route to IOMX-0675



D) Highly differentiated binding profile of IOMX-0675



E) Disruption of the HLA-G – LILRB1/2 ligand-receptor interaction

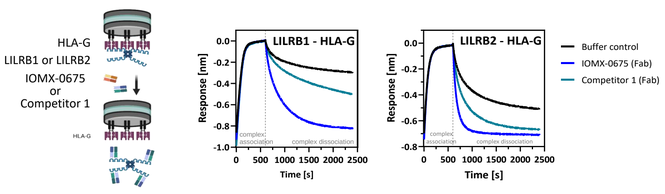


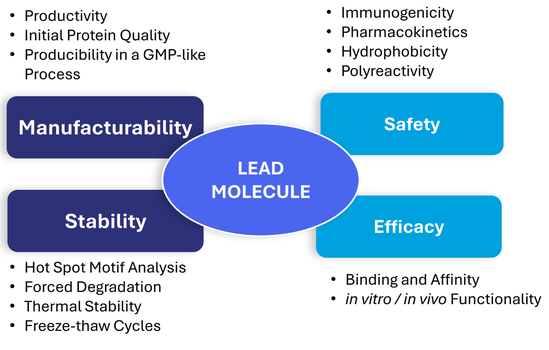
FIGURE 1

A) Crystal structure derived surface representation model of the interaction between HLA-G subdomains (MHC-class-I, α3 and β2m) (*grey*) with the two N-terminal Ig-like domains of LILRB1 (*blue*) and superimposed with the respective AlphaFold predicted structures for LILRB2/A1/A3 domains D1 and D2 (*light blue/purple/green*). **B)** AlphaFold predicted cartoon representation model of the corresponding domains of LILRB1, LILRB2, LILRA1 and LILRA3 demonstrate a high structural homology. **C)** Strategy to funnel down *de novo* panning output to IOMX-0675 as lead molecule. **D)** Differential binding profile of IOMX-0675 against LILRB1/2 and LILRA1/3 compared to clinical competitor antibody (Competitor 1). Monovalent affinities of both antibodies to LILRB1/2 and LILRA1/3 were measured by biolayer interferometry (BLI) on an Octet Red96e and fitted using a 1:1 binding model. **E)** Fab fragments from IOMX-0675 or Competitor 1 and assay buffer control were used to examine the displacement of precoated LILRB1/2 from the HLA-G interaction site by BLI as shown in the graphical assay illustration.

Developability Assessment: the Idea behind

- Developability assessment of biotherapeutics is considered as a powerful risk mitigation tool; data are used to select and deselect candidates and assess the risks for developing the lead molecule
- Developability is a crosstalk of data gathered by all discovery disciplines addressing manufacturability, stability, safety and efficacy (Fig. 2A)
- Manufacturability and stability data in early discovery phase are focused on sequence analysis and conditions present during the technical generation of clinical state material in a CMC process (Fig. 2B)
- Efficacy and safety data rely on prediction models forecasting the potential behavior of a drug candidate in humans and are substantiated by cell culture-based experiments and animal studies

A) Developability Assays to Identify Robust Lead Molecules



B) Manufacturability and Stability Parameters Challenged during Basic Production Processes

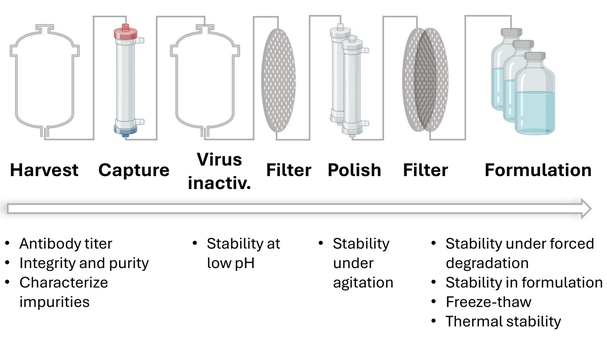


FIGURE 2

A) Selection of the lead molecule is based on manufacturability and stability, safety and efficacy in early discovery and development. Each discipline addresses different characteristics of the molecule. **B)** Schematic drawing of a GMP Process and parameters to be addressed in **manufacturability** and stability assays that could be derived from conditions present in a GMP process.

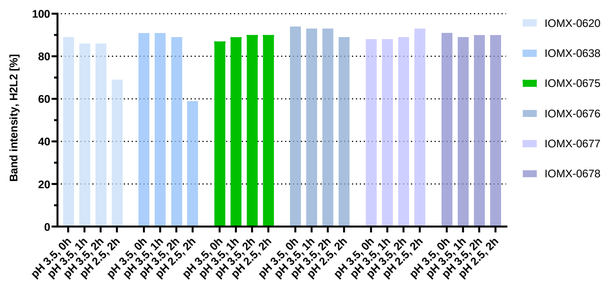
Manufacturability & Stability

- Amongst the final six lead molecules, IOMX-0675 showed highly preferrable quality and yield already after production in discovery scale platforms (Fig. 3A)
- IOMX-0675 showed no liabilities after incubation at low pH as determined using SDS-PAGE (Fig. 3B), HP-SEC, BLI (Octet) (*data not shown*) mimicking the virus inactivation step in an industrial scale GMP DSP
- Lead candidates passed forced degradation and stability study without significant findings and confirmed histidine-based buffer as suitable starting point for formulation development (Fig. 3C)

A) Productivity and Quality Assessment After Affinity Capture

Candidate ID	HEK cells		CHO cells	
	Monomer content [%]	Vol. yield [mg/L]	Monomer content [%]	Vol. yield [mg/L]
IOMX-0620	95.7	263	96.2	141
IOMX-0638	95.8	63.6	97.4	133
IOMX-0675	97.9	257	97.3	110
IOMX-0676	98.8	25.6	96.5	140
IOMX-0677	NA	185	98.2	131
IOMX-0678	91.8	257	99.4	135

B) Stability and Integrity at low pH



C) Forced Degradation and Stability Study

Candidate ID	FORCED DEGRADATION (incubation at up to 40 °C for 28 days in histidine-based buffer)				STORAGE STABILITY - Freeze/Thaw	MECHANICAL STABILITY - Shear Stress	Overall Assessment
	Relative active concentration (Octet)	Aggregate formation (HP-SEC)	Fragmentation and integrity (SDS-PAGE)	Hot spot modifications (MS analysis)			
	Potency loss ≤10% on both targets [yes/no]	HMW ≤10% [yes/no]	Proteolytic products ≤10% [yes/no]	Affected cleavages or modifications observed [yes/no]			
IOMX-0620	yes	yes	yes	yes	+	+	+
IOMX-0638	yes	yes	yes	n.a.	+	+	+
IOMX-0675	yes	yes	yes	yes (Fc only)	+	+	++
IOMX-0676	yes	yes	yes	n.a.	+	+	+
IOMX-0677	yes	yes	yes	yes	+	+	+
IOMX-0678	yes	yes	yes	yes	+	+	+

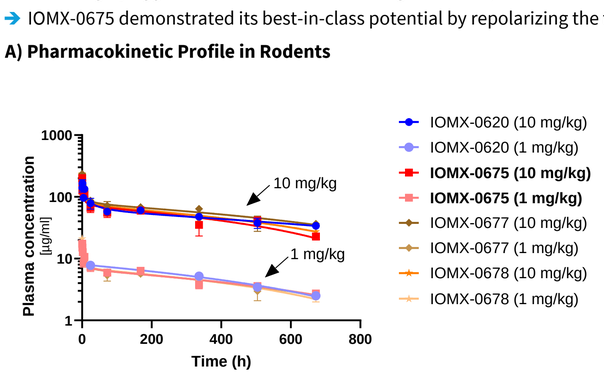
FIGURE 3

A) Production data (HEK and CHO, n=1) after Protein A purification: monomer content as described by main target peak derived from analytical HP-SEC and volumetric yield as calculated from area under the curve (Protein A affinity chromatography). n.a.: data not available **B)** Lead molecules were subjected to pH 3.5 conditions for up to two hours to mimic their behavior in an industrial scale downstream process during virus inactivation step. Percentage of intact IgG (H2L2)-species in pH-treated samples assessed in SDS-PAGE under non-reducing conditions is shown. Sample conditioning at pH 2.5 for two hours served as maximum stress control. **B)** Lead molecules were formulated in Histidine-based buffer and incubated at elevated temperatures for up to 28 days. Aliquots were subjected to repeated freeze/thaw cycles and shear stress was applied by orbital shaking for 48 hours at room temperature. Samples were analyzed for functional integrity (relative active concentration, Octet), aggregation, fragmentation and degradation propensity (SDS-PAGE, HP-SEC) and affected PTM sites (mass spectrometry).

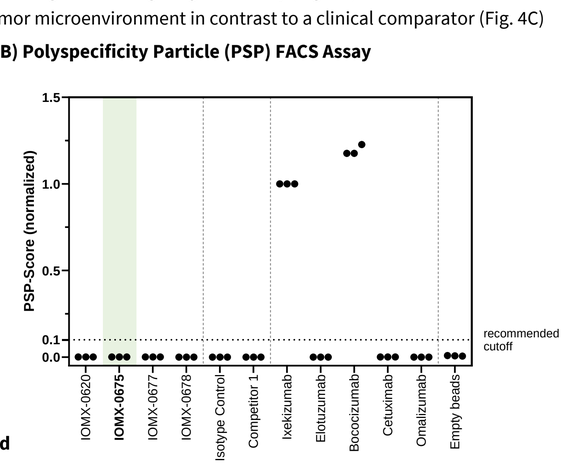
Safety & Efficacy

- Lead candidates showed low immunogenicity risk in line with human antibody sequences as determined using Lonza's Epibase *in silico* T cell epitope prediction platform (*data not shown*)
- All tested lead molecules demonstrated a linear, dose-dependent pharmacokinetic profile in rodents (Fig. 4A)
- Lead molecules showed comparable hydrophobicity profile to naturally occurring immunoglobulins (*data not shown*)
- In comparison to reference and control molecules, iOmx leads showed no interaction in Polyreactivity FACS assay (Fig. 4B)
- Inhibition of the LILRB1/2 pathway by IOMX-0675 or competitor 1 enhanced T cell activation *in vivo* (Fig. 4C)
- In the highly aggressive A375 melanoma xenograft model, IOMX-0675 showed significant single-agent activity (Fig. 4C)
- IOMX-0675 demonstrated its best-in-class potential by repolarizing the tumor microenvironment in contrast to a clinical comparator (Fig. 4C)

A) Pharmacokinetic Profile in Rodents



B) Polyspecificity Particle (PSP) FACS Assay



C) *In vivo* anti-tumor study in a CD34⁺ fully humanized, myeloid engrafted A375 melanoma model

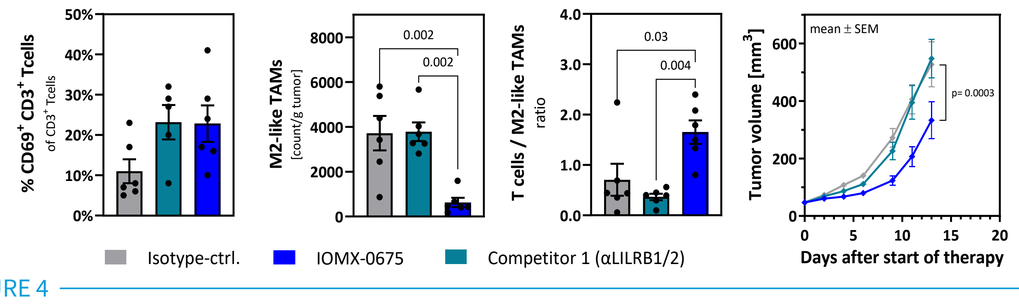


FIGURE 4

A) Pharmacokinetics analysis for single intravenous administration of 1 or 10 mg/kg of IOMX-0675 in C57Bl6 animals. EDTA plasma samples from three animals per time point were analyzed by ELISA. **B)** Summary of Polyspecificity Particle (PSP) FACS Assay (Makowski et al.) results, with calculated normalized PSP-scores of all tested lead molecules as well as isotype control, competitor molecule and internal assay references (Ixekizumab, Elotuzumab, Bococizumab (*inhouse generated material*), Cetuximab and Omalizumab (*obtained from pharmacy and rebuffered to PBS*), as well as empty protein A beads). Samples were measured in triplicates. **C)** A375 melanoma tumor cells were implanted subcutaneously into NOG-EXL mice, fully humanized with CD34⁺ stem cells from two donors. Randomized animals were treated twice weekly with 20 mg/kg IOMX-0675, competitor 1, or corresponding isotype control antibody. Tumor infiltrating immune cells were analyzed by flow cytometry. Activated CD69⁺ CD3⁺ T cells, CD68⁺CD11c⁻HLA-DR⁻ M2-like macrophages and the ratio of CD3⁺ T cells to M2-like macrophages are shown as mean ± SEM.

mailto: Stefan.Kaden@iomx.com

QR code

References

Makowski, E. K., Wu, L., Desai, A. A., & Tessier, P. M. (2021). Highly sensitive detection of antibody nonspecific interactions using flow cytometry. mAbs, 13(1). https://doi.org/10.1080/19420862.2021.1951426

Abbreviations

LILR: inhibitory leukocyte immunoglobulin-like receptors; CMC: chemistry, manufacturing and control; technical process to develop biotherapeutics; GMP: good manufacturing practice; Virus inactiv.: virus inactivation step; low pH incubation to inactivate enveloped viruses; SDS-PAGE: sodium dodecyl sulfate based polyacrylamide agarose gel electrophoresis; HP-SEC: analytical high performance size exclusion chromatography; BLI: bio-layer interferometry determined using Octet (Sartorius), DSP: downstream processing; HEK: human embryonal kidney cells; CHO: Chinese hamster ovarian cells; MS: mass spectrometry; PTM: posttranslational modification, PSP: Polyspecificity article, Fig.: figure