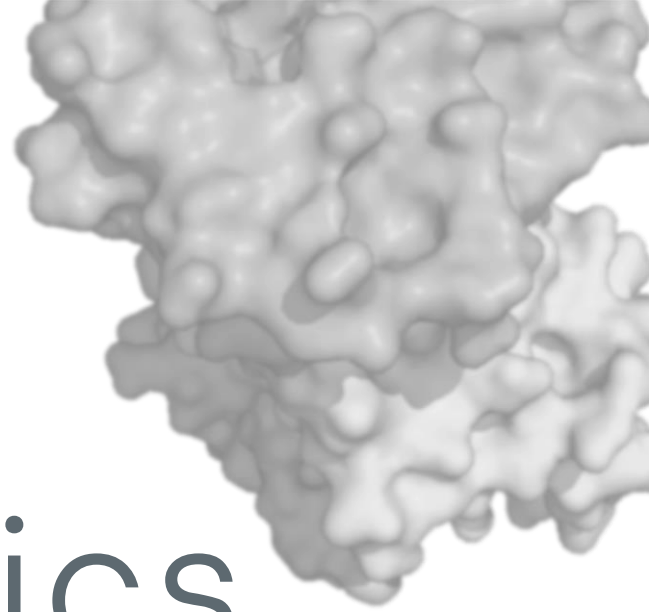


Toleris Biotherapeutics

AutoImmunity Modifying Biologicals - inspired by pregnancy

non-confidential slide deck 06/2025

overview

Toleris Biotherapeutics: about us

- established January 2024
  spin-off from the University of Würzburg, Germany
- fully owned by founders
  Prof. Jürgen Engel (CEO), Dr. Valentin Bruttel (CSO), Prof. Jörg Wischhusen (Chief Scientific Advisor)
- assets: worldwide license for innovative tolerization platform
  AutoImmunity Modifying Biologicals (AIM Bios)
- proof of concept in 6 animal models in 3 indications
- development candidates for MS/MOGAD, NMOSD and type 1 diabetes
- intellectual property:
  - platform patent filed in major countries in 2017 (granted in KR, CN, AU)
  - MOGAD/MS, NMOSD, PD and type 1 diabetes applications in 2022 (nationalized ~30 countries) / 2023

Toleris team & funding

Toleris management team

Jürgen Engel

CEO

strategic consultant, former CEO of Nasdaq listed company, successful development of several drugs, in- and out-licensing, M&A, public financing

Valentin Bruttel

CSO

immunologist and bioengineer, co-inventor AIM platform technology

Jörg Wischhusen

CSA

chief scientific advisor, PI, co-inventor AIM platform technology, Scientific founder Catalym

Markus Haake

SVP Preclin Dev

drug discovery and non-clinical development, co-founder Catalym

funding/awards:

eXIST

Bundesministerium für Wirtschaft und Klimaschutz

m⁴ Award

Bayerisches Staatsministerium für Wirtschaft, Landesentwicklung und Energie

Innovationspreis 2022

BioRegionen in Deutschland

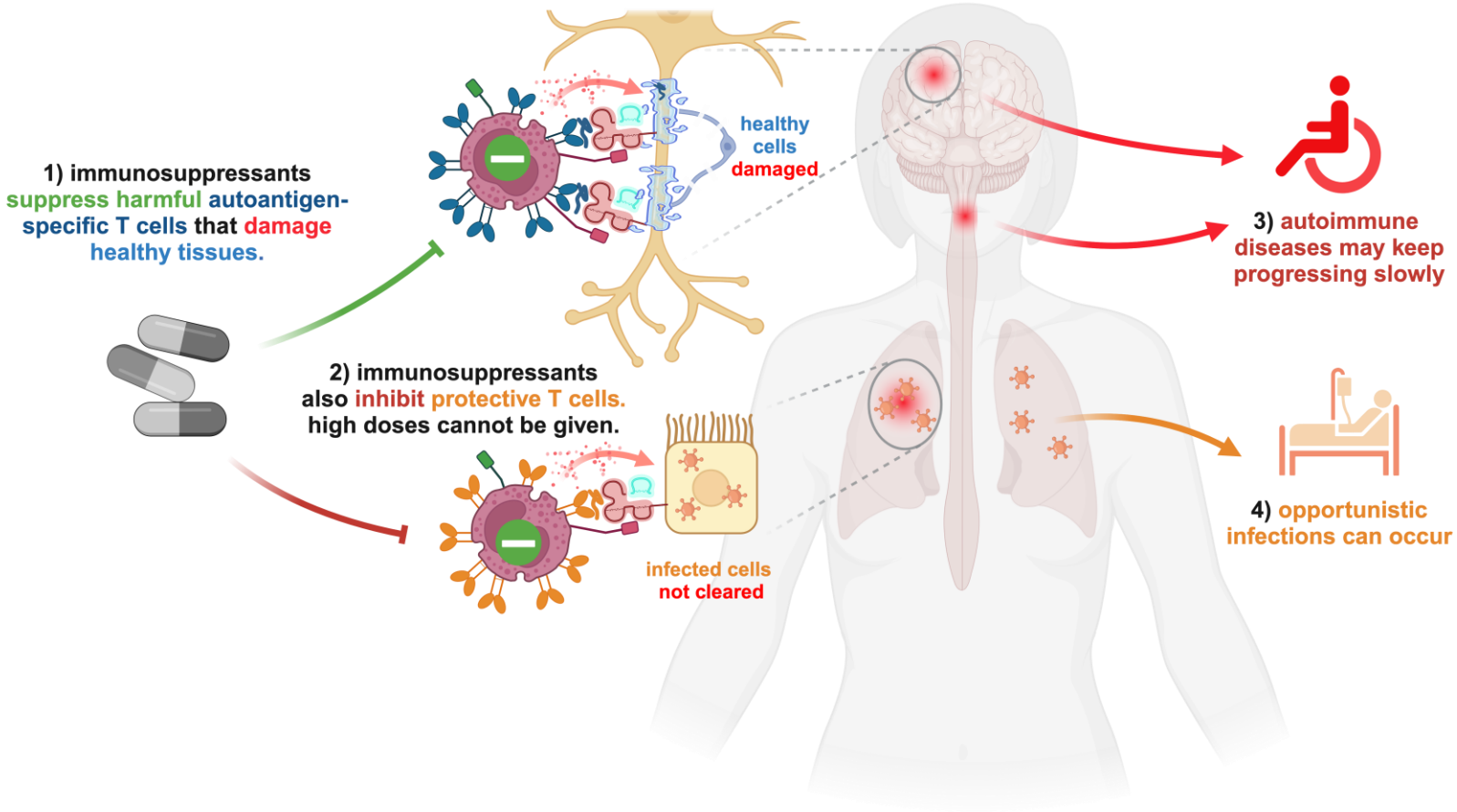
Collaboration partners: Prof. Michael Levy (Harvard Medical School), Prof. Friedemann Paul (Charité Berlin)

autoimmune disease burden

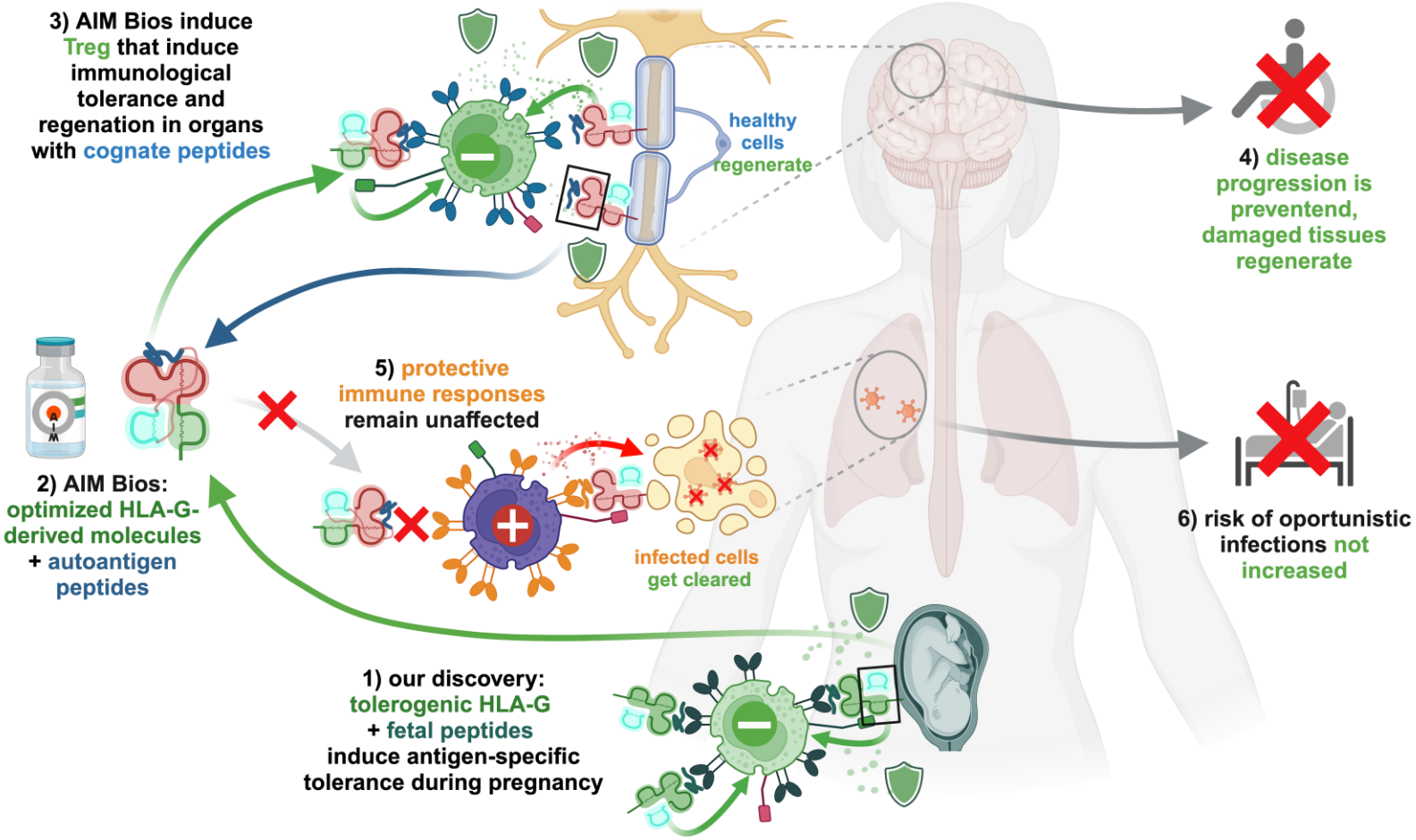
- **>80 recognized autoimmune diseases** (https://www.niaid.nih.gov/diseases-conditions/autoimmune-diseases)
- **~10% of the world population are affected** (10.1136/annrheumdis-2023-eular.4269)
- **growing incidence and prevalence** (https://pubs.sciepub.com/ijcd/3/4/8/index.html)
- **significant economic and health impacts,** morbidity and disability (PMID: 28121529)
- most **common** autoimmune diseases include:
  - rheumatoid arthritis (RA)
  - systemic lupus erythematosus (SLE)
  - **type 1 diabetes (T1D)**
  - **multiple sclerosis (MS) and related diseases (MOGAD, NMOSD)**
  - autoimmune thyroid diseases

introduction & idea

the key challenge in autoimmune therapy: precision

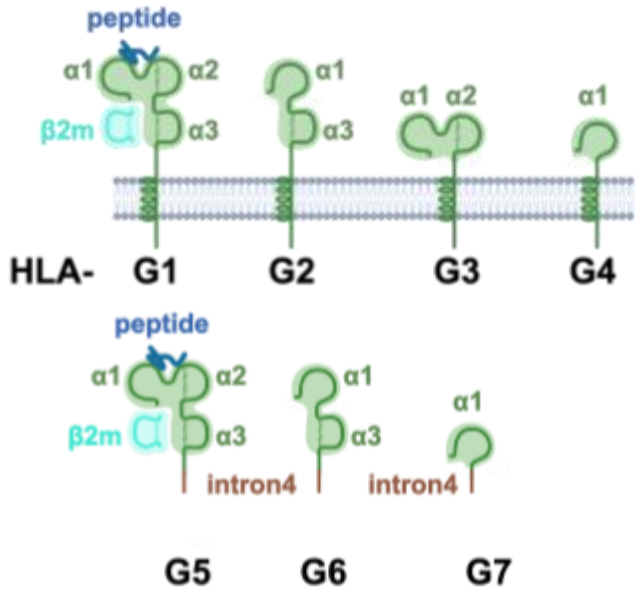


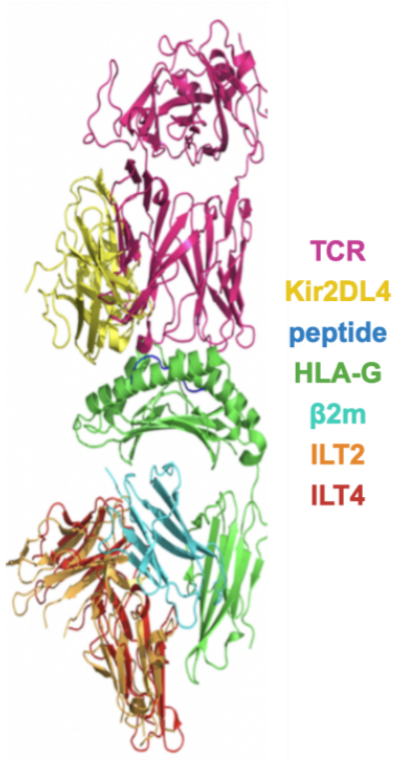
our solution: mimicking natural targeted tolerance

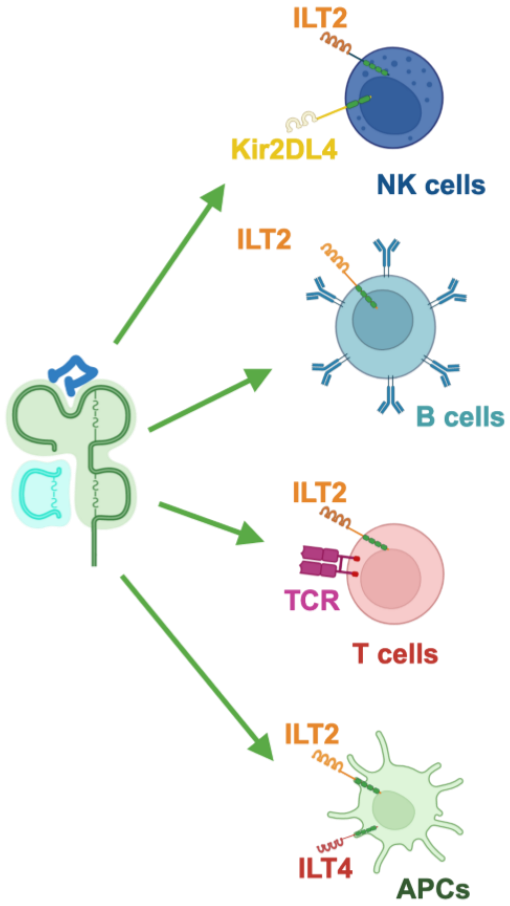


HLA-G: isoforms and known effects on immune cells

HLA-G has numerous published immunosuppressive effects on immune cells. Unlike other MHCs, HLA-G possesses hardly any allelic variants that affect the protein sequence. Both membrane-bound and soluble variants are known.







inhibition of
- **proliferation**
- **cell-mediated lysis**
- **IFN-γ secretion**

induction of transendothelial migration

inhibition of antibody secretion

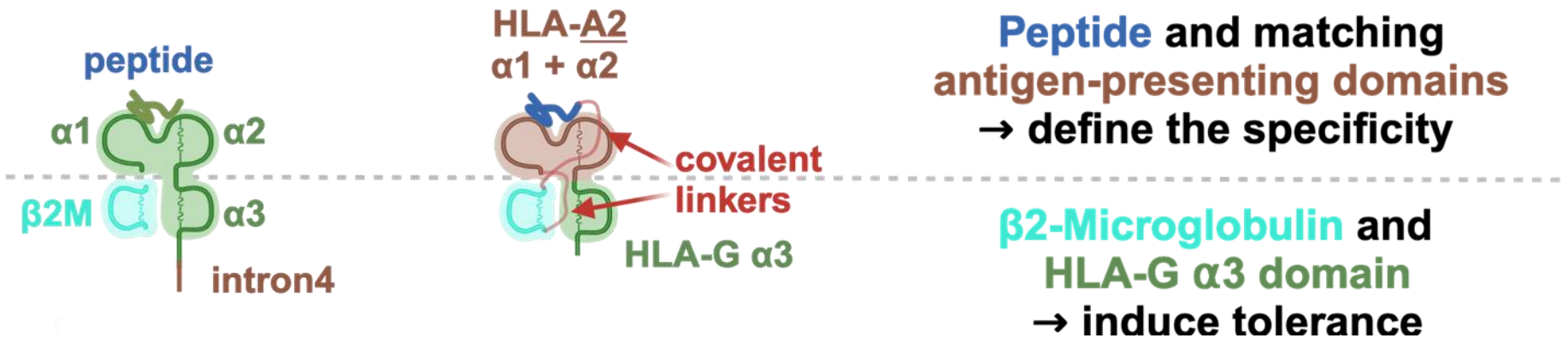
inhibition of
- **proliferation**
- **cell-mediated lysis**
- **IFN-γ secretion**

induction of
- **TH2-type cytokine profile**
- **Treg**

induction of tolerogenic APC

AIM Bios: adaptable to induce tolerance to any protein

AIM Bios are soluble, HLA-G derived molecules in which the variable presented peptide antigen, the presenting domains, β2-microglobulin and the tolerance-inducing HLA-G α3 domain are covalently linked.



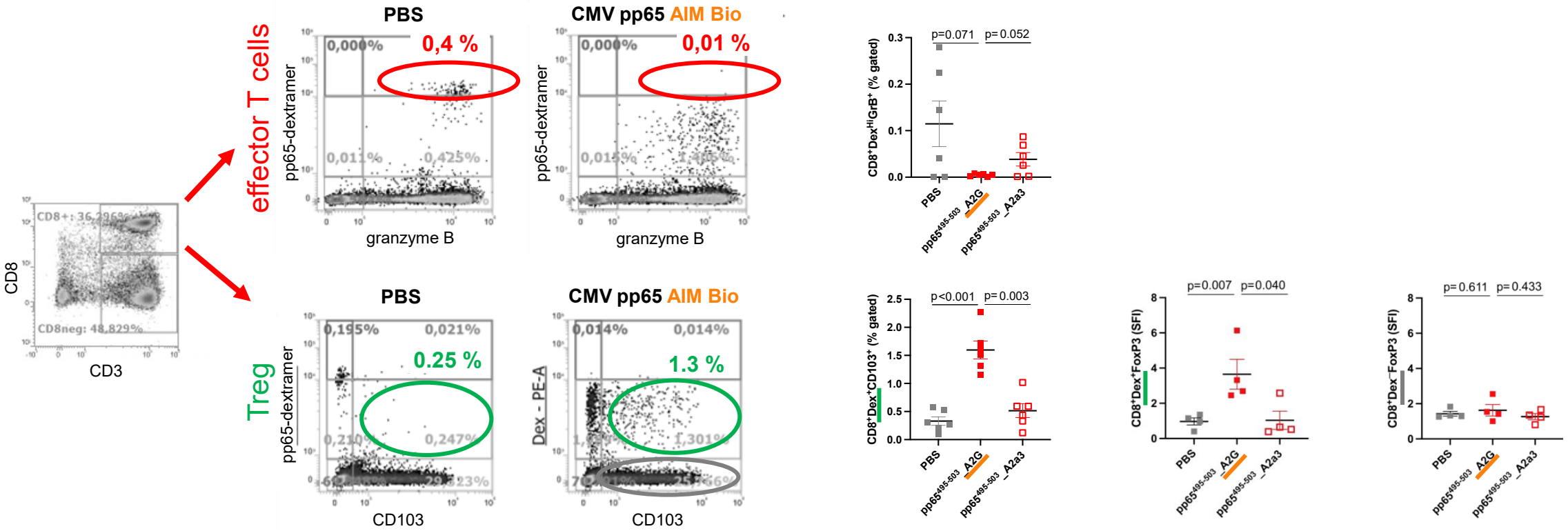
HLA-G5

chimeric
AutoImmunity
Modifying Biological
(AIM Bio)

mode of action & proof of concept

AIM Bios reduce human cognate Teff and induce Treg

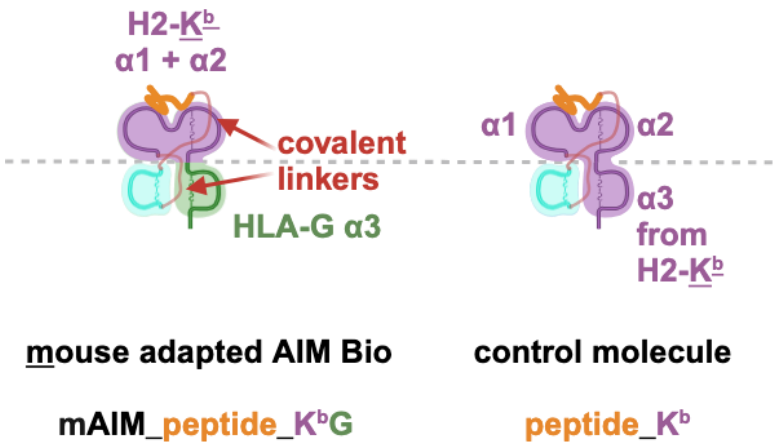
To investigate AIM Bio effects on human T cell responses, healthy blood donors were screened for established CD8⁺ T cell responses against a commonly targeted viral peptide. 5 µg/ml AIM Bios presenting this peptide ($pp65^{495-503}$_A2G) or control molecules lacking the immunosuppressive HLA-G α3 domain ($pp65^{495-503}$_A2a3) were added to PBMCs of such blood donors. Treatment with **AIM Bios strongly reduced the frequency of cognate effector T cells and induced cognate Foxp3⁺ Treg cells**. In non-cognate T cells, Foxp3 was not induced.



PoC: AIM Bios induce protective, antigen specific Treg

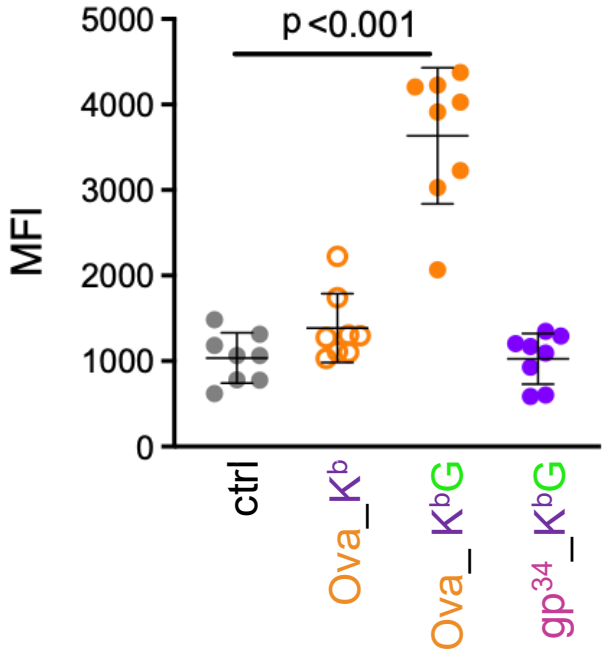
mouse-adapted AIM Bios

Different human T cells can detect billions of antigens. In contrast, in certain transgenic mice, all T cells recognize the same antigen, which simplifies studies. We thus constructed mouse-adapted AIM Bios and control molecules.



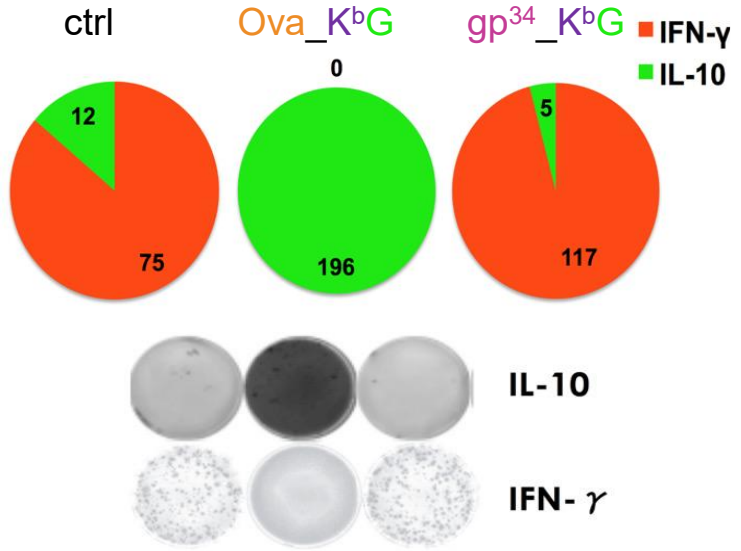
AIM Bios induce CD8+ Treg

OT-I splenocytes treated with mouse-adapted AIM Bios upregulate Treg markers CD122+ (below), FoxP3 and CD103 (not shown).



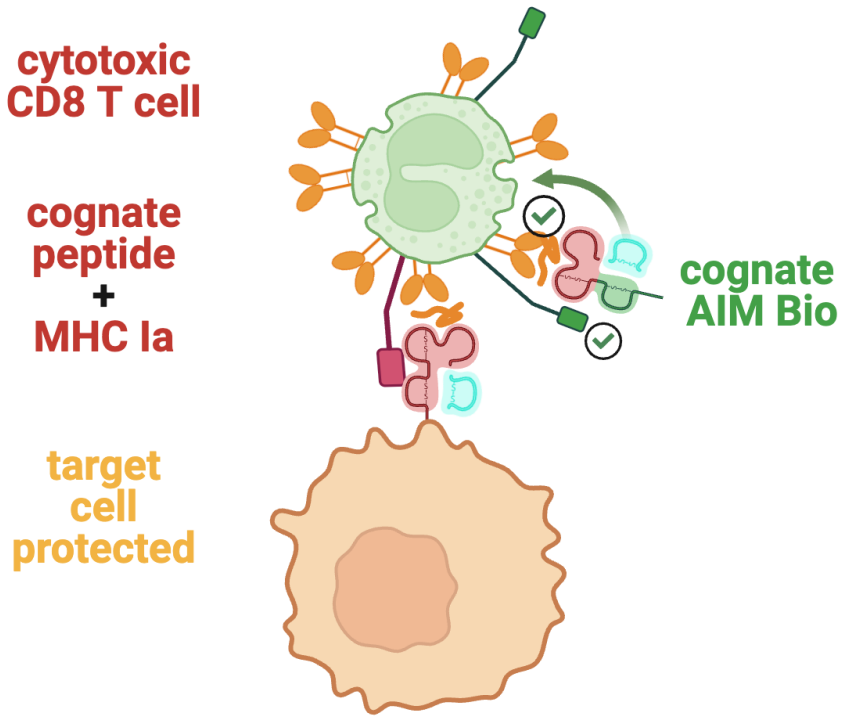
only cognate AIM Bios induce anti-inflammatory cytokines

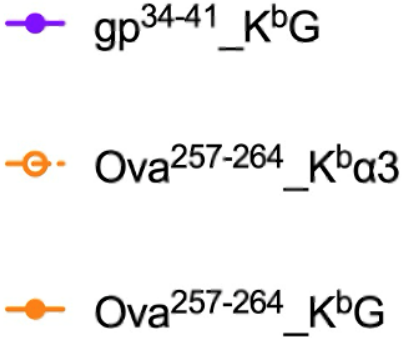
OT-I splenocytes treated with 5 µg/ml Ova AIM Bios for 14 days secrete anti-inflammatory IL-10 rather than pro-inflammatory IFN-γ (ELISpot)

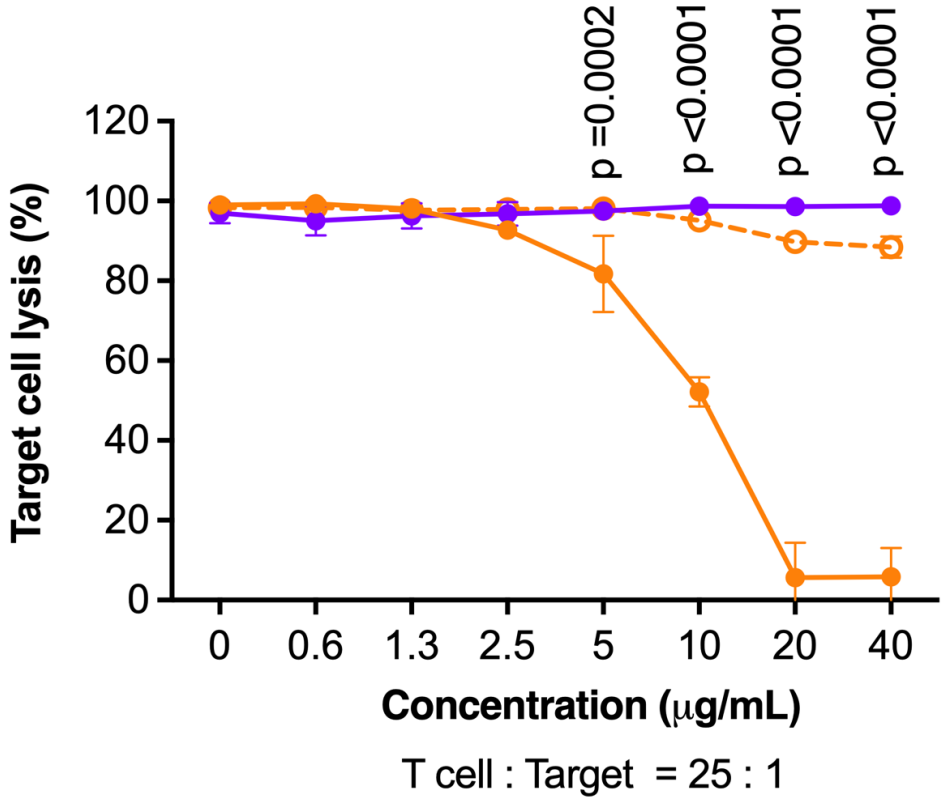


AIM Bios inhibit cytotoxic effects

Activated effector T cells lyse target cells presenting the correct MHC molecules and loaded with cognate peptides. OT-I **T cell-mediated target cell lysis can be completely inhibited in a dose-dependent manner by AIM Bios loaded with the cognate peptide**, while AIM Bios with a different peptide had no effect.

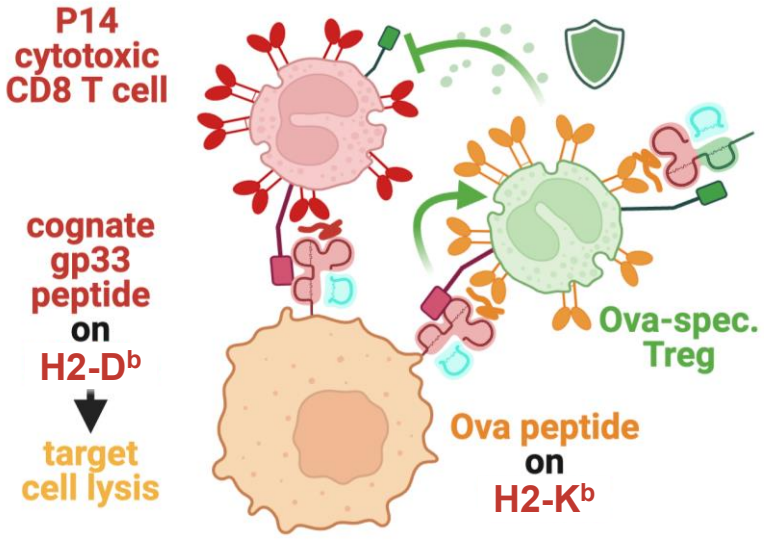




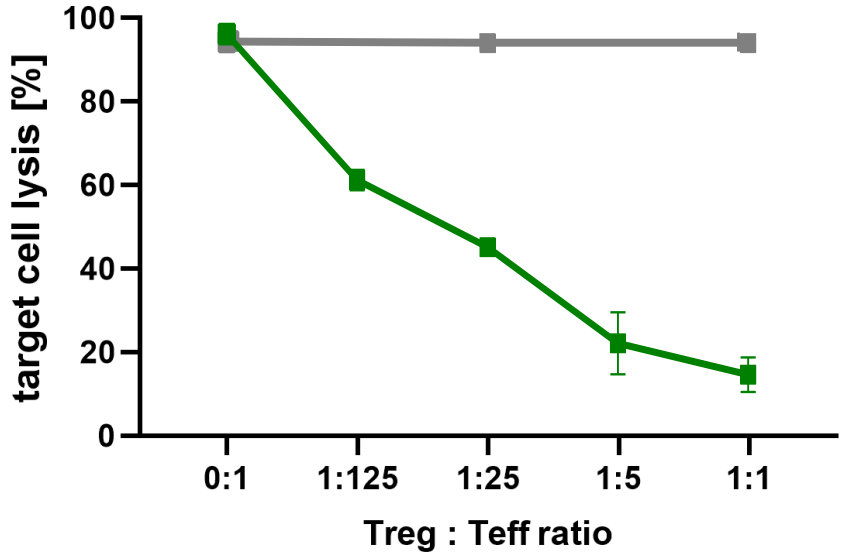


AIM Bios induce potent, antigen-activated Treg

In a second experiment, Ova-tolerance-inducing Tregs were generated via AIM Bios and added to viral gp33 peptide-specific P14 effector T cells. Remarkably, **P14-mediated target cell lysis was strongly suppressed by AIM Bio-induced Tregs, even at extremely low Treg-to-Teff ratios (1:125).** However, this suppression was observed only when the target cells were loaded with the peptide recognized by the Tregs.(bystander-suppression)



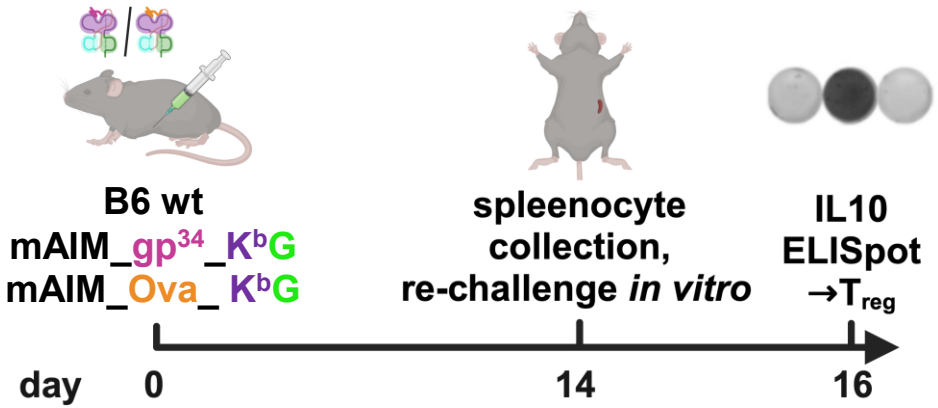
Teff	Treg	peptide on targets
P14	AIM_Ova-OT1	gp^{33}
P14	AIM_Ova-OT1	gp^{33} + Ova



AIM Bios induce antigen specific Treg *in vivo*

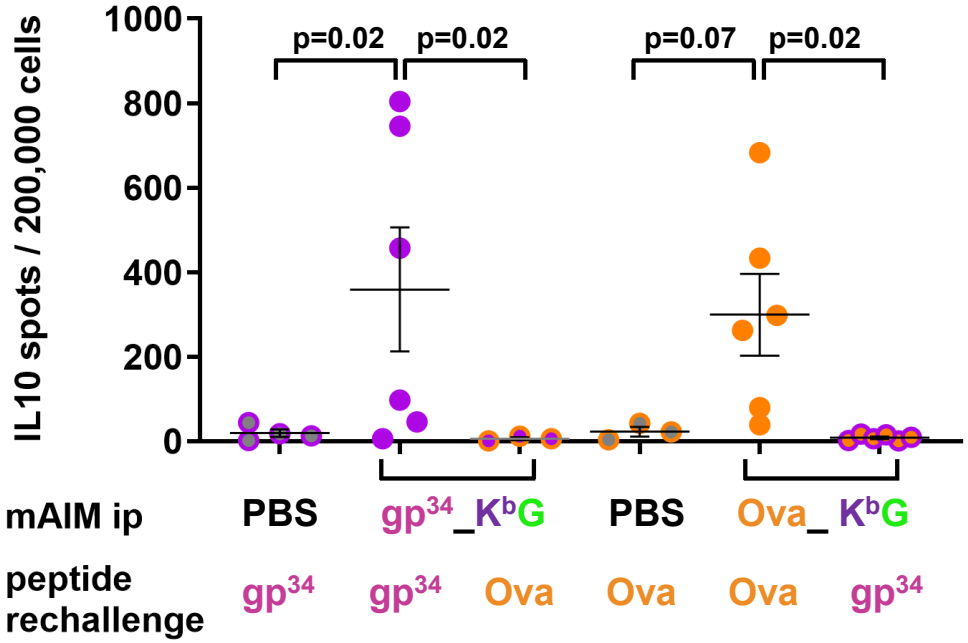
Experimental setup

Mice were injected with AIM Bios inducing tolerance to one of two model peptide antigens.



Results

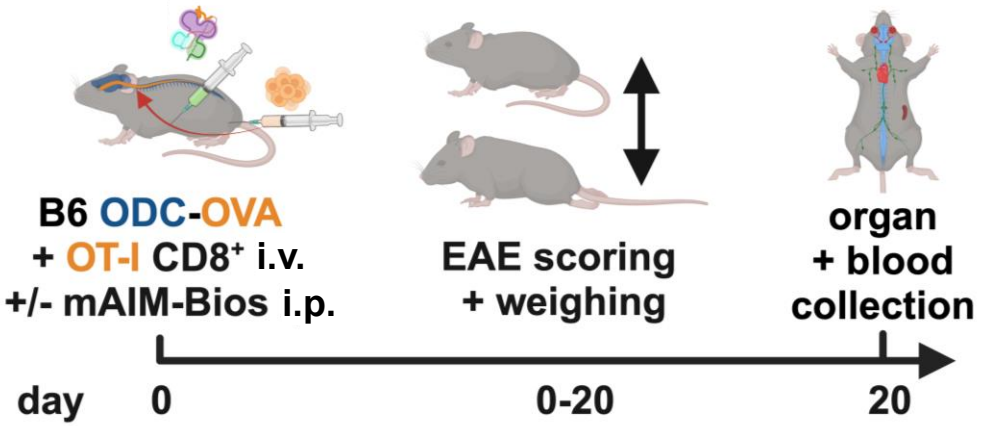
After 14 days, **splenocytes collected from the treated mice secreted immunomodulatory IL10 when re-challenged with the same antigen** as presented on AIM Bios



AIM Bios fully prevent MS symptoms in mice

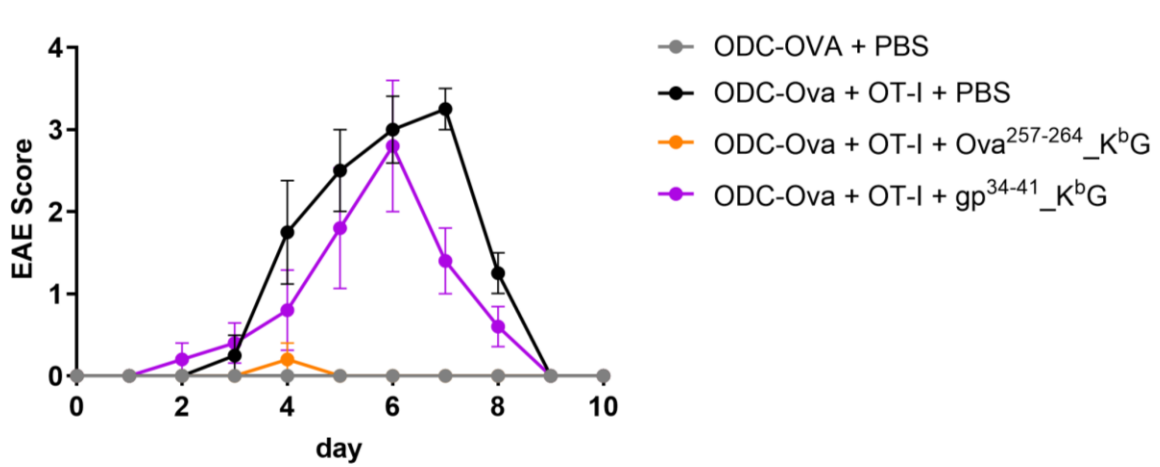
Experimental setup

Oligodendrocytes are attacked in MS patients. Mice expressing the Ova model antigen in oligodendrocytes were injected with Ova-specific T cells and mouse-adapted AIM Bios.



Results

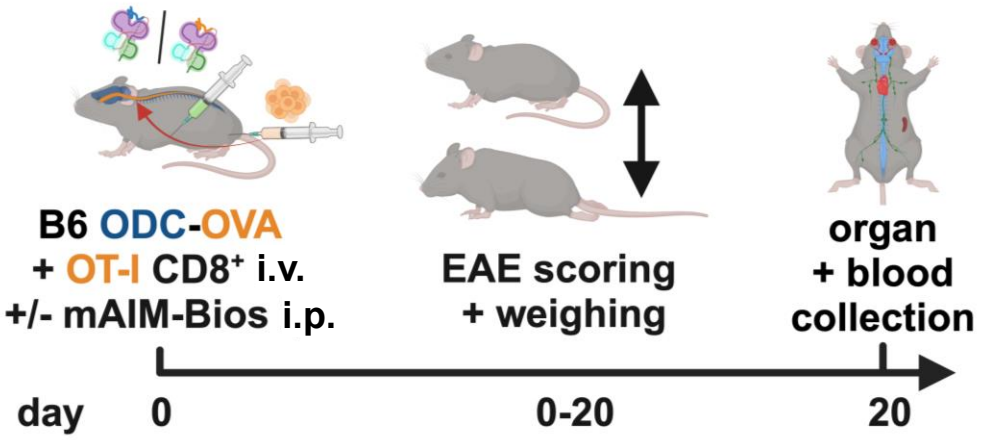
Mice treated with AIM Bios presenting the targeted Ova peptide were completely protected from paralysis (EAE).



AIM Bios induce dominant, organ-specific tolerance

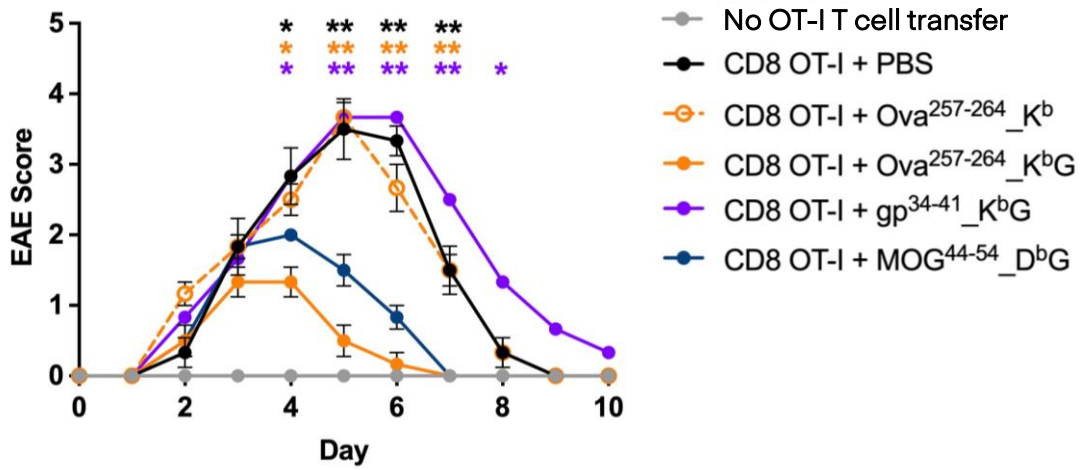
Experimental setup

Oligodendrocytes are attacked in MS patients. Mice expressing the Ova model antigen in oligodendrocytes were injected with Ova-specific T cells and 100µg mouse-adapted AIM Bios.



Results

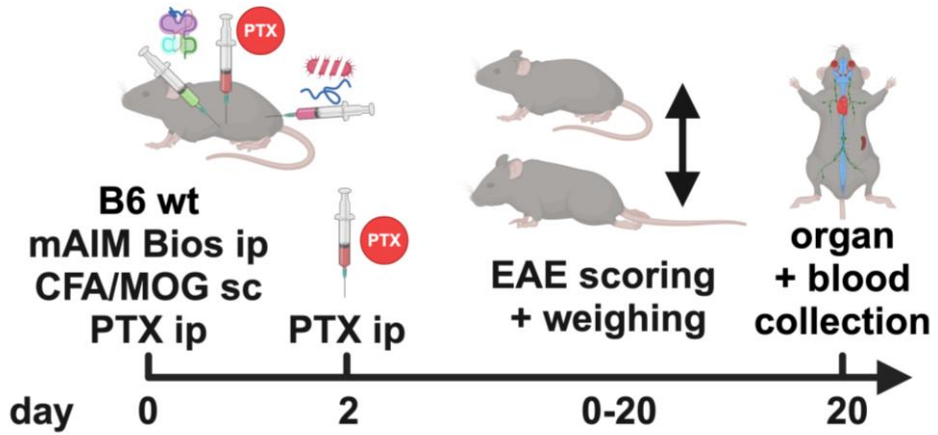
An **AIM Bio inducing tolerance for MOG** (MOG44-54) **conferred almost the same level of protection as an OVA-specific AIM Bio**. Protection depends on the antigen and the HLA-G a3 domain.



murine MOG AIM Bios prevent MOG autoantibodies

Experimental setup

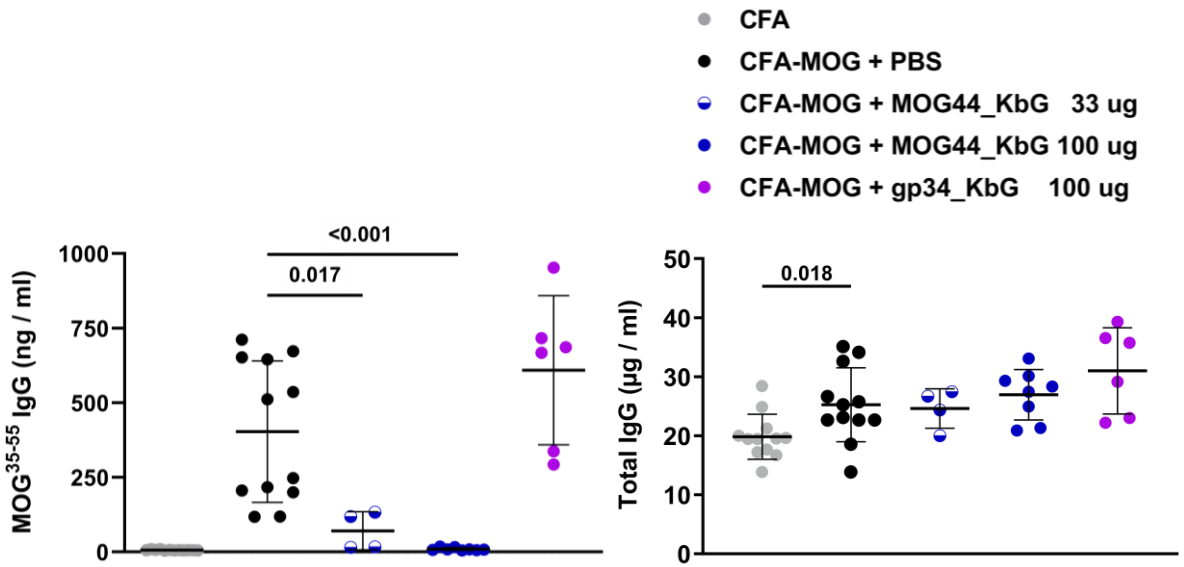
Wildtype mice in which MS-like symptoms (experimental autoimmune encephalitis, EAE) is induced through a MOG peptide (MOG35-55) given in combination with a strong adjuvant and a toxin that opens the blood-brain-barrier were treated with MOG-tolerance inducing and control AIM Bios.



Geraldes, R. et al.,
Nat Rev Neurol **20**, 620–635 (2024)

Results

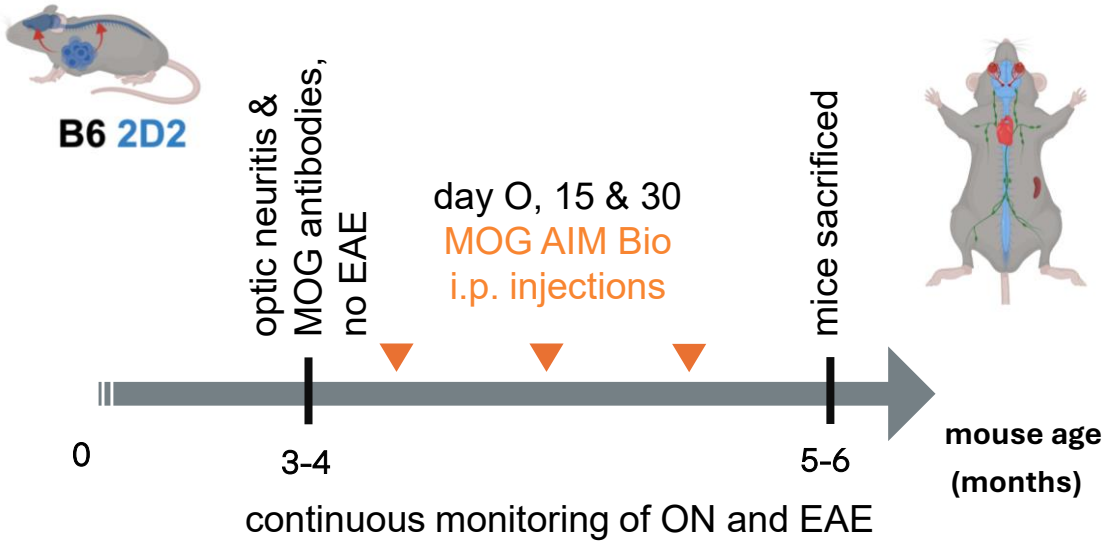
Mice treated with MOG-tolerance inducing AIM Bios developed significantly less symptoms (not shown) and **did not develop MOG-specific autoantibodies**. AIM Bio treatment did not reduce protective antibodies (total IgG).



murine MOG AIM Bios have therapeutic effects

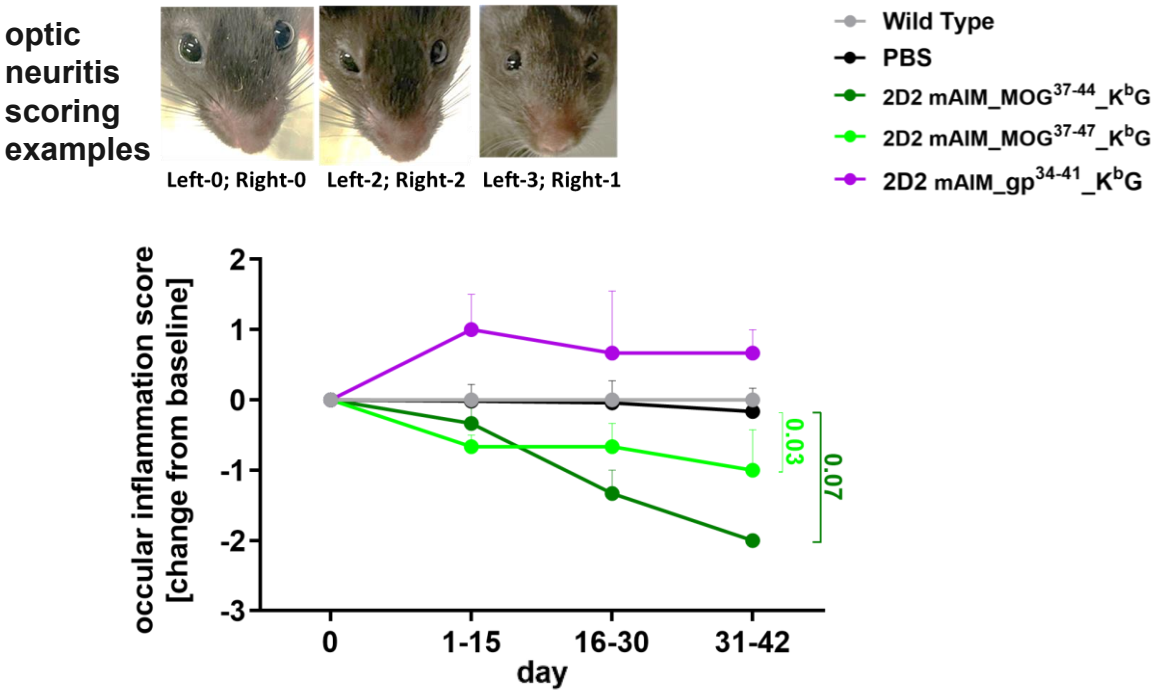
Experimental setup

2D2 transgenic mice express an MHC class II-restricted MOG-specific T cell receptor on all T cells. They spontaneously develop MOG Antibody Disease (MOGAD)-like symptoms including paralysis and optic neuritis (ON). These mice were treated with MOG tolerance-inducing AIM Bios after ON onset (3µg/g).



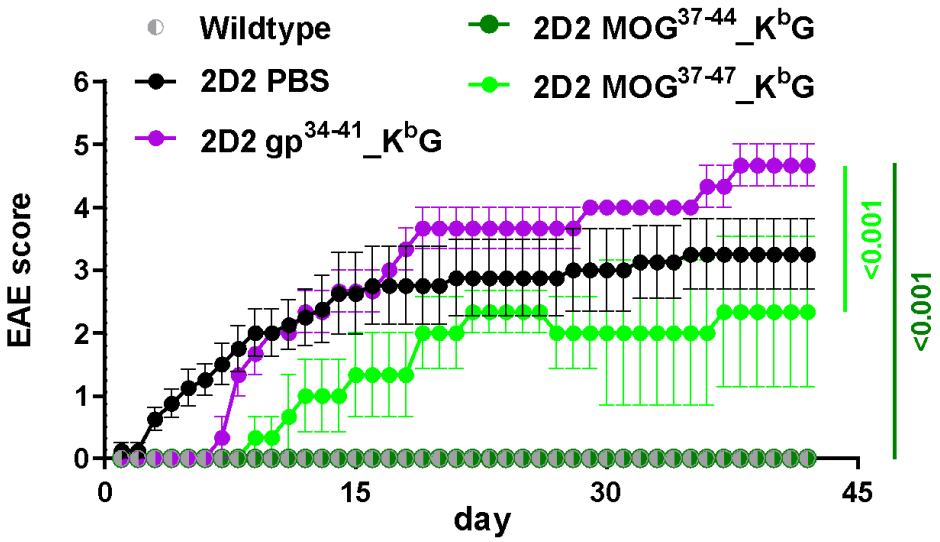
Results

Even after significant swelling of the eyes, a **MOG-tolerance inducing AIM Bios reduced pre-existing optic neuritis** in 2D2 mice.

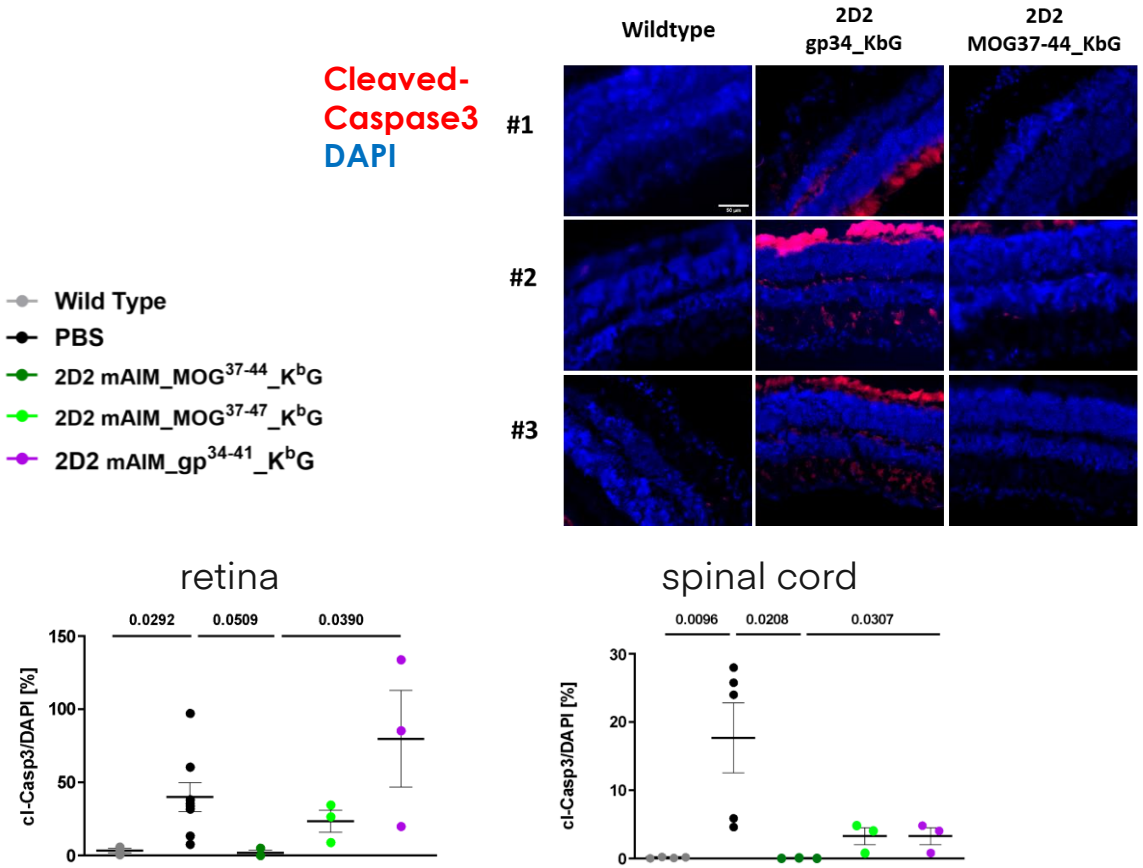


MOG AIM Bios prevent neuron loss and EAE

Correspondingly, **MOG AIM Bios completely protected against later MS symptoms** (EAE)

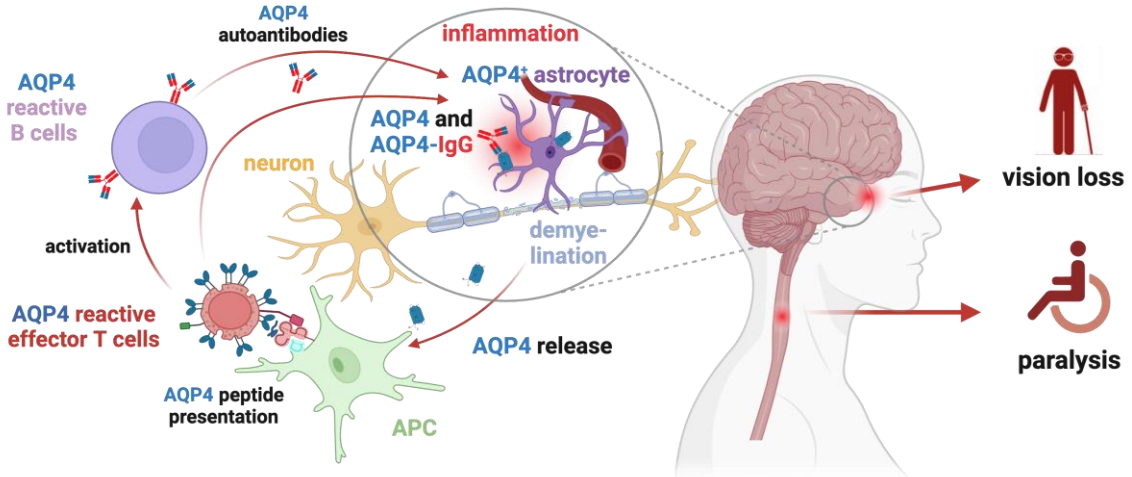


MOG AIM Bios completely prevented cell death (red = apoptosis) in all organs predominantly affected by MS/MOGAD in 2D2 mice.

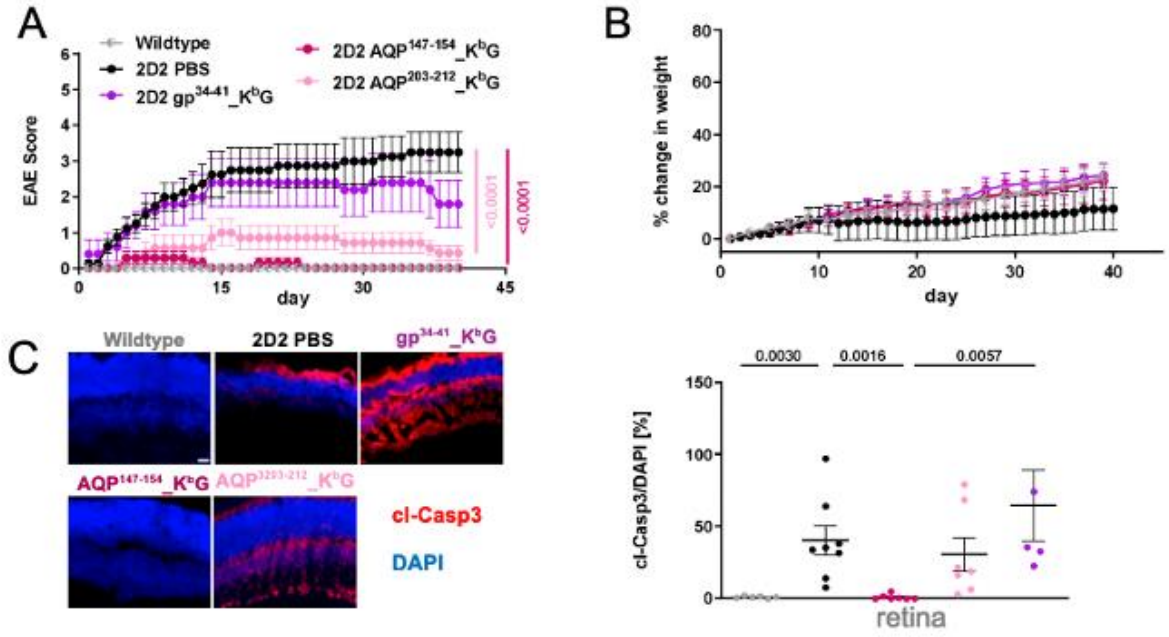


NMOSD AIM Bios prevent EAE and neuron loss

Neuromyelitis Optica Spectrum Disorder (NMOSD) is a severe autoimmune condition caused by aquaporin-4 (AQP4) specific immune cells and antibodies. These drive astrocyte damage and cause smoldering disease despite acute treatment.



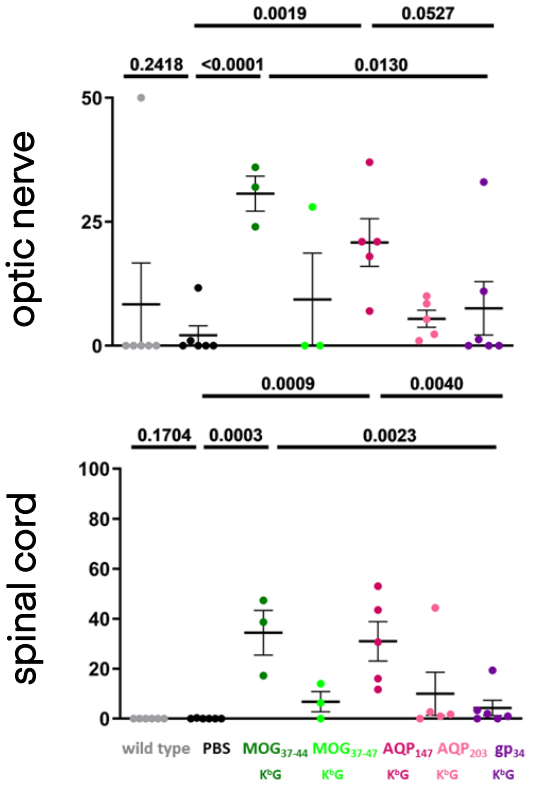
AQP4-specific AIM Bios completely prevent EAE symptoms (A), **weight loss** (B) **and neuron loss in the retina** (C) **and optic nerve and spinal cord** (not shown) in the 2D2 optic neuritis mouse model.



AIM Bios induce Foxp3⁺ CD8⁺ Tregs in target tissues

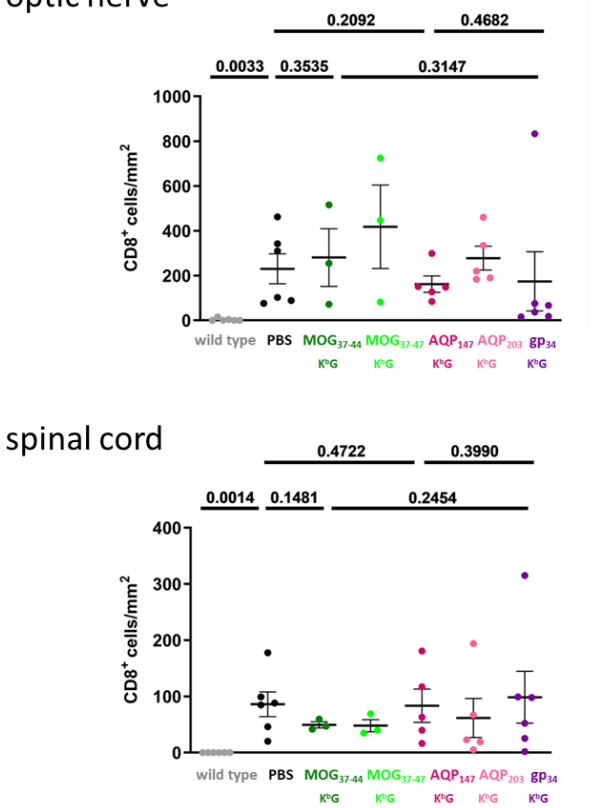
2D2 Mice treated with therapeutic AIM Bios showed a **significant increase in CD8⁺ FoxP3⁺ Treg in spinal cord** (IF).
The highest local Treg induction was achieved by the most effective AIM Bios (MOG_{37-44}_K^bG and AQP_{147}_K^bG.)

CD8⁺ FoxP3⁺ Treg/total CD8⁺ [%]

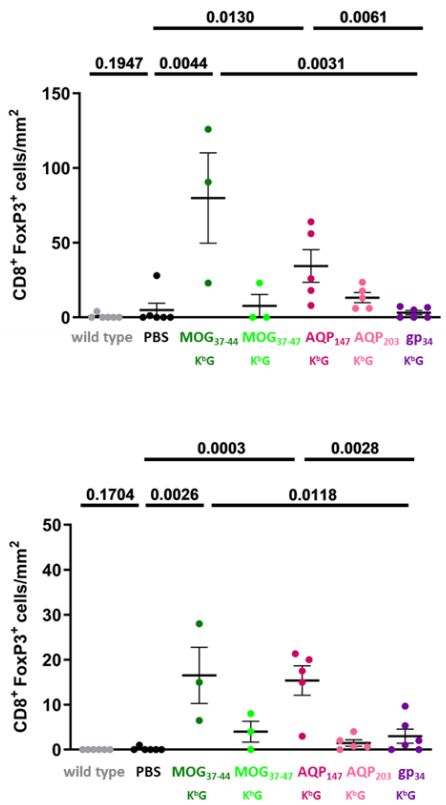


→ total counts

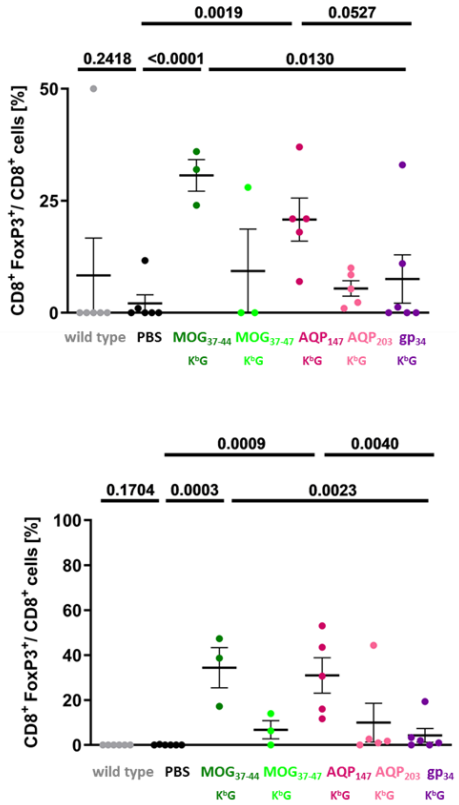
infiltrating CD8⁺ (all)



infiltrating Foxp3⁺CD8⁺

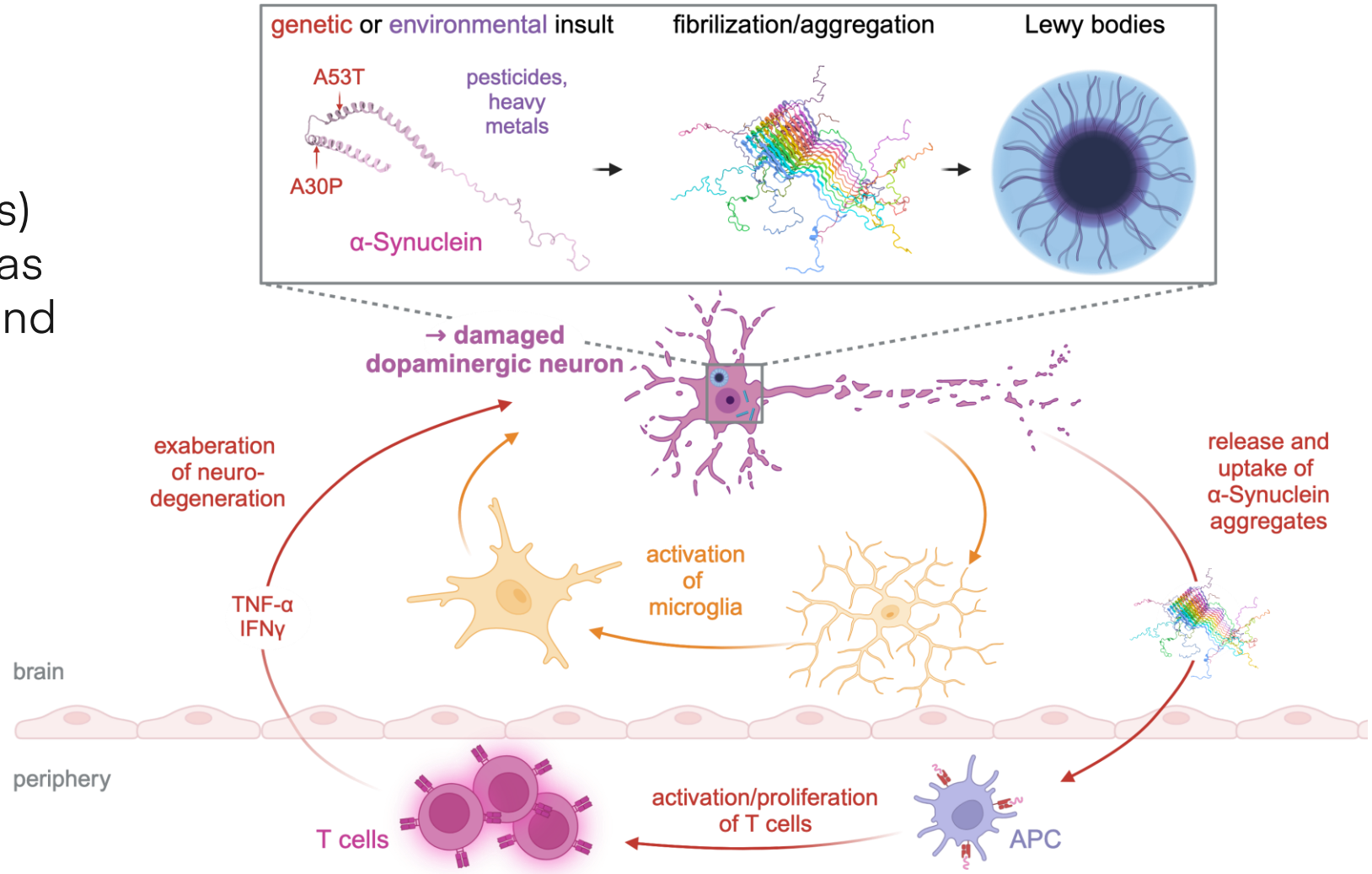


Foxp3⁺CD8⁺/total CD8⁺ [%]



aSyn specific T cells in Parkinson's disease

PD is a neurodegenerative disease characterized by **accumulation of α-Synuclein** (aSyn) aggregates (Lewy bodies) and neuroinflammation. aSyn has immunostimulatory functions, and **aSyn-specific effector T cells** precede disease onset.

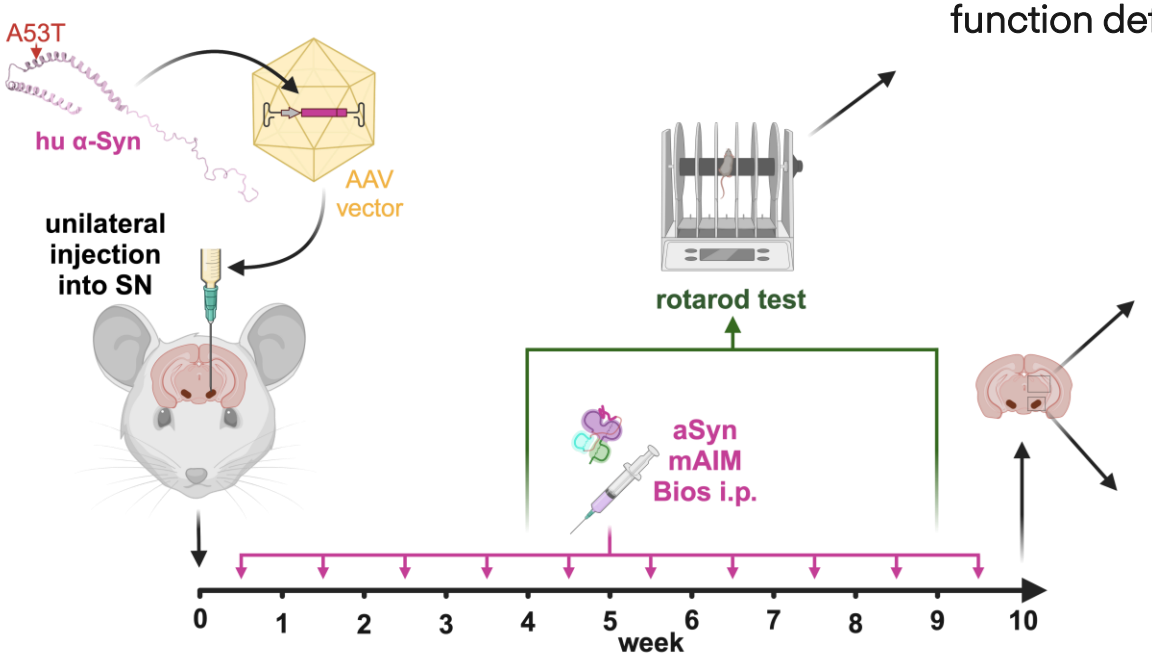


aSyn AIM Bios completely prevent PD symptoms

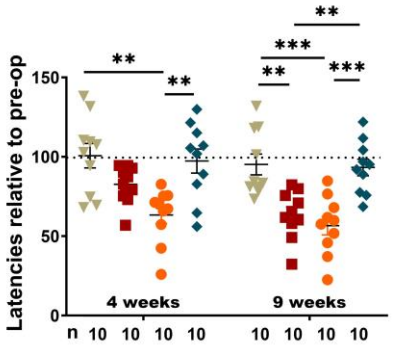
Unilateral AAV aSynA53T Parkinson's model

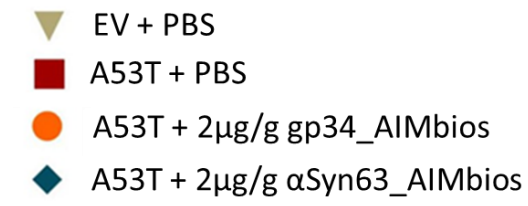
see Karikari, ..., Bruttel, ..., Wischhusen, ..., Ip ,

Brain, Behavior, and Immunity 101 (2022) 194–210

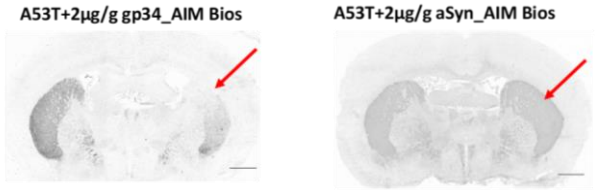


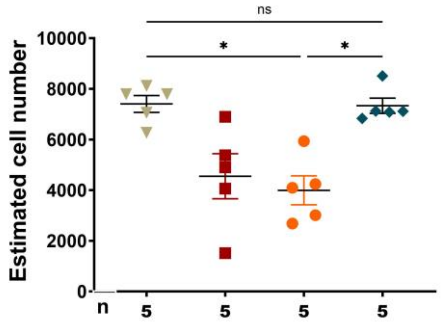
aSyn AIM Bios prevent motor function deficits



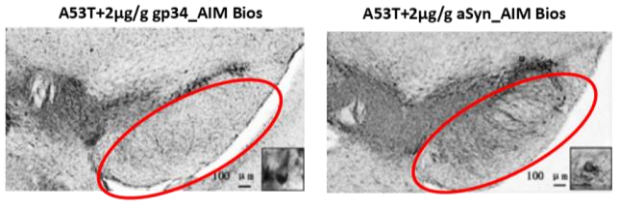


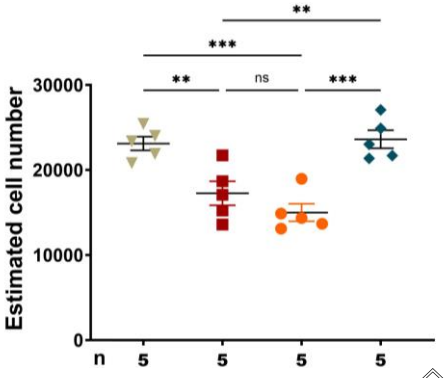
aSyn AIM Bios prevent loss of dopaminergic terminal fibers





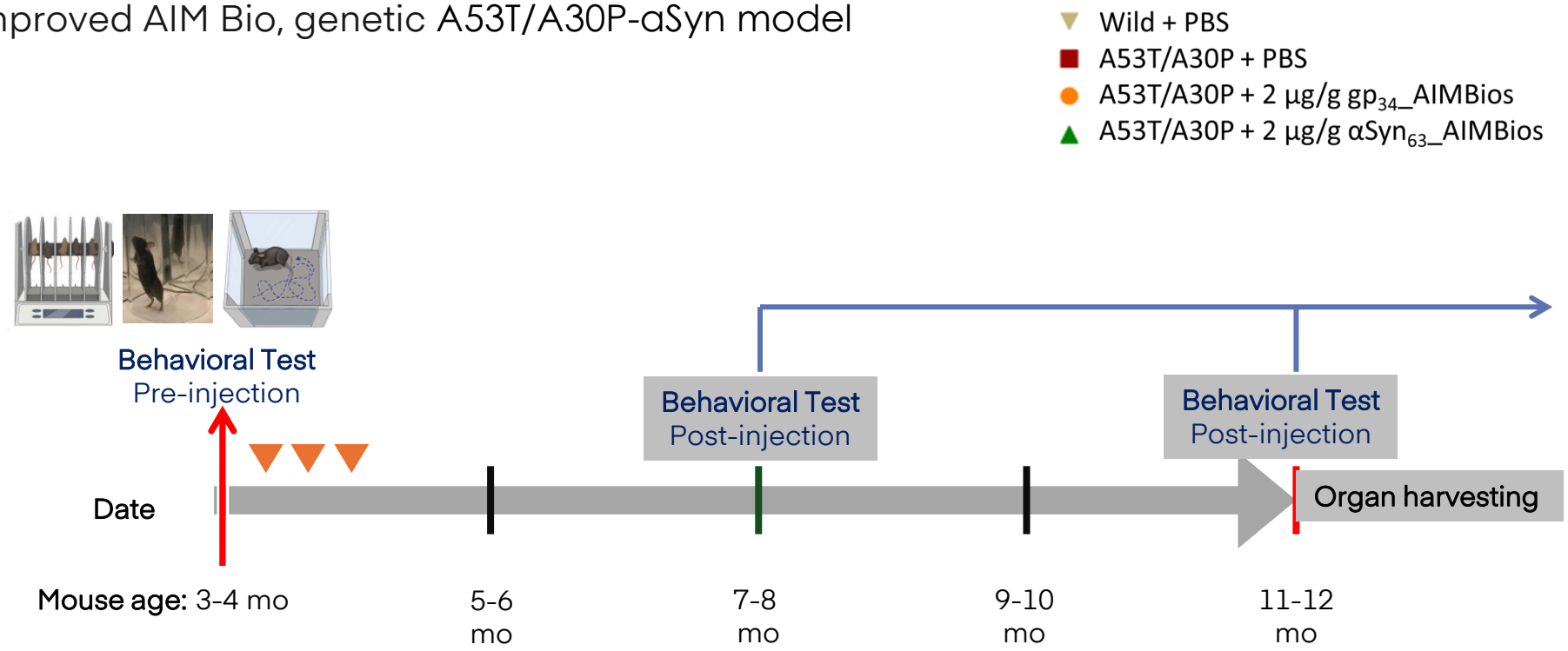
aSyn AIM Bios prevent loss of substantia nigra neurons

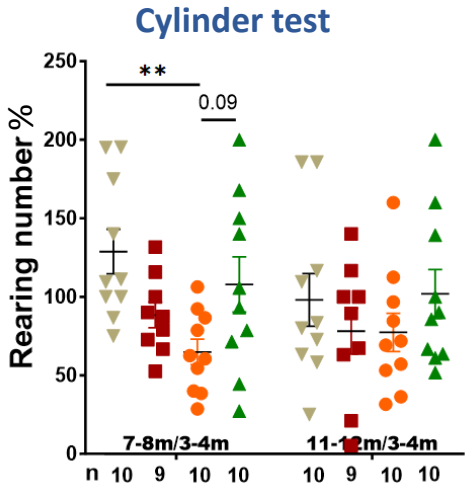




aSyn AIM Bios completely stop PD symptoms

Improved AIM Bio, genetic A53T/A30P-aSyn model

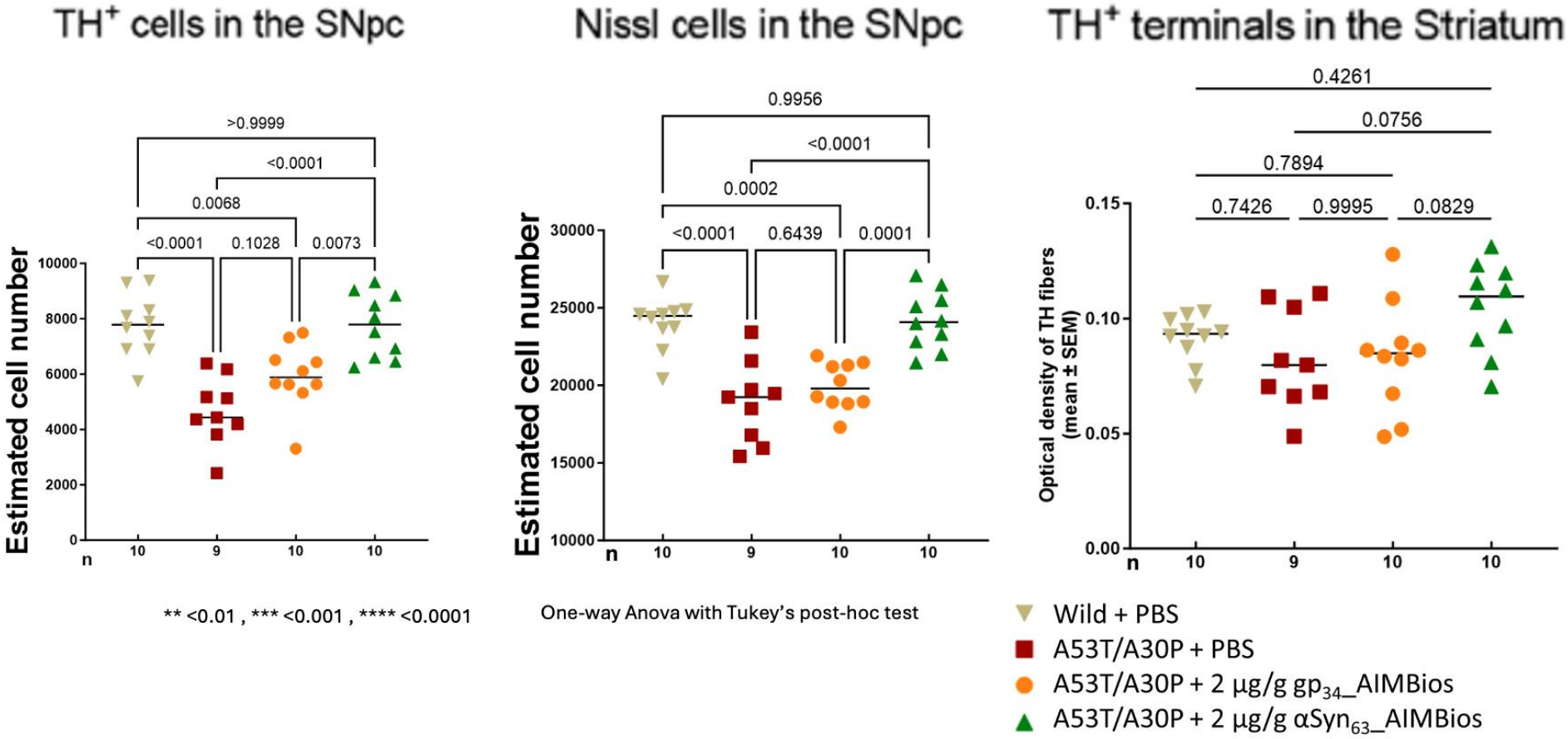




▼ At 3-4 months of age, 3 AIM Bio injections were given in two-week intervals

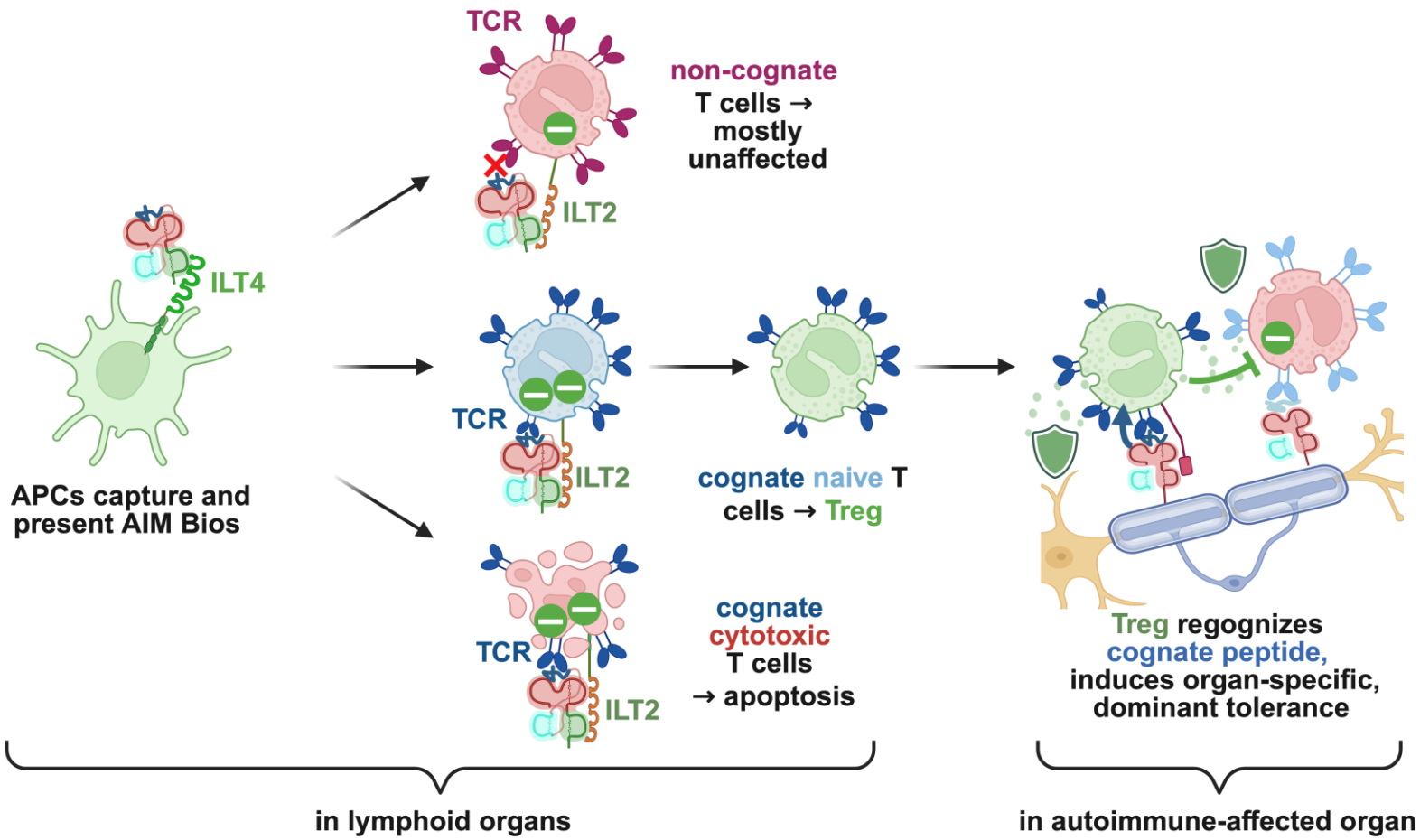
aSyn AIM Bios induce long-term neuroprotection

Improved AIM Bio, genetic A53T/A30P-aSyn model



soluble HLA-G / AIM Bios: anticipated mode of action

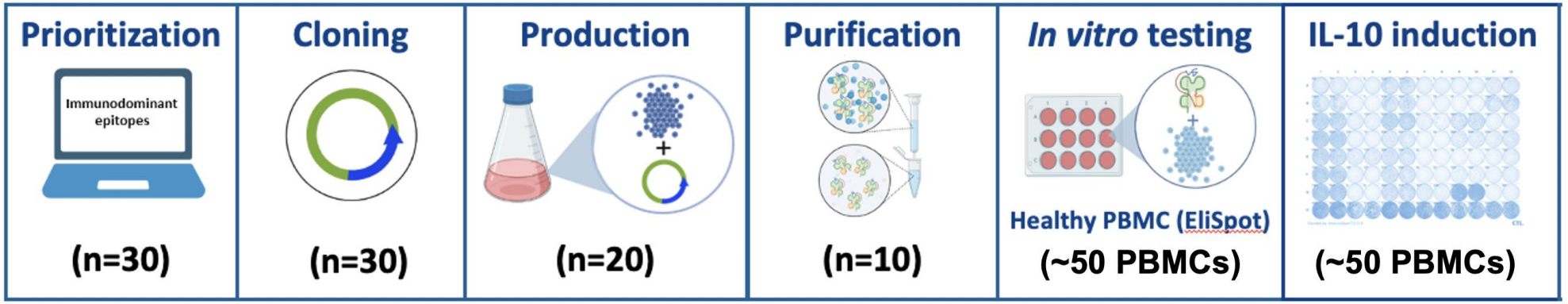
Soluble **AIM Bios** are **captured by antigen-presenting cells** in lymph node via ILT4. Non-cognate effector T cells interacting will not be affected by AIM Bios, while cognate, highly activated effector T cells undergo apoptosis. **Naïve cognate T cells** are **polarized to become tolerogenic Treg**, which induce potent local immuno-suppression after recognizing their cognate peptide in an autoimmunity affected organ.



human candidate development

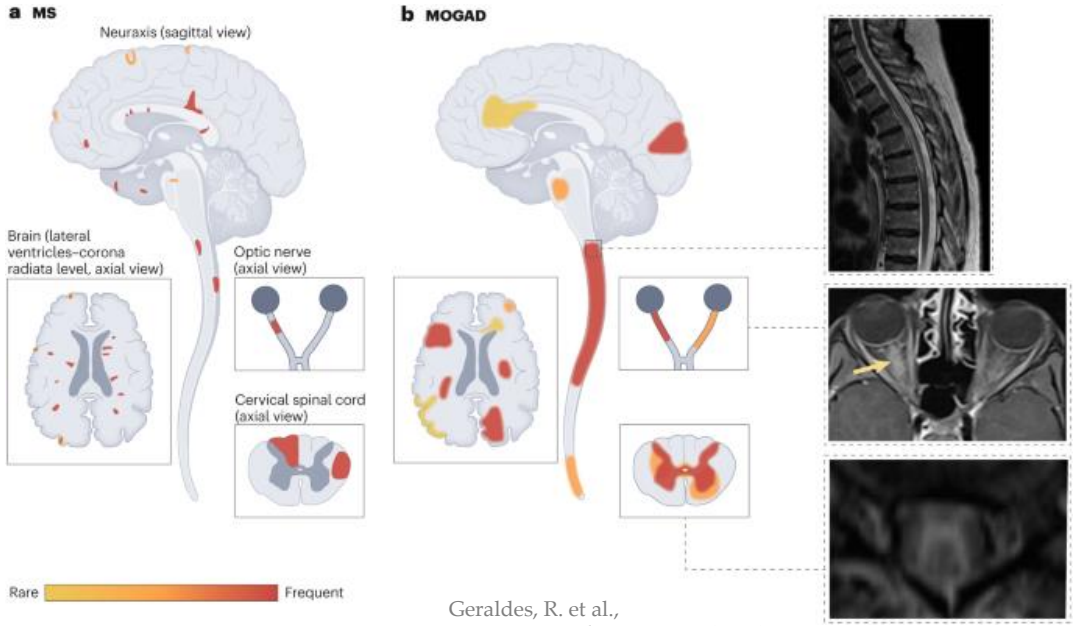
AIM Bio human candidate production & prioritization

Immunodominant and functional HLA-G or HLA-A2 restricted epitopes in autoimmunity target proteins are predicted in silico. AIM Bios are cloned into mammalian expression vectors. Transient transfection in suspension cell lines produces similar yields and purities as seen in monoclonal antibodies. Candidates are then prioritized based on yields, thermal stability, HLA-G receptor binding and the capacity to induce Treg in healthy donor or patient PBMCs.



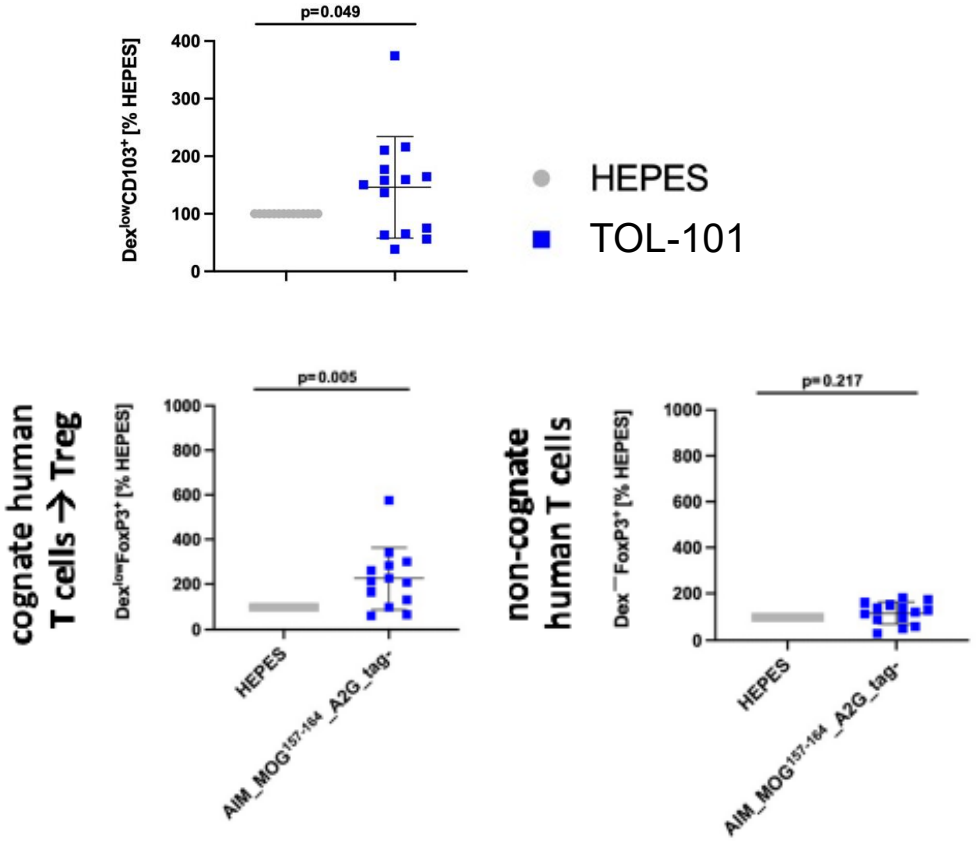
TOL-101 induces MOG-specific Treg

Multiple Sclerosis (MS) and MOG Antibody Disease (MOGAD) are demyelinating disorders in which oligodendrocytes are targeted. Challenges include targeting chronic damage, managing progression, and monitoring low-grade inflammation. Autoantibodies drive the pathogenesis.



Geraldes, R. et al.,
Nat Rev Neurol **20**, 620–635 (2024)

The MOG tolerance inducing lead AIM Bio TOL-101 induces MOG-specific Foxp3⁺ CD103⁺ CD8⁺ Treg in healthy donor and MS/MOGAD patient PBMCs. Non-cognate (dextramer-neg.) CD8⁺ T cells showed no induction.



manufacturability assessment at CDMOs

Developability assessments, including initial stability studies*

- transient expression in CHO (up to 3.0 L)
- good yields after purification (AEX,SEC) (>250mg/L)
- promising product qualities (monomer: >99%)
- Stability is acceptable at 2 - 8°C temperature in buffer (pH 7.4) and under light basic conditions (pH 9.0)

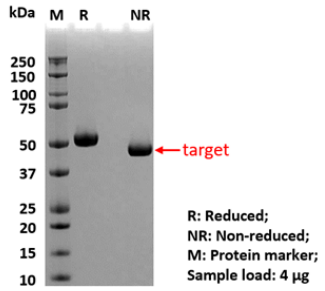
*Similar results at AsymBio

WuXi Biologics
Global Solution Provider

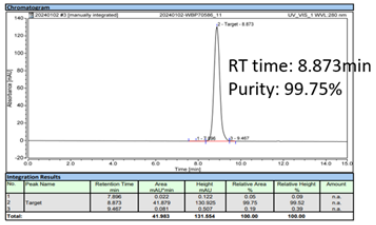
Final QC Data Results for AIM Bio TOL101 (MOG157)

Molecule information	
WuXi ID	WBP70586_11_MOG157_A2G_fs_tagless
Product lot#	20240102-WBP70586_11
Client ID	MOG157_A2G_fs_tagless
Molecular type	Tagless protein
Extinction coefficient	1.97
Molecular weight (kDa)	48.91
pI	6.00
Expression	
Cell line	CHO-K1
Production volume(L)	3.00
Harvest days	D7
Expression scientist	Xuelian Liu
Purification & QC	
Purification steps	Q HP-Capto Butyl ImpRes-UF/DF
Purity of SEC-HPLC(%)	99.75
Formulation Buffer	20 mM Hepes, 150 mM NaCl, pH7.5
Concentration(mg/mL)	1.23
Endotoxin(EU/mg)	0.068
HCP ratio(ppm)	36157
Volume(mL)	584.50
Final amount (mg)	718.94
Final yield (mg/L)	239.65
Number of vials	102(0.1 mL/vial*20 vials, 2 mL/vial *20 vials, 2.5 mL/vial*3 vials, 4.5 mL/vial*10 vials, 10 mL/vial*49 vials)
Storage temperature	-80℃
Purification scientist	Tian Li
Shipping Date	2024-01-18

SDS-PAGE



SEC-HPLC



➢ GMP-compliant manufacturing at CDMOs feasible

estimated production needs for FIH study

Following these specifications, preliminary discussions with GMP-compliant CDMOs were initiated:

- Non-GMP production:

  DS: one batch in 200L scale.
  DP: one batch a' 2,000 vials (5 mg/mL, 1 mL filling volume per vial).

- GMP production:

  DS: one batch in 500L scale.
  DP: one batch a' 2,000 vials (5 mg/mL; 1 mL filling volume per vial).

regulatory development

A lean IND-filing program for TOL101 was supported by the German regulatory authority (PEI) for AIM Bio lead program TOL101 (MOGAD) in September 2024:

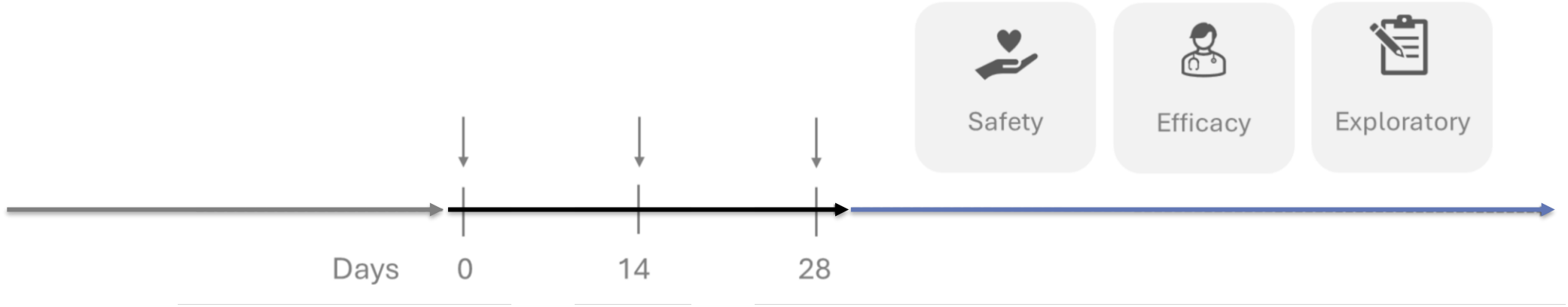
- Preclinical proof of principal in suitable *in vivo* and *in vitro* in systems already provided
- One binding assay sufficient for release and stability testing
- PK/TOX with mouse-adapted AIM Bio in mice sufficient
- Maximum recommended starting dose (MRSD) to be derived from *in vitro* and *in vivo* experiments
- Quality development largely comparable to other biologics (antibodies)

Similar regulatory developments should apply to our other AIM Bio programs such as NMOSD or Parkinson's disease

(meeting materials can be shared under CDA)

MOGAD FIH study plan

TOL101 MOGAD Phase I study design:



inclusion criteria:

- adult, 1st MOGAD event
- HLA-A2+
- MOG-Ab+
- no immunotherapy
- optional: adaptive design healthy volunteers first

treatment:

- 3 treatments, 2 week interval
- 4 treatment groups
- 3+3 dose escalation design

key endpoints (up to 1y):

- EDSS score
- MOG-Ab levels
- TOL101 Treg in blood/LNs (?)
- lesions (MRI)

Toleris AIM Bio platform pipeline overview

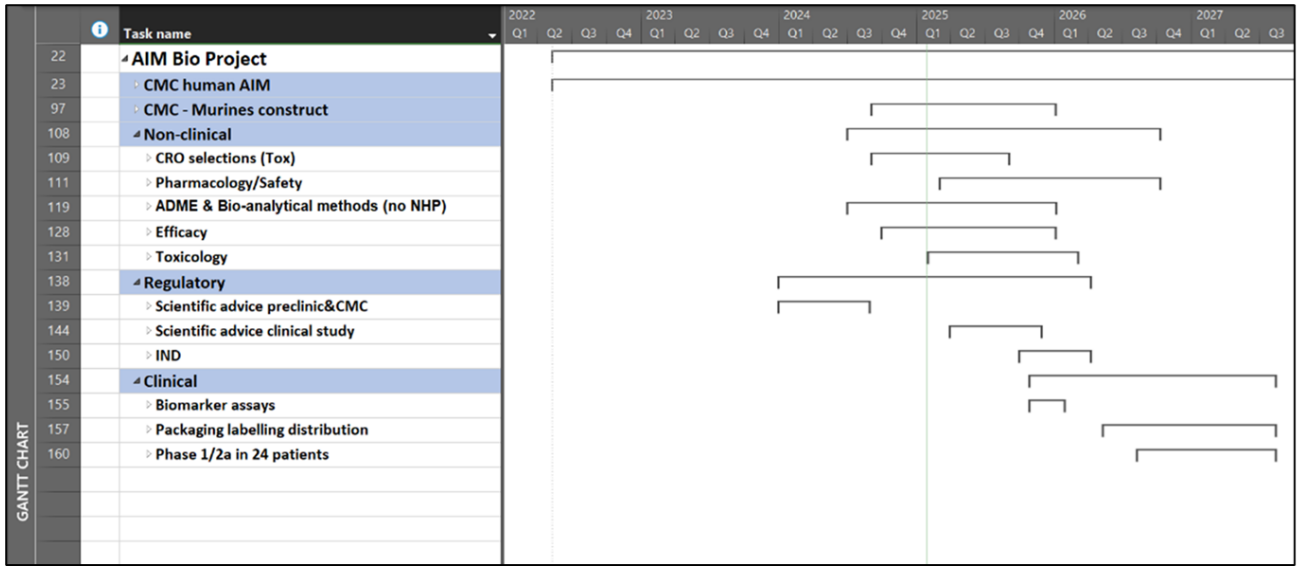
disease	candidate	predicted candidates	prioritized candidate	PoC *in vivo*	*IND filing*	Phase I	Phase II	Phase III
MS/MOGAD*	TOL101							
NMOSD	TOL201							
Parkinson's disease	TOL301							
type 1 diabetes	several							
myasthenia gravis	several							
pemphigus & others	predicted							

*current status: Toleris IND-filing program for TOL101 supported by Paul-Ehrlich-Institute in scientific advice meeting.

investment case

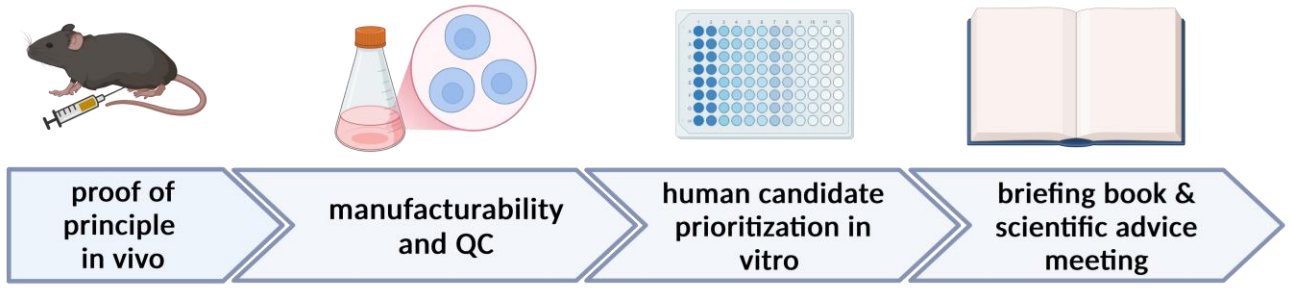
investment opportunities

Toleris' next key milestone to be achieved with a Series A round is the completion of a first clinical Phase 1a/b study in MOGAD or an alternative lead indication. This requires ~15 Mio € per indication.



Budget estimate	
CMC:	~5M€
Non-clinical:	~3M€
Regulatory:	~1M€
Clinical:	~4M€
Other Activities: (IP,SAB):	~1M€

In parallel, we aim to complete *in vivo* proof of principle studies and human candidate prioritization in 1-2 additional indications. This requires ~1-1.5 Mio € per indication.



autoimmune disease market landscape

- AIM Bios are a platform technology with **potential to revolutionize treatment options** in numerous multi-billion $ disease markets (MS, PD, T1D, RA, IBD, ...)
- MOGAD and NMOSD are orphan diseases (prevalence 1.3–2.5 100,000[1]) with **high medical need**, and in the case of MOGAD **no approved therapeutics**. This should facilitate clinical entry and accelerate approval.
- extension of use of the MOGAD compound for MS and NMOSD feasible according to KOLs.
- MS, T1D and MG are common autoimmune diseases with a high medical need (~10 years lower life expectancy in T1D[3]). Current therapeutics strongly impair the daily lives of patients. AIM Bios could stop disease progression and extend intervals between treatments to several months.

References: 1 PMID: 37789888; 2 PMID: 32296622; 3 PMID: 36804193

risk mitigation strategy

A comprehensive risk assessment focusing on the most common causes of failure in first-in-human (FIH) clinical trials was conducted for the MOGAD program. Wherever possible, mitigation strategies were identified and already implemented.

key risk	likelihood:	mitigation strategy
• species differences:	low to medium	in vitro exp./human molecule
• poor translatability of disease models:	low	numerous, diverse models
• unexpected human-specific toxicities:	low	expressed during pregnancy
• PK/PD mismatch:	low	HLA-G half-life known
• immunogenicity:	low	fully human components
• unanticipated drug-drug interactions:	low	most likely irrelevant/synergistic
• formulation/delivery issues:	low	successful pilots at CDMOs

Further details and supporting information available in backup slides.

USPs: robust, physiological, potent & targeted

	therapeutic approach	effective	targeted	complexity/cost
AG-specific therapeutics	**antigen** alone	-	+/ -	+ +
	tolerogenic **vaccination**	+/ -	+	+/ -
	regulatory **cells**	+	+	-
	physiological 2-signal protein	+	+	+

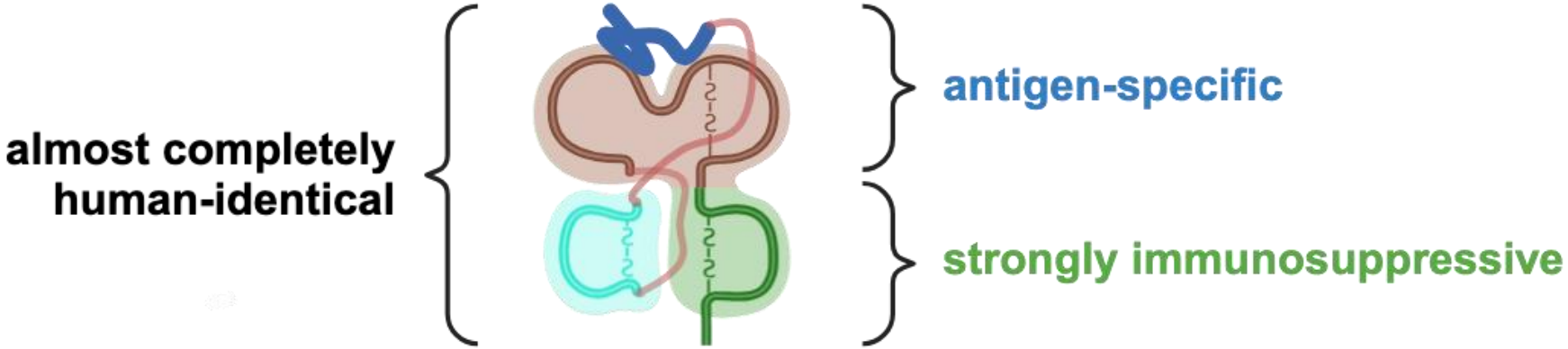
key benefits of AIM Bios:
- induce tissue-reactive, antigen-specific $CD8^+$ Tregs
- potent HLA-G–pathway
- nearly physiological, robust biomolecules

competitors:
antigen-agnostic CD8 Treg: *Mozart Therapeutics;* ***antigen alone:*** *Apitope, ImCyse;* ***via liposomes:*** *ActoBio, Anokion, AnTolRx;* ***red blood cells:*** *Cellerys;* ***nanoparticles****: Topas, Selecta Biosciences, Cour Pharmaceuticals;* ***RNA-based:*** *Biontech* ***tolerogenic cells****: Dendright, Idogen;* ***artificial antigen presenting cells:*** *Parvus;* ***antigen-specific tolerogenic biomolecules:*** *Cerberus Therapeutics, Cue Biopharma*

thank you for your attention!

AIM Bio USPs:

- physiological molecules and tolerance mechanism
- selective prevention of autoantibodies
- precision through antigen-specific $CD8^+$ Treg
- organ-specific bystander protection
- efficacy and specificity demonstrated *in vitro* and *in vivo*



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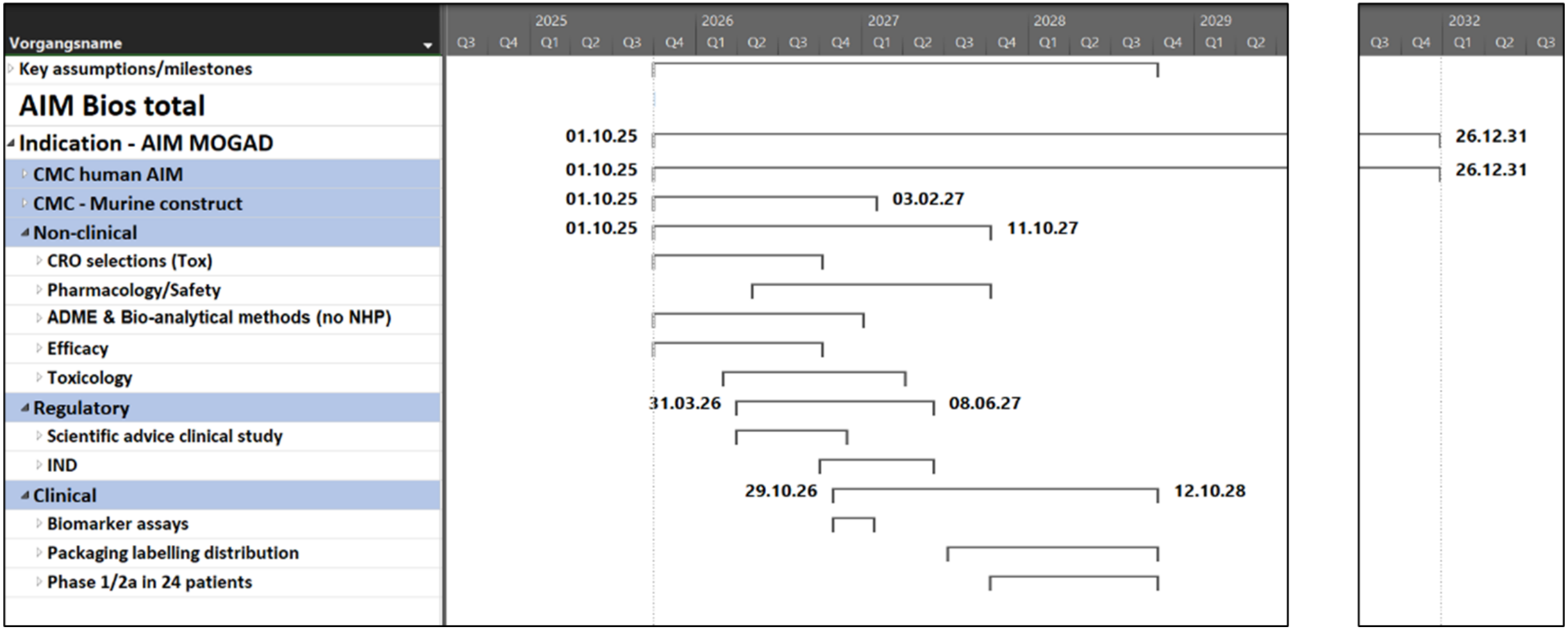
Please visit www.toleris.com for further information. Some figures generated with biorender.com .

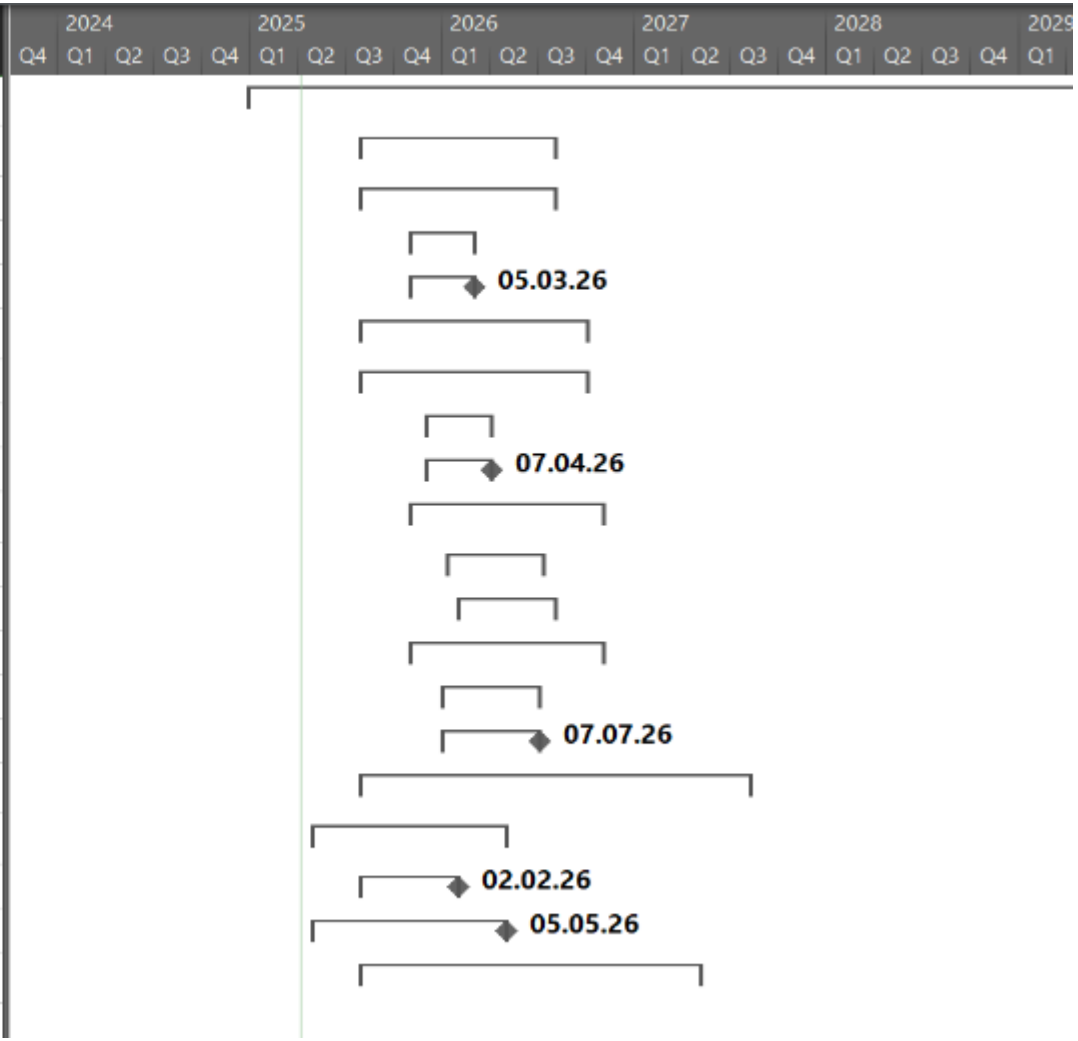
supplementary slides

S1: Project plan MOGAD



S2: Project Plan NMOSD,T1D,MG

Vorgangsname	Start	Finish
TOL101-MOGAD/MS	Wed 01.01.25	Tue 28.10.31
TOL201-NMOSD	Fri 01.08.25	Wed 05.08.26
Research & Development	Fri 01.08.25	Wed 05.08.26
Regulatory	Tue 04.11.25	Thu 05.03.26
Scientific advice preclinic&CMC ready	Tue 04.11.25	Thu 05.03.26
TOL401-Type 1 Diabetes	Fri 01.08.25	Tue 06.10.26
Research & Development	Fri 01.08.25	Tue 06.10.26
Regulatory	Thu 04.12.25	Tue 07.04.26
Scientific advice preclinic&CMC ready	Thu 04.12.25	Tue 07.04.26
TOL501-Myasthenia Gravis	Mon 03.11.25	Thu 05.11.26
Research & Development	Mon 12.01.26	Tue 14.07.26
In vivo POC	Tue 03.02.26	Wed 05.08.26
TCR analysis	Mon 03.11.25	Thu 05.11.26
Regulatory	Fri 02.01.26	Tue 07.07.26
Scientific advice preclinic&CMC ready	Fri 02.01.26	Tue 07.07.26
Preclinical Research	Fri 01.08.25	Tue 10.08.27
Preclinical Assay Development	Thu 01.05.25	Tue 05.05.26
PK Assay development	Fri 01.08.25	Mon 02.02.26
Bioassay development	Thu 01.05.25	Tue 05.05.26
Clinical Assay development	Fri 01.08.25	Mon 10.05.27

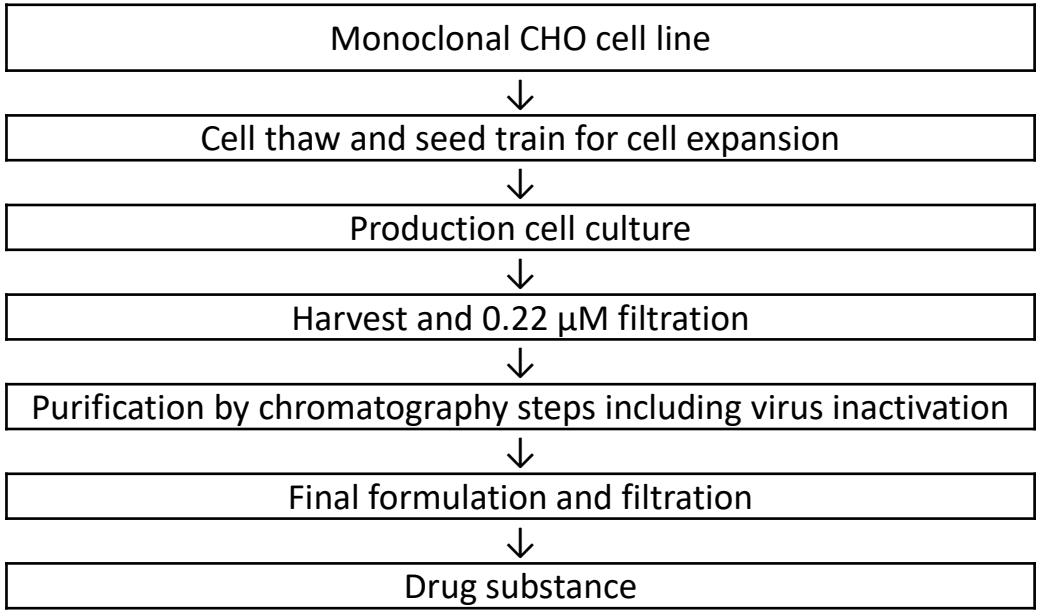


S3: pre-clinical development program

Quality development-Potency Assay	
• Binding assay of TOL101 to ILT2 or ILT4 or TCR	
***In vivo* studies with MOG37:**	***In vitro* studies with TOL101**
• Maximum tolerated dose (MTD) / Dose range finding (DRF) • Pivotal 4-week repeated dose toxicity RDT (GLP) • T-Dependent Antibody Response (TDAR) (separate or included into pivotal RDT) • Safety Pharmacology CNS (separate) • Safety Pharmacology Respiratory (separate)	• Tissue Cross Reactivity solid tissue (GLP) • Tissue Cross Reactivity liquid tissue • Cytokine Release Assay • Membrane Proteome Array
Dose finding for maximum recommended starting dose (MRSD)	
Modelling and Simulation: Use existing data (binding, PK, PoC, dose dependency of AIM Bios) to build a mathematical mouse model Add human data (binding, physiological HLA-G levels etc) to predict behaviour of MOG157 in humans	
Not to be performed:	
• NHP PK/Tox • Genotoxicity and Carcinogenicity (inappropriate for biologicals according to ICH S6 R1 (2011)) • DART (later; if appropriate for the study population) • Local Tolerance (to be integrated in pivotal RDT) • Alanine Peptide Scan: MOG peptide is covalently bound to MOG157 and unlikely to be displaced	

S4: manufacturing process and QTPP of TOL101

Planned DS manufacturing process



Initial Quality Target Product Profile (QTPP)

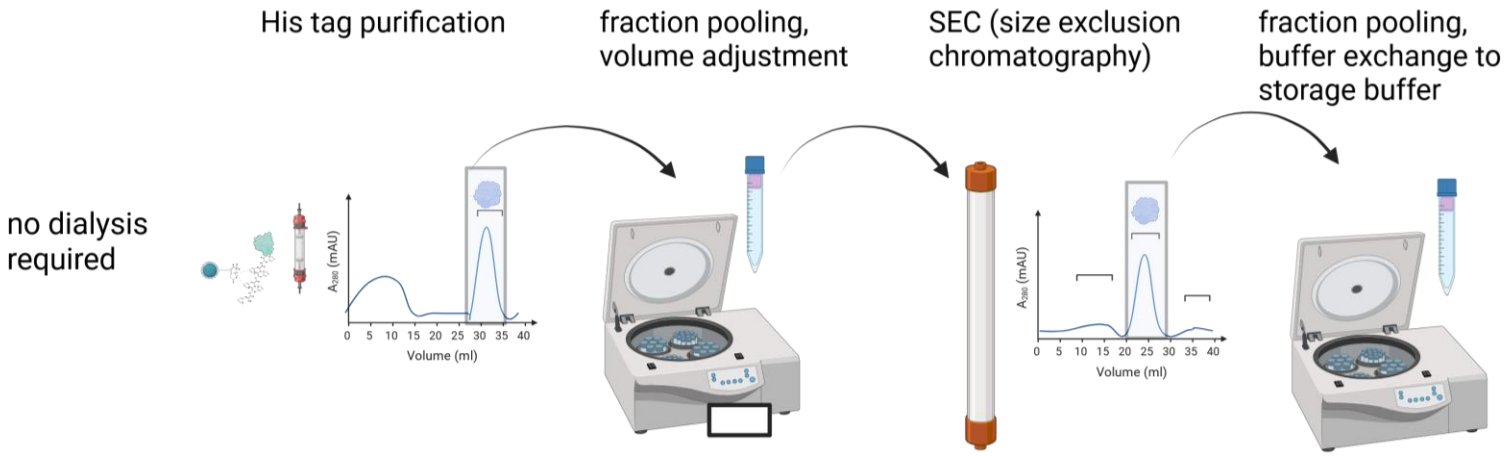
Product properties	Target
Indication	MOGAD (Orphan disease)
Treatment duration	Intermittent
Delivery mode	Intravenous infusion
Dosage form for FIM	Prefilled vials with liquid
Regimen/ Frequency	TBD
Dosage strength(s)	TBD
DS/DP concentration	Depending on dosage needs, e.g. 5 mg/mL
DP container closure system	Glass vials for DP, e.g. 2R
DS intended storage conditions	≤ -60°C
DP intended storage conditions	2-8°C
DP intended shelf-life	≥ 24 months
Product quality	Appropriate preliminary acceptance criteria will be implemented

S5: established purification protocols

HIS tag purification

→ immobilized metal affinity chromatography (IMAC)

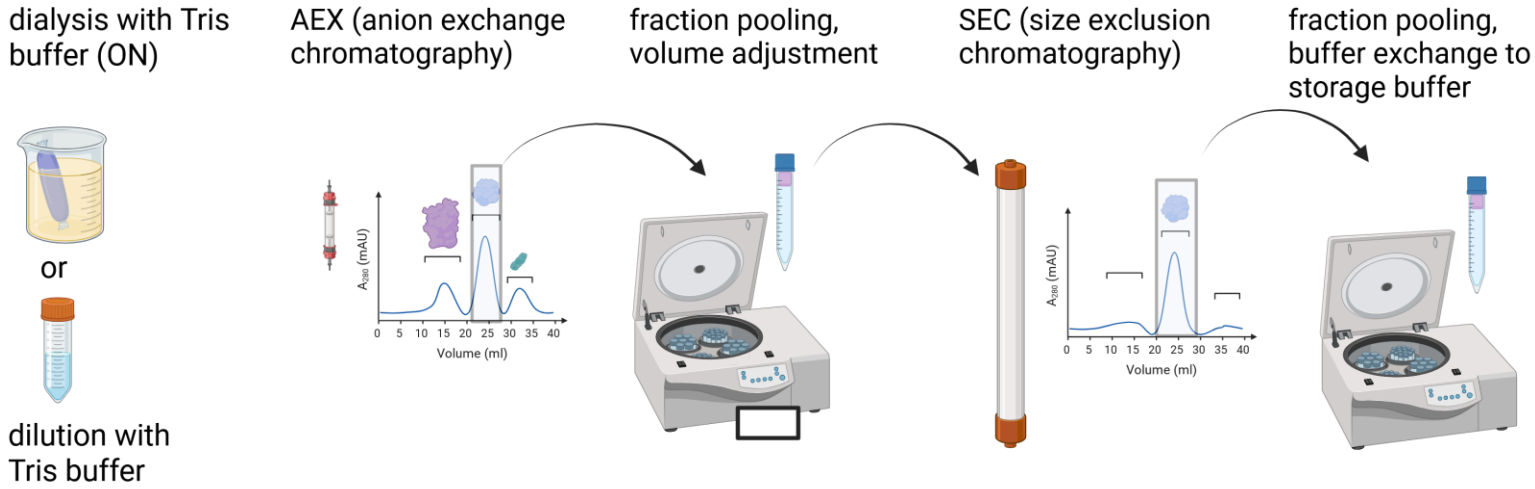
→ size exclusion chromatography



tagless purification

→ anion exchange chromatography

→ size exclusion chromatography



S6: competitor analysis: antigen-specific tolerance

Relevant competitors such as Apitope or ImCyse simply administer peptides in the absence of co-stimulation, which is known to work in mice and in allergies (hyposensitation), but thus far failed to induce sustained tolerance in autoimmune disease patients. We believe that our active tolerization approach will be considerably more potent.

Administration of antigen via liposomes (ActoBio, Anokion, AnTolRx), red blood cells (Cellerys), or nanoparticles targeting the liver/immature dendritic cells/monocytes (Topas/Selecta Biosciences/Cour Pharmaceuticals) works well when tolerance can be established in a naïve immune system, but many such "antigen only" approaches have failed to induce tolerance in patients.

Artificial materials used in some of these nanoparticles may cause problems as observed with iron-containing nanoparticles. BioNTech´s RNA-based antigen delivery may activate the immune system via TLR3 as desired for classical vaccines.

Administration of tolerogenic dendritic cells, as developed by Dendright and Idogen, requires an individually tailored cell therapy product for every patient. The use of artificial antigen specific cells (Parvus) will likely still require further optimization. Comparable cellular products are very expensive and challenging to produce, as seen with CAR T cells. Here, an off-the-shelf biomolecule offers clear advantages.

Mozart Therapeutics also targets CD8 Tregs, but not in an antigen-specific way. Cerberus Therapeutics and Cue Biopharma use antigen-specific biomolecules, but do not rely on physiological constructs or tolerance pathways.

AIM Bios are unique in combining both the potent tolerance-inducing HLA-G domain and the specific antigen domains in an almost completely physiological molecule, which evolved over millions of years to safely and reliably induce immunological tolerance during pregnancy.

S7: CVs Founder team

Jürgen Engel

Jürgen Engel is a serial biotech entrepreneur and co-founder of Toleris Biotherapeutics GmbH. His achievements include over 100 licensing agreements, company formations, sales, and successful dilutive and non-dilutive financing strategies. During 48 years at pharmaceutical companies ASTA Medica and AEterna Zentaris he has successfully co-invented and developed new drugs for the benefit of patients, e.g. Cetrotide, Impavido, Milteforan, Katadolon, Azelastine, Ifosfamide/Mesna, Lobaplatin and Retigabine as well as a medical device the dry powder inhaler known as novolizer. He led AEterna Zentaris Inc. as President and CEO up to his retirement in 2013.

Thereafter prior to joining Toleris, Jürgen served at AEterna Zentaris as strategic advisor from 2019 until 2023 and as Chairman of the Board of Directors of Cell BT Inc., playing a key role in a merger with a private biotech company. He was also a Strategic Advisor for Ergomed PLC and involved in their IPO at the AIM stock market in London. He led the restructuring and sale of Oncoscience AG. Additionally, he was a member of the advising counsel for GIG Berlin and an advisor for River Rock/Immodulon. He has also been a long-time board member of various German pharmaceutical and chemical associations.

In addition to his career in pharmaceutical industry Jürgen is Honorary Professor at the Technical University of Dresden since 1993, and adjunct Professor School of Pharmacy University of Regensburg since 1990.

Jürgen started his career as organic chemist at TU Braunschweig. He has authored over 250 scientific publications, several books and numerous patent applications, earning several awards for his contributions to pharmaceutical research including the renowned Galenus von Pergamon Award for the development of Miltex in oncology.

LinkedIn linkedin.com/in/prof-dr-dr-juergen-engel-a328a228

Valentin Bruttel

Valentin Bruttel is a CSO and co-founder of Toleris Biotherapeutics GmbH. He holds a Bachelor of Science in Biomedicine from Julius-Maximilians-Universität Würzburg and a Master of Science in Molecular Medicine from Trinity College, Dublin. He earned his PhD from Julius-Maximilians-Universität Würzburg in 2016 in the field of tumor immune escape. Between 2016 and 2023, he developed the AutoImmunity Modifying Biologicals platform for targeted immunosuppression at the University and University Hospital Würzburg.

This project was funded by the Bavarian State Ministry (m4 Award) and the Federal Ministry of Economics (EXIST Research Transfer) as well as through licensing agreements. Valentin has published numerous peer reviewed papers, is inventor of 9 granted and pending patents and received several awards and fellowships.

LinkedIn linkedin.com/in/valentin-bruttel-2b08b791

S8: CVs Founder team

Jörg Wischhusen

Jörg Wischhusen is a co-inventor and co-founder of Toleris Biotherapeutics GmbH and the scientific founder of CatalYm GmbH. He studied biochemistry in Tübingen, Germany, and (simultaneously) piano chamber music in Winterthur, Switzerland. During his thesis on mechanisms of immune escape in glioma (mentored by Michael Weller and Hans-Georg Rammensee) he performed early work in the field of experimental immune checkpoint blockade. In 2005 he was recruited as a junior research group leader to the University of Würzburg where he became a professor in 2013. His group explores whether pregnancy-related mechanisms for immune tolerance can be targeted or exploited for immunomodulation in cancer or autoimmunity. He is author on >100 peer-reviewed publications, inventor on 16 patent families. Next to Toleris Biotherapeutics GmbH, CatalYm GmbH, where he served as acting CSO for the first 2.4 years, is a successful spin-off from his lab.

LinkedIn linkedin.com/in/joerg-wischhusen-5698b9b7

Markus Haake

Markus Haake joined Toleris Biotherapeutics GmbH in July 2024. He has more than 20 years of experience in building Biotech companies. Beforehand, he was Head of Preclinical Development and Research and Vice President Pharmacology at CatalYm GmbH, München.

Markus is a chemist with a diploma and a PhD achieved at the Department of Physiological Chemistry, University of Würzburg. After his PhD, he became Research Scientist and Project Manager of TeGenero AG, working on immunological disorders. After spending a short period as a Senior scientist at Ganymed Pharmac. AG, he went to Kenta Biotech AG, Bern, Switzerland as a member of the management with focus on the development of fully human antibodies for the treatment of nosocomial infections. Back at University of Würzburg, he was a co-founder of CatalYm GmbH, which is a spin-off from Jörg Wischhusen´s lab, and was strongly involved in the development of Visugromab into the clinics for several years. CatalYm has meanwhile developed into a promising player in the immuno-oncology space. Markus contributed to drug discovery and development projects in immunological disorders, cancer and infectious disease and successfully developed therapeutic antibodies, that entered clinical trials and late-stage development (e.g. Tosatuxumab, Aerumab, Visugromab).

LinkedIn linkedin.com/in/markus-haake-670185164