Supplemental Materials & Methods

A patient-specific antigen-selection engine for personalized pMHC-II tolerance therapy in eosinophilic esophagitis

Preclinical-stage program. Predicted-binding values index presentation potential only; immunodominance, natural processing, and tolerogenic outcome are established experimentally, not inferred. All epitopes below are computational predictions; no tetramer or TCR validation has been performed for any epitope. Not medical advice.

Table S1. Worked lead epitopes on HLA-DRB1*07:01 (antigen-selection engine)

Allergen (UniProt)	**Lead peptide (15-mer)**	**Core / note**	**Predicted IC_{50} (nM)**	**Strong binders, antigen × DRB1*07:01**	**Validation status**
Dairy — β-casein (P02666)	QDKIHPFAQTQSLVY (aa 61–75)	FAQTQSLVY core (aa 67–75); milk is the most common EoE trigger [5,6]	131.9	32 (β-casein on this allele)	Computational prediction; tetramer/ TCR validation pending (no prior experimental epitope; see note)
Dairy — β-casein (P02666)	PQSVLSLSQSKVLPV (aa 174–188)	Engine's top-ranked β-casein core (distinct region)	13.4	(same 32)	Computational prediction
Wheat — α/β-gliadin (P18573)	SRCQAIHNVVHAIIL	HNVVHAIIL core	6.4	37 (gliadin on this allele)	Computational prediction
Soy — β-conglycinin (P25974)	ALLLPHFNSKAIVIL	—	2.1	114 (β-conglycinin on this allele)	Computational prediction

*Strong-binder counts are per antigen on HLA-DRB1*07:01 (IC_{50} < 500 nM across all 15-mers of that allergen), not per listed peptide; the two β-casein rows share the same antigen-level count of 32. Predictions: mhcnuggets (class II) [15], the same predictor used for Figure 1. DRB1*07:01 is one of five HLA-DRB1 alleles scanned (*01:01, *03:01, *04:01, *07:01, *15:01); the full landscape (924 unique 15-mers → 4,640 evaluations → 832 strong binders) is in Figure 1. The dairy antigen is β-casein (P02666); the FAQTQSLVY core occurs in β-casein and is absent from β-lactoglobulin, correcting an earlier internal mislabel. Note: a founding-brief reference to an experimentally validated 'eoeTCR-4' clone for this epitope could not be located in the literature (PubMed/OpenAlex) and has been withdrawn; the dairy lead is prioritized on the documented presence of milk-responsive $CD4^+$ T cells in EoE [5,6], not on a validated epitope–allele–TCR triad.*

Table S2. AlphaFold-multimer structural prediction metrics

Construct	Format	pLDDT	ipTM (interface)	Mean PAE (Å)	Max PAE (Å)	RMSD to PDB 1S9V (Å)
Dairy pMHC-II	Complex	0.859	0.872	1.8	3.2	1.2
Wheat pMHC-II	Complex	0.884	0.896	2.1	3.8	1.4
Soy pMHC-II	Complex	0.878	0.891	1.9	3.5	1.3
Dairy single-chain	Single-chain	0.824	N/A	N/A	N/A	1.5

AlphaFold [13] in multimer mode [14]. Acceptance gate: ipTM ≥ 0.87 with full 15-residue in-groove engagement; pLDDT > 0.70 high-confidence; interface PAE < 5 Å acceptable. All pMHC-II complexes clear the gate. Anchor placement (P1/P4/P6/P9) verified against the predicted binding core.

Table S3. Nanoparticle design specifications

Parameter	Value	Rationale / notes
Core material	Fe_3O_4 (magnetite)	Biodegradable; precedent in tolerogenic delivery [11]
Core diameter	20 nm	Splenic retention; <100 nm avoids renal filtration
Surface coating	PEG_2?-maleimide	~75 reactive sites; stoichiometric conjugation
pMHC copies per NP	5	≈1.4% surface occupancy (17.5 nm^2 of ~1,257 nm^2); sub-saturating
Inter-epitope spacing	5.8 nm	Within range for TCR engagement / Tr1 priming
Avidity gain	~24×	Relative to monovalent; within published 10–50× tolerogenic window
Manufacturing (PACT™)	Shared backbone, swappable cassette	Standardized once; only peptide changes per patient [12]

Surface occupancy corrected to 1.4% (geometry: 17.5 nm^2 footprint on a ~1,257 nm^2 20 nm-diameter sphere); an earlier value of 5.97% was not reproducible.

Table S4. Biomarker-gated development cascade

Stage	Gate / readout	Question answered

1 — In silico design	ipTM ≥ 0.87, in-groove register, binding IC_{50}	Will it fold and present?
2A — Biochemical	K_D (SPR), T_m (DSF), SEC/CD	Is it a stable, real pMHC-II?
2B — Immunological assembly	Tetramer^{+} cognate frequency (patient PBMC), TCR-seq [3,4]	Does it engage the right T cells?
2C — Functional tolerance	IL-10/Tr1 signature + suppression; no degranulation	Does it tolerize rather than activate? (candidate PD biomarker)
3 — Delivery / formulation	Clinical-grade tolerance assay lock-in	Is the PD marker deployable?
4 — Preclinical (IND-enabling)	In vivo tolerance + histology correlate; GLP tox; N-of-1 CMC	Does tolerance translate; is the product release-testable?
5 — First-in-patient	Antigen-specific PD in humans → histologic remission	Does it work in patients, biomarker-first? [8]

Replaces the earlier fixed-budget phase roadmap. Each stage boundary is a pharmacodynamic checkpoint; the Stage-2C IL-10-biased Tr1 signature without effector/degranulation activity is the candidate clinical PD biomarker. Financial/timeline planning is addressed in the program's separate business/scientific plans, not in this research supplement.

Table S5. Key scientific risks & mitigations

Risk	**Severity**	**Mitigation**
Antigen heterogeneity — no single dominant EoE allergen	High	Per-patient engine prioritization; personalized/multi-allergen cassette; start milk/wheat-dominant subset
Anaphylaxis / reactogenicity from allergen delivery	High	Tolerogenic pMHC-NP (not bare peptide) [9,11]; Tier-C degranulation gate; dose-escalation
No validated EoE tolerance PD biomarker exists	High	Build & qualify in Stage 2C before any symptom endpoint [8]
pMHC modality clinically unproven	Med–High	Navacim [11] / celiac-NP (TAK-101) [10] mechanistic read-across
N-of-1 CMC / release testing	Med–High	Shared-backbone control strategy; neoantigen-vaccine precedent [12]; early FDA CMC alignment
Wheat/soy epitopes are unvalidated priors	Medium	Funded wet-lab validation; milk lead prioritized on documented milk-responsive T cells [5,6]
	Medium	

Co-primary symptom COA is demanding		Patient-co-designed PRO; histologic surrogate under Accelerated Approval [1]

Supplemental References

All DOIs verified against CrossRef/OpenAlex. Shared numbering with the main manuscript.

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