Scientific Development Plan — Esophageal Adenocarcinoma (EAC)

Dual-arm program: GUCY2C T-cell engager (treatment) + DKK1 neutralizing trap (interception)

Generated by the Claude Science drug-program pipeline. Every claim is traceable to a retrieved source (cBioPortal, Open Targets, ClinicalTrials.gov, Drugs@FDA) or a generated computational result. In-silico results and clinical assumptions are flagged as open questions with go/no-go gates.

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0. Program summary

- **Indication / scope:** Esophageal adenocarcinoma — full trajectory (Barrett's → LGD/HGD → intramucosal/early EAC → locally advanced → metastatic). Strategy intent: interception through treatment.
- **Lead targets:**
- **GUCY2C** (guanylyl cyclase C) — treatment arm, advanced/peri-operative EAC
- **DKK1** (Dickkopf-1) — interception arm, Barrett's → dysplasia progression
- **Evidence basis:** Somatic, chromosomally-unstable adenocarcinoma (TP53 mutated 87%, CDKN2A homozygous-deleted 39%, CCND1 amp 35%, ERBB2 amp 15%; cBioPortal esca_tcga_pan_can_atlas_2018, n=182). Novelty steer deprioritized HER2/EGFR/VEGFA/PD-1/CLDN18.2/FGFR2b. GUCY2C selected on GI-luminal restriction (therapeutic window); DKK1 on secreted Wnt-axis progression biology with DKN-01 clinical precedent.
- **Modality:** Engineered antibody-based binders (two formats). GUCY2C → anti-GUCY2C × anti-CD3 T-cell engager. DKK1 → neutralizing binder / ligand-trap.
- **Top patient priorities addressed:** eating/swallowing preservation; QoL-weighted survival; minimize toxicity (targeted > cytotoxic); reduce uncertainty via biomarkers; well-tolerated interception to avoid/defer esophagectomy.
- **One-line thesis:** Attack EAC at two points on its trajectory with tumor-selective biologics — intercept malignant progression in Barrett's by neutralizing secreted DKK1, and treat established EAC by redirecting T cells against the GI-restricted surface antigen GUCY2C — chosen specifically to sidestep the crowded HER2/PD-1/VEGF field.

1. Computational design engine

- **Target structure source:** AlphaFold DB — GUCY2C (UniProt P25092, model v6), DKK1 (O94907, v6).
- **Preparation:**
- GUCY2C extracellular domain isolated (residues 24–430; well-folded, pLDDT>70 across 50–425). Intracellular kinase/cyclase domain (~490–1073) excluded — irrelevant for a surface binder.
- DKK1 CRD2 domain isolated (residues 178–256), the LRP6-binding functional region and the high-confidence structured segment; disordered N-terminus/linkers excluded.
- **Hotspot/epitope definition:** surface-exposed, high-confidence residue patches selected by a neighbor-count + pLDDT heuristic — GUCY2C ECD patch [239,240,267,422,424,425]; DKK1 CRD2 patch [181,182,183,194,195].
- **Design method:** RFdiffusion binder backbones (Complex_base checkpoint, noise=0), 8 backbones/target, binder length 70–100 aa (GUCY2C) / 60–90 aa (DKK1) → ProteinMPNN inverse folding (v_48_020, binder chain, 8 sequences/backbone at T=0.1).

- **In-silico validation:** Boltz-2 fold-back of each best-per-backbone binder in complex with the target (--use_msa_server on the target chain, binder single-sequence, 5 diffusion samples). Metrics: interface ipTM (pass >0.5), complex pLDDT (fold >0.7).
- **Selected leads:**

Arm	Lead	Binder len	ipTM	Complex pLDDT	Pass rate
Treatment (GUCY2C)	gucy2c_bb2	71 aa	**0.915**	0.896	7/8
Interception (DKK1)	dkk1_bb1	63 aa	**0.858**	0.908	8/8

- **Sequence-level liabilities (ESM / next step):** run ESM-2 embeddings and a developability screen (charge patches, hydrophobicity, N-glyco motifs, unpaired Cys) on the top leads; not yet done — flagged as open question O-1.

2. In-vitro assay cascade

1. **Expression & QC** — express binders as His-tagged domains (E. coli/mammalian); GUCY2C arm re-formatted into an anti-CD3 bispecific (knob-in-hole or scFv-based). SolubleMPNN redesign available if aggregation appears. Purity/identity by SEC + intact MS.

2. **Biophysics / binding** — SPR/BLI affinity + kinetics against recombinant GUCY2C ECD and DKK1 CRD2; thermal (nanoDSF) and colloidal stability.

3. **Functional / cellular** —

- **GUCY2C:** T-cell–redirected cytotoxicity (TDCC) against GUCY2C+ EAC lines / organoids; cytokine release; specificity vs. GUCY2C-low normal intestinal epithelium (window confirmation).
- **DKK1:** TCF/LEF Wnt-reporter rescue; DKK1 neutralization in Barrett's-derived organoids; migration/invasion readouts.
- **Go/no-go per tier:** advance only if KD ≤ 50 nM, Tm ≥ 55 °C, and a functional EC50 in the disease-relevant assay with ≥10× selectivity over normal-tissue control.

3. Delivery & formulation

- **GUCY2C T-cell engager:** IV, bispecific antibody format; step-up dosing to manage CRS; half-life extension (Fc or albumin-binding) for dosing convenience.
- **DKK1 trap:** IV or SC neutralizing antibody/Fc-fusion; interception setting favors a well-tolerated, infrequent SC schedule (patient-priority: minimize burden).
- Standard antibody formulation (histidine/trehalose, polysorbate); stability program to support the chosen route.

4. Preclinical package

- **Proof-of-concept models:** GUCY2C — EAC cell-line/PDX xenografts + human T cells, or humanized-CD3 transgenic; GUCY2C+ organoids. DKK1 — Barrett's/EAC organoid progression models and, where available, a surgical/reflux or L2-IL1β-type Barrett's mouse model for interception PoC.
- **PK/PD & biodistribution:** target-engagement PD (see §7); tumor vs. normal-GI distribution is the key GUCY2C safety readout.

- **GLP tox:** on-target GI toxicity is the central risk for GUCY2C (normal enterocyte expression) — dedicated GI histopathology; DKK1/Wnt modulation requires bone (Wnt→osteo) and intestinal monitoring.
- **Limitations:** GUCY2C's luminal restriction in *normal* gut is a species- and polarity-dependent argument; mouse Gucy2c expression differs from human — flag O-2.

5. CMC & IND strategy

- Standard recombinant antibody manufacturing (CHO); bispecific adds pairing/assembly QC. Platform framing: both assets are antibody-class, sharing expression/analytics/formulation platforms.
- Sequence the IND-enabling package treatment-arm first (clearer regulatory precedent for advanced EAC); interception arm follows with a prevention-oriented package (§8, regulatory stage).

6. Companion diagnostic

- **GUCY2C:** IHC/RNA for GUCY2C positivity on tumor tissue for patient selection.
- **DKK1:** plasma/tissue DKK1 level as enrichment biomarker; Barrett's segment length + p53 IHC + dysplasia grade for interception-arm eligibility. Cytosponge-based molecular triage is an emerging, less-invasive option aligned with patient priorities.

7. Translational PD / biomarker strategy & go/no-go gates

- **PD / target-engagement:** GUCY2C — on-treatment T-cell infiltration + GUCY2C occupancy in tumor biopsies; DKK1 — free vs. bound DKK1 and Wnt-pathway transcriptional signature.
- **Efficacy-anchoring (patient-priority-linked):** dysphagia-free interval and swallowing function (treatment); histologic dysplasia regression / progression-free interval (interception) — both map to the eating/QoL priorities from Stage 2.
- **Decision gates:**
- **G1 (developability):** leads pass ESM/biophysics liability screen → else redesign.
- **G2 (binding):** KD ≤ 50 nM + confirmed epitope → else affinity maturation.
- **G3 (function + selectivity):** potent + ≥10× normal-tissue window → else kill/reformat.
- **G4 (in-vivo PoC):** tumor control (treatment) / dysplasia regression (interception) at tolerated dose.
- **G5 (tox):** acceptable GI (GUCY2C) / bone-GI (DKK1) safety margin → IND.

8. Open questions & required validation

- **O-1 (compute, near-term):** ESM-2 embedding + developability/liability screen on top leads; orthogonal fold-back (Chai-1/AF2-multimer) for confidence consensus. *Not yet done this session.*
- **O-2 (preclinical):** GUCY2C normal-gut therapeutic window is the make-or-break question — needs human normal-tissue IHC + TDCC selectivity data; mouse cross-reactivity caveat.
- **O-3 (clinical):** whether a GUCY2C T-cell engager's on-target GI toxicity is manageable at efficacious doses (CRS + enterocyte risk).
- **O-4 (clinical, interception):** DKK1-neutralization must demonstrate dysplasia regression on a timescale and safety profile acceptable for a (largely pre-malignant) Barrett's population — a high regulatory bar. *Requires clinical validation.*
- **O-5:** epitope hotspots were chosen by structural heuristic, not experimental functional-site mapping — confirm the DKK1 binder blocks the LRP6 interface and the GUCY2C binder targets an accessible, tumor-exposed epitope.

Traceability: driver genomics — cBioPortal esca_tcga_pan_can_atlas_2018 (n=182). Target association/tractability — Open Targets (EAC MONDO_0005028, Barrett's MONDO_0013662). Competitive landscape — ClinicalTrials.gov (351 EAC / 106 active / 203 Barrett's interventional) + Drugs@FDA. Design leads — RFdiffusion→ProteinMPNN→Boltz-2 (this session).