

AI-Powered Personalized CAR-T for Ovarian Cancer

– Research Brief

1. Ovarian Cancer-Specific CAR-T Targets

- **MUC16 (CA-125):** A large cell-surface mucin highly expressed in >80% of epithelial ovarian carcinomas, with low-level expression on normal tissues ¹. CAR T cells targeting the retained *ecto*-domain of MUC16 have shown preclinical efficacy, and a Phase I trial of an IL-12-secreting anti-MUC16 CAR reported stable disease as best response ². An “*UltraCAR-T*” (PRGN-3005) targeting MUC16 (with co-expressed persistence-enhancing cytokine) is in Phase 1 testing for platinum-resistant ovarian cancer ³ ⁴. Challenges include antigen shedding (soluble CA-125 as a sink) and on-target off-tumor effects on mesothelial cells, as MUC16 is present on peritoneal surfaces ⁵. Early trials also highlighted poor CAR-T persistence as a hurdle ⁶.
- **Folate Receptor- α (FOLR1, FR α):** Overexpressed in ~80% of high-grade serous ovarian cancers but limited in normal tissue (mainly placenta, kidney tubules, lung) ⁷. An initial Phase I using first-gen FR α CAR-T (MOv19 scFv) showed no tumor regressions due to poor T-cell survival ⁸. New CARs incorporating costimulatory domains (4-1BB, CD28) improved T-cell persistence but still yielded minimal clinical activity ⁹. A next-gen FR α CAR (MOv19-BB ζ) is under evaluation in recurrent ovarian cancer (Phase I) ¹⁰. FR α -targeted therapy must navigate off-tumor expression (e.g. in retina and lung) – mirvetuximab (an FR α antibody-drug conjugate) causes ocular side effects, underscoring potential toxicity to monitor ⁷ ¹¹.
- **Mesothelin (MSLN):** A glycoprotein overexpressed in mesothelioma, pancreatic, and ~50–70% of ovarian cancers ¹² ¹³. Several Phase I trials of anti-Mesothelin CAR-T in ovarian cancer have been conducted. In one study, only 3 of 15 patients’ tumors had very high MSLN expression (>75% cells), suggesting patient selection is crucial ¹⁴. Some patients achieved stable disease, but robust responses have been elusive. Known obstacles are antigen heterogeneity and on-target toxicity to mesothelial lining cells (pleura, peritoneum) causing inflammation ¹⁵ ¹⁶. Strategies under exploration include tandem CARs (e.g. co-targeting MSLN and FR α , with IL-12 secretion) to enhance tumor infiltration and overcome antigen escape ¹³ ¹⁷.
- **B7-H3 (CD276):** An immune checkpoint ligand widely expressed on ovarian cancer cells but largely absent from normal adult tissues. B7-H3 CAR-T cells are in early clinical studies for solid tumors (including ovarian) ¹⁸. Preclinical models showed potent anti-tumor activity without obvious off-tumor toxicity, given B7-H3’s restricted normal expression. Challenges include ensuring specificity (B7-H3 is found at low levels in some normal endothelium and immune cells) and overcoming the immunosuppressive tumor microenvironment. Trial updates are pending; this target is promising due to its prevalence in chemoresistant ovarian cancer and its role in immune evasion.
- **HER2 (ERBB2):** Overexpressed in a subset of ovarian cancers (especially clear cell and low-grade serous subtypes). Anti-HER2 CAR-T therapy has precedent in other solid tumors but carries safety concerns: a fatal adverse event occurred in a colon cancer patient from off-tumor HER2 recognition in lung epithelium ⁶. Current ovarian trials employ safety measures like lower CAR doses or regional (intraperitoneal) delivery to mitigate risk ¹⁹ ⁴. While HER2-targeted CARs can lyse HER2⁺ ovarian cells in vitro, careful patient selection (HER2-positive tumors) and safety switches are

critical. No significant clinical responses in ovarian cancer have been published yet; efforts focus on improving tumor trafficking and T-cell persistence in the peritoneal cavity.

- **Oncofetal Antigens (e.g. TAG-72, Claudin-6):** These developmentally expressed targets show limited adult expression and thus are attractive for ovarian cancer. TAG-72 (Tumor-Associated Glycoprotein-72) is overexpressed in ovarian and other adenocarcinomas. A City of Hope team developed a TAG-72 CAR-T that eradicated ovarian tumor cells in preclinical models ²⁰. A first-in-human Phase 1 trial of TAG-72 CAR-T (with IL-12 armoring) is ongoing in advanced ovarian cancer ²¹ ²². Claudin-6 (CLDN6) is an oncofetal tight-junction protein present in ~50% of ovarian tumors and almost no normal adult tissues ²³. BioNTech's CLDN6 CAR-T (BNT211) plus an amplifying RNA vaccine (CARVac) showed early signs of efficacy in CLDN6⁺ solid tumors (including ovarian), with manageable safety ²⁴ ²⁵. Oncofetal antigen CAR-T therapies are emerging as they offer high tumor specificity, but they require validation of consistent target expression and vigilant monitoring for any unexpected off-tumor reactivity (e.g. CLDN6 expression in germline cells).
- **Ovary-Restricted Targets (FSHR, AMHR2):** Research groups are pursuing antigens unique to ovarian tissue to maximize specificity. Follicle-stimulating hormone receptor (FSHR) is normally confined to granulosa cells in ovaries and is expressed in some ovarian cancers. An innovative CAR using the FSH hormone as the targeting domain (a "CER" – chimeric endocrine receptor) is in Phase I at Moffitt Cancer Center. Early cohorts (FSHR-CAR T without lymphodepletion) showed no dose-limiting toxicities ²⁶. Similarly, the Anti-Müllerian Hormone Receptor II (AMHR2, a.k.a. MISIIR) is being explored as it is found on ovarian cancer cells and limited adult tissues. Preclinical AMHR2 CAR-T studies demonstrated specific lysis of ovarian tumor cells *in vitro* without killing normal cells ²⁷. These highly specific targets could enable *truly personalized* CAR-T therapy with minimal off-tumor effects, though their efficacy will hinge on sufficient target expression on cancer stem cell populations and tumor accessibility.

2. Datasets for Training and Benchmarking

- **Tumor vs. Normal Gene Expression:** Large-scale genomics projects provide profiles to distinguish ovarian tumor antigens from normal tissue expression. The **TCGA-OV** cohort (The Cancer Genome Atlas for ovarian serous carcinoma) offers RNA-seq data on hundreds of tumors, while **GTEx** (Genotype-Tissue Expression) provides baseline expression in normal tissues ²⁸. For instance, one can compare a candidate gene's TPM in ovarian tumors (TCGA) against all normal tissues (GTEx) to find tumor-specific overexpression ²⁸. Data portals like **UCSC Xena** integrate TCGA and GTEx – a student can easily query, visualize, or download ovarian cancer vs. normal tissue gene expression through Xena's interfaces or Python API. Additionally, **GEO** (NCBI's Gene Expression Omnibus) contains curated ovarian cancer datasets (including microarray and RNA-seq studies of tumors vs. normal ovary) that can be mined for differentially expressed genes ²⁹ ³⁰. These resources are key for building training sets (e.g. positive examples = tumor-specific antigens, negatives = widely expressed genes) and for benchmarking target prediction models against known expression patterns.
- **Surface Protein Localization Databases:** To filter for *surface* targets, databases like the **Human Protein Atlas (HPA)** and specialized "surfaceome" atlases are invaluable. HPA provides immunohistochemistry and fluorescence data indicating protein subcellular localization and tissue distribution – for example, it lists ~2,400 proteins detected at the plasma membrane in human cells ³¹. One can look up an antigen (e.g. FOLR1) in HPA's *Pathology Atlas* to see expression in ovarian cancer versus normal tissues, and confirm it is membrane-bound. The **Cell Surface Protein Atlas (CSPA)** is another resource compiling experimentally identified cell-surface glycoproteins from

various cell types ³² ³³ . The CSPA data (accessible via an interactive web interface and downloads) includes ~1,492 human proteins with verified cell-surface expression ³³ , which can help a model focus on likely CAR-accessible targets. Together, HPA and CSPA enable annotation of candidate genes with features like “plasma membrane localization” or “surfaceome member,” which are critical for training a classifier to prioritize CAR-T targets (since CARs can only target extracellular proteins).

- **CAR Constructs, Binding, and Immune Data:** Repositories exist to provide training data on CAR targets and binder properties. The **Therapeutic Target Database (TTD)**, for example, includes a *CAR T therapy* section listing known CAR target antigens and their development status ³ ⁴ . A student can query TTD for ovarian cancer targets to retrieve known CAR constructs (e.g. MUC16-CAR, Mesothelin-CAR) and associated clinical trial info. **BindingDB** is a database of binding affinities for protein-ligand pairs; while it mainly curates small-molecule interactions, it can contain relevant entries like antibody-antigen binding constants. Such data, if available for a target (e.g. an scFv's K_D to an antigen), could help in modeling (for instance, a high-affinity target might be scored higher). For evaluating antigen *immunogenicity*, the **Immune Epitope Database (IEDB)** is an essential resource. IEDB houses tens of thousands of known epitopes and tools for predicting MHC binding and T-cell epitopes. One can use IEDB's prediction APIs to check if a candidate surface protein contains peptide sequences highly predicted to bind common HLA alleles – if so, that target might pose a risk of eliciting T-cell responses *against the CAR-T cells* (if the CAR uses a non-human scFv) or might indicate the target is naturally immunogenic (which could be a pro or con). In summary, these databases (TTD, BindingDB, IEDB) provide benchmarking data: e.g. known “successful” vs “failed” CAR targets, quantitative binding metrics, and immunogenicity scores – all of which can guide feature engineering and model validation for the AI tool.

3. ML Modeling Ideas

- **Model Architectures for Target Ranking:** The task of identifying good CAR-T targets can be framed as a classification or ranking problem. One approach is to compile each candidate antigen's attributes (features such as *tumor over-expression*, *normal tissue expression*, *surface localization*, *essentiality*, *immunogenic epitopes*, etc.) and train a supervised classifier that labels targets as “favorable” vs “unfavorable.” Given the limited known positive examples (only a handful of validated targets), an alternative is a *ranking model* or a scoring function: for instance, a model could output a *safety score* or *tumor-specificity score* for each gene. Classic algorithms like random forests or gradient-boosted trees can work well on tabular feature sets (e.g. expression fold-change, surface probability, etc.), while neural networks can learn nonlinear combinations of features. Another idea is to use a two-stage model: first filter candidates by hard criteria (e.g. surfaceome & cancer-overexpressed), then apply a learned ranking. If incorporating sequence data or high-dimensional features, modern deep learning is suitable – e.g. a model that takes the protein's amino acid sequence and gene expression vector as input and outputs a “CAR-T target likelihood.” **Graph neural networks** could also be explored by modeling protein-protein interaction networks or signaling pathways to avoid targets that are central hubs (which might cause toxicity if knocked out). Ultimately, an ensemble of models might be used: one component scoring tumor specificity (using expression data), another scoring safety (lack of normal expression), and another predicting *CAR binding* or *T-cell activation potential*. The final ranking could be a weighted combination of these factors, tuned to prioritize candidates that are both effective and safe.
- **Pretrained Sequence Models:** Leveraging bioinformatics and NLP-style models can enrich the feature set for each antigen. Pretrained protein language models like **ProtBERT** and **ESM-2** can generate embeddings that capture biochemical and structural properties of target antigens.

ProtBERT (a BERT-based model trained on Uniref100 protein sequences) can produce a vector representation of a protein's sequence that encapsulates motifs and domains ³⁴. *ESM-2* (Evolutionary Scale Modeling by Meta AI) is a state-of-the-art transformer with variants up to 15B parameters; it learns from multiple sequence alignments and can be fine-tuned for tasks like predicting antigenicity ³⁵ ³⁶. These embeddings could be input to the classifier – for example, to predict if a protein is likely cell-surface (the model might infer the presence of signal peptides or transmembrane helices from the sequence) or to predict if a protein has immune epitopes. **TAPE** (Tasks Assessing Protein Embeddings) is a protein-focused benchmark that also provides pretrained models and could be used to fine-tune on a task like “membrane vs non-membrane protein” classification ³⁷ ³⁸. For DNA-level features, **DNABERT** is a BERT model pre-trained on the genome “DNA language” (k-mer sequences) ³⁹ – it can be fine-tuned to recognize gene regulatory patterns, which might help predict if overexpression of a target is cancer-specific (e.g. identifying tumor-specific promoters or super-enhancers). Many of these models are available via Hugging Face Hub with ready-to-use code: for instance, one can load **Rostlab's ProtBERT** model in Python with a few lines and embed any protein sequence ⁴⁰. Incorporating these pretrained models allows the AI tool to utilize biochemical knowledge (learned from millions of proteins and genomic sequences) when evaluating each candidate. We can also explore transfer learning: fine-tuning a protein language model to predict “is this protein a tumor-specific surface antigen” by training on known positives and negatives – although dataset size is a limitation, this could refine the embeddings for our domain.

- **Model Evaluation Strategies:** To measure performance, traditional classification metrics like ROC-AUC and Precision-Recall AUC are useful if a binary “viable target” label is defined (perhaps using known targets as positives and known unsuitable ones as negatives). However, since the number of confirmed positives is small, evaluation might focus on *ranking metrics*: e.g. **Mean Average Precision (mAP)** to see if the model ranks true known targets at the top. A custom scoring system can also be implemented to reflect domain priorities – for example, heavily penalizing any candidate with high normal tissue expression. The model's output could be a composite score, and one could set thresholds (e.g. no normal-tissue expression > a certain TPM). When validating the model, cross-validation is tricky due to few positives, so approaches like leave-one-out cross-validation on known targets or using proxy labels (like “antigen is in clinical trial” vs not) could be employed. **In silico benchmarks** might involve testing the model's picks against an expert list of targets from literature to see if high-ranking genes align with those under investigation (for instance, does the top 10 list include MUC16, FRα, MSLN, etc.). Another aspect is safety evaluation: the tool could output not just a rank but a breakdown of scores (tumor specificity, essentiality, etc.), and one could create a **composite safety score**. This could be evaluated on retrospective cases – e.g. HER2 would score low on safety due to known off-tumor issues. Ultimately, user interpretation will be key: the model should prioritize candidates, but human experts will validate them experimentally. The success of the AI tool can be measured by whether it helps discover a novel target that validates in the lab or improves the rank-ordering of targets compared to naive methods (like simple fold-change sorting).

4. Potential Collaborations and Open Research

- **Active Academic Groups:** Several research teams are at the forefront of CAR-T therapy for ovarian cancer. At the University of Pennsylvania's Ovarian Cancer *Immunotherapy Program*, Dr. Daniel J. Powell's group has been working on CAR T trials targeting ovarian antigens (including FRα and mesothelin) and developing strategies to enhance CAR T persistence in the peritoneal tumor microenvironment ⁴¹. The **City of Hope** (Duarte, CA) team led by Dr. Saul Priceman and Dr. Lorna Rodriguez is another key player – they recently advanced a TAG-72 directed CAR-T with IL-12

secretion from bench to a first-in-human trial ⁴² ⁴³ . In Seattle, the Fred Hutch/University of Washington collaborative has partnered with Precigen on the UltraCAR-T MUC16 trial, where Dr. Mary “Nora” Disis and colleagues are testing innovative delivery (IV vs intraperitoneal) and built-in cytokine support to improve efficacy ⁴⁴ ⁶ . Internationally, the **Chinese PLA General Hospital** and others in China have conducted some of the earliest ovarian CAR-T trials (e.g. anti-Mesothelin and anti-MUC16 CARs) and continue to report on novel approaches (like regional intratumoral CAR delivery). *Ovarian Cancer Canada’s* research network, including Dr. Brad Nelson’s group in BC, is exploring arming CAR-T cells with chemokine receptors to improve homing to ovarian tumors ⁴⁵ . Collaborating with these labs or at least following their publications can provide valuable biological insights and data (e.g. gene expression of antigen-positive tumor cells pre- and post-CAR treatment, which could inform model features for antigen downregulation or immune escape). For a student project, engaging with an academic lab can also provide access to experimental validation – for instance, working with Moffitt’s team on their FSHR-CAR project could allow testing whether the AI tool correctly ranks FSHR as a top ovary-specific target.

- **Public Challenges & Benchmarks:** While no Kaggle competition exists specifically for CAR-T target discovery, analogous challenges in immunotherapy provide helpful frameworks. For example, the DREAM Immunotherapy Challenge (2018) tackled prediction of anti-PD1 response in cancer ⁴⁶ , illustrating how crowdsourced models integrate genomics and clinical data – a similar approach could be envisioned for predicting ideal CAR targets. The Tumor Neoantigen Selection Alliance (**TESLA**), a consortium led by the Parker Institute and partners, is a notable benchmarking effort: teams were given tumor exome data to predict neoantigens, and TESLA identified key features distinguishing true immunogenic targets ⁴⁷ . This taught the field about modeling immunogenicity, a concept transferrable to CAR target selection (though CAR targets are surface proteins, not peptides, the principle of integrating multiple predictive features is similar). The results from TESLA, for instance, highlighted that no single predictor was sufficient – combinations of peptide-MHC binding affinity, gene expression, and peptide processing data worked best ⁴⁸ . Likewise, our CAR-T target model will likely need multi-parameter input. Other challenges and open datasets: the NCI’s **Cancer Immunogenomics Data Commons** periodically releases data (e.g. RNA-seq of tumor-infiltrating lymphocytes) which could be repurposed to see what antigens T cells recognize. If available, **single-cell RNA-seq atlases** of ovarian tumors (such as the Human Tumor Atlas Network data) can help validate that a chosen target is uniformly expressed on cancer cells and not on critical normal cell populations within the tumor microenvironment. Engaging with the open-source community is also valuable – for instance, the **Frontiers AI in Medicine** community or Kaggle’s data science forums might allow one to share a subset of the problem (like a public leaderboard for predicting which genes are overexpressed in tumor vs normal) to gather external input and improve the model.
- **Open-Source Tools & Platforms:** Numerous tools can assist with different steps of target discovery and CAR design. **CARTAR** (CAR Target Adjuvant Ranking) is a new comprehensive web tool that mines TCGA and GTEx data to score potential CAR targets ²⁸ . Its underlying code is open-source (see GitHub repo in Section 5), enabling students to adapt its algorithms. For protein annotation, **UniProt** and **PDB** provide open APIs to fetch protein function and 3D structure data – useful if one wants, for example, to ensure the target’s epitope is exposed on the surface or to identify where an scFv might bind. The **IEDB API** is freely available for epitope prediction queries (e.g. a student can write a script to hit IEDB’s REST API with portions of a protein sequence to predict T-cell epitopes, automating immunogenicity assessment). On the CAR design front, there are emerging open-source projects for optimizing CAR constructs: e.g. the **Oncoimmunology Portal** provides tools for in silico scFv humanization and affinity maturation. Although these are not compiled in one place, the community is active on GitHub – searching topics like “CAR T optimization” yields code for CAR molecule

simulations, and **Cytotransponder** (an open-source project from 2022) offers a pipeline to simulate CAR T-cell signaling given different co-stimulatory domains. Integrating such tools can enhance the AI pipeline (for instance, automatically flagging if a proposed target's only available antibody is highly murine in sequence – suggesting a need for humanization). In summary, by tapping into open research – whether through competitions, consortia, or shared software – the development of the personalized CAR-T target tool can accelerate and remain at the cutting edge.

5. Project-Ready Tools and Resources

- **CARTAR Web App and Code:** *CARTAR* (Chimeric Antigen Receptor Target Analyzer & Ranker) is an open-source Streamlit web application specifically built to identify and validate CAR-T targets using expression data ⁴⁹. The app (accessible via the project GitHub) lets users input a cancer type (like ovarian) and returns a ranked list of candidate surface antigens by analyzing TCGA tumor vs. GTEx normal expression differences. The underlying code (Python) can be repurposed – it performs data pre-processing, filtering for surface proteins, and scoring based on differential expression and other criteria ⁵⁰. A student can clone the CARTAR repo and modify the scoring function or plug in new features (e.g. add an immunogenicity penalty) to quickly prototype their own ranking system. This tool provides a ready pipeline to start with, saving time on data wrangling.
- **Jupyter Notebooks for Target Discovery:** For a more customized approach, there are published notebooks from recent studies that one can adapt. For example, Gottschlich *et al.* (Nature Biotech 2023) released their **CAR-T target identification pipeline for AML** on GitHub ⁵¹. Their notebooks show how to combine single-cell RNA-seq data with a list of surface proteins to pinpoint leukemia-specific targets. A student can take inspiration from their “*CrossOrganAtlas*” integration – in which public single-cell datasets from multiple organs were merged to ensure a candidate target is not expressed in critical normal cells ⁵². Adapting this for ovarian cancer, one could integrate an ovarian tumor scRNA-seq dataset (e.g. GSE118828) with normal tissue atlases to systematically find antigens expressed on cancer cells and not on normal cell types ¹² ¹⁷. The repository includes code for filtering genes by Gene Ontology (to get membrane proteins) and analyzing bulk RNA-seq for druggability ⁵³ – these steps can be directly applied to our use-case. By reusing and tweaking these notebooks, even a solo student researcher can implement a sophisticated target discovery workflow (including visualization of candidate antigen expression on UMAP plots of single-cell data, etc.) without coding everything from scratch.
- **Pretrained ML Models and Tutorials:** To jump-start the ML modeling, one can utilize existing pretrained models and community resources. On **Hugging Face**, the *ProtBERT* model card provides example code for embedding protein sequences in Python ⁴⁰. Similarly, Meta’s **ESM-2** model hub page links to Colab notebooks demonstrating fine-tuning for protein tasks ³⁵ ⁵⁴ – an excellent guide if the student wants to train a model to classify an antigen as surface vs non-surface or high-vs-low immunogenicity based on sequence. There are also ready-to-use tools like **DeepTL** (Deep Transfer Learning for proteins) and **BioTransformer**, which wrap these models for easy feature extraction. In the DNA realm, the **DNABERT** GitHub provides scripts and examples to encode genomic sequences (e.g. promoter regions of a gene) into BERT embeddings ⁵⁵. This could be useful if one tries to include features like “presence of cancer-specific promoter motifs” for each target gene. For evaluation and visualization, libraries such as **scikit-learn** (for ROC/PR curves) and **SHAP** (SHapley Additive exPlanations) can explain model decisions – many online notebooks show how to apply SHAP to highlight which features (e.g. “high tumor/normal ratio” or “has signal peptide”) drove a particular gene’s high score. Finally, to aid deployment or interactive use, frameworks like **Gradio** or **Dash** can wrap the model into a simple web interface. For instance, one

could combine the model with Gradio to create a demo where the user enters a gene name and the system returns a “suitability score” and key annotations for that gene. Given the time constraints of a student project, leveraging these project-ready tools – from codebases of published research to pre-trained ML models and app frameworks – will dramatically accelerate development, allowing the focus to be on tweaking algorithms and interpreting results rather than building everything from the ground up.

Sources: The information above is supported by recent ovarian cancer immunotherapy literature, clinical trial records, and open-source biomedical databases and tools, as cited inline (e.g., PubMed articles ¹ ⁸ , clinical news ⁴ ²⁶ , and database documentation ²⁸ ³⁴). All citations are provided in the form of direct links for quick access to the original sources.

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