From Target Identification to Therapeutic Antibody Development and Characterization

Abstract

The pre-clinical development of therapeutic monoclonal antibodies requires the integration of rational target identification, antibody discovery, structural and functional characterization, and formulation-driven stabilization. This establishes such an integrated pipeline through a series of connected yet distinct domains spanning the fields of bioinformatics, antibody discovery, higher-order structure monitoring, and biotherapeutic formulation. Collectively, these studies aim to advance the discovery and development of novel targeted therapeutics, particularly in the context of breast cancer.

The first workflow focused on the **systematic identification and validation of omics-identified therapeutic biomarkers** using breast invasive carcinoma (BRCA) datasets from The Cancer Genome Atlas (TCGA). A comprehensive analysis was performed on a cohort of approximately one thousand breast cancer patients using an integrated mapping strategy that combined transcriptomic and proteomic data. A robust bioinformatic pipeline was designed to prioritize potential therapeutic targets based on differential expression, with refinement guided by logFC and adjusted *p* values derived from both mRNA and protein abundance analyses. This approach identified 23 intersected omics biomarkers, which were further bioinformatically evaluated for tumour specificity, cell-surface localization, and expression across tumour and normal cell lines. Additional validation through literature mining, database searches, and GTEx-supported normal tissue expression analysis strengthened the biological and therapeutic relevance of the shortlisted candidates. Among these, **Discoidin Domain Receptor 1 (DDR1)** emerged as a promising transmembrane therapeutic target and candidate for antibody generation and characterization. A naïve human phage display library was screened to obtain DDR1-specific single-chain variable fragments, which were subsequently reformatted into full-length human IgG1 antibodies. These antibodies were expressed, purified, and subjected to biochemical, biophysical, and functional evaluation. Several candidates demonstrated high-affinity binding to native DDR1 on breast cancer cells and showed tumour cell lysis activity through antibody-dependent cell cytotoxicity (ADCC). This workflow establishes a direct translational connection between bioinformatics-guided target identification and experimental antibody development, highlighting the feasibility of integrating publicly available omics resources with *in vitro* antibody discovery strategies to generate novel therapeutic candidates.

Recognizing that successful antibody development also depends on structural integrity and product quality, the second workflow focused on **higher-order structure (HOS) characterization and chemometric-enabled platform for analytical monitoring** of monoclonal antibodies, using trastuzumab as a model antibody. Since comprehensive HOS characterization is a regulatory requirement for therapeutic antibodies, particularly for biosimilarity assessment, a novel multi-technique spectroscopic platform was developed to monitor structural heterogeneity during thermal degradation of **trastuzumab**. This analytical platform integrated UV-visible spectroscopy, far-UV circular dichroism, intrinsic and extrinsic fluorescence spectroscopy, and Fourier-transform infrared (FTIR) spectroscopy with chemometric analysis. While size-exclusion chromatography confirmed progressive formation of soluble aggregates, the multi-technique spectroscopic approach provided richer structural insight, revealing early tertiary destabilization and hydrophobic exposure immediately following thermal stress, preceding the global backbone unfolding

detected by circular dichroism. Data fusion using principal component analysis, biplots, and dendrogram analysis enabled high-resolution mapping of conformational transitions over the thermal stress timeline. This platform demonstrates the utility of combining orthogonal spectroscopic methods with multivariate data analysis to sensitively detect early-stage structural heterogeneity in bulk antibody preparations and provides a robust analytical framework for stability studies and biosimilarity evaluation.

The third workflow addressed **formulation optimization through high-throughput screening of unexplored excipients**, with the goal of improving monoclonal antibody stability under stress conditions. Fourteen FDA-approved inactive ingredients were screened using trastuzumab as a model monoclonal antibody under forced thermal stress conditions in accordance with ICH Q5C principles. Among the excipients tested, **myo-inositol, isomalt, and choline chloride** were identified as the most effective stabilizers, producing approximately 40–50% lower aggregation than the control without excipients. Spectroscopic analyses further confirmed improved retention of higher-order structure and increased melting temperature in formulations containing these excipients. Their stabilizing effects were maintained across low and high concentrations, different temperatures, and extended exposure durations, while remaining below recommended parenteral intake limits. In addition, excipient-containing formulations showed less than ~5% homolysis than polysorbate-80 and buffer only controls, indicating favourable biocompatibility. This platform highlights the potential of underexplored FDA-approved excipients to enhance the physical stability of monoclonal antibodies and supports their future inclusion in next-generation biologic formulations.

Overall, this presents a connected and multidisciplinary framework in which each study functions as a separate but complementary platform within the therapeutic antibody development pipeline. From omics-driven target identification and antibody generation to advanced structural monitoring and formulation stabilization, the work demonstrates how integrated strategies can strengthen the discovery, characterization, and stabilization of antibody therapeutics. These findings provide both conceptual and practical advances for breast cancer-targeted therapy therapeutic development, analytical biophysics, and biologic formulation science.