Novel Antibody-Drug Conjugate Formulation and Method for Treating Her2-Low and Her2-Ultralow Breast Cancer with Enhanced Efficacy and Reduced Toxicity

New York General Group
October 10, 2025

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Technical Field

The present invention relates to the field of oncology therapeutics, specifically to an improved pharmaceutical composition comprising an antibody-drug conjugate targeting human epidermal growth factor receptor 2 and a method for treating patients with hormone receptor-positive or hormone receptor-negative, HER2-low or HER2-ultralow metastatic breast cancer. The invention further relates to a dosing regimen that maximizes therapeutic efficacy while minimizing adverse events, particularly interstitial lung disease and left ventricular dysfunction.

Background of the Invention

Breast cancer represents a heterogeneous disease characterized by varying expression levels of molecular markers including human epidermal growth factor receptor 2 and hormone receptors such as estrogen receptor and progesterone receptor. Historically, breast cancer has been classified as HER2-positive when tumors demonstrate an immunohistochemistry score of 3+ or an immunohistochemistry score of 2+ with positive in situ hybridization, or as HER2-negative when tumors show an immunohistochemistry score of 0, 1+, or 2+ with negative in situ hybridization. Recent clinical evidence has established that a substantial proportion of tumors previously classified as HER2-negative actually express low levels of HER2 protein, a category now designated as HER2-low, defined as immunohistochemistry 1+ or immunohistochemistry 2+ with negative in situ hybridization. Approximately 65% of tumors originally classified as HER2-negative meet HER2-low criteria, representing a significant patient population with previously limited targeted treatment options.

The DESTINY-Breast04 clinical trial demonstrated that trastuzumab deruxtecan, an antibody-drug conjugate comprising a HER2-directed humanized immunoglobulin G1 monoclonal antibody linked to a topoisomerase I inhibitor payload, significantly improves overall survival and progression-free survival compared with physician's choice chemotherapy in patients with HER2-low metastatic breast cancer. The updated analysis with a median follow-up of 32.0 months confirmed a median overall survival of 22.9 months for trastuzumab deruxtecan versus 16.8 months for chemotherapy in the overall cohort, representing a 31% reduction in risk of death. In the hormone receptor-positive cohort, median overall survival was 23.9 months for trastuzumab deruxtecan compared with 17.6 months for chemotherapy, while in the hormone receptor-negative cohort, median overall survival was 17.1 months versus 8.3 months, demonstrating a 42% reduction in risk of death.

Despite these significant clinical benefits, current antibody-drug conjugate therapies are associated with notable safety concerns. In the DESTINY-Breast04 trial, adjudicated drug-related interstitial lung disease or pneumonitis occurred in 12.1% of patients receiving trastuzumab deruxtecan, with grade 3 events in 1.1% and grade 5 fatal events in 1.1% of patients. Left ventricular dysfunction was observed in 5.1% of patients, including grade 3 events in 0.3% of cases. Gastrointestinal toxicities including nausea occurred in 76.0% and vomiting in 40.7% of patients. These adverse events necessitate treatment discontinuation in 16.7% of patients and limit the therapeutic window of current formulations.

Furthermore, the DESTINY-Breast04 trial revealed that patients achieving complete response to trastuzumab deruxtecan demonstrated substantially prolonged survival outcomes, with median progression-free survival of 25.3 months and overall survival not yet reached at the time of analysis. This observation suggests that optimizing drug delivery to maximize complete response rates while minimizing toxicity represents a critical unmet medical need. Additionally, exploratory analyses demonstrated that trastuzumab deruxtecan efficacy is independent of estrogen receptor expression level, with similar median overall survival and progression-free survival observed in patients with estrogen receptor expression greater than 10% compared with estrogen receptor-low-positive expression of 1% to 10%, indicating that HER2 expression rather than hormone receptor status is the primary determinant of therapeutic response.

The DESTINY-Breast06 trial further extended the clinical utility of trastuzumab deruxtecan to patients with HER2-ultralow breast cancer, defined as immunohistochemistry 0 with faint or barely perceptible membrane staining in 10% or fewer tumor cells. This trial demonstrated significant improvement in median progression-free survival for trastuzumab deruxtecan compared with chemotherapy in both HER2-low and HER2-ultralow populations, suggesting that even minimal HER2 expression is sufficient to facilitate antibody-drug conjugate uptake and therapeutic efficacy. However, accurate identification and differentiation of HER2-low and HER2-ultralow samples from HER2 immunohistochemistry 0 samples with no staining remains challenging, with concordance rates of only 78% between local and central laboratory assessments, highlighting the need for improved diagnostic methodologies and therapeutic approaches that are effective across the spectrum of low HER2 expression.

Current antibody-drug conjugate formulations deliver the cytotoxic payload through a linker that is cleaved within the tumor microenvironment, releasing the active drug. While this mechanism provides some degree of tumor selectivity, systemic exposure to the released payload contributes to off-target toxicities. The bystander effect, whereby released payload diffuses to neighboring cells, enhances antitumor efficacy but also increases the risk of damage to normal tissues, particularly in organs with high cell turnover such as the lungs and gastrointestinal tract. The median time to onset of interstitial lung disease in the DESTINY-Breast04 trial was 129 days, with a range of 26 to 710 days, indicating that pulmonary toxicity can occur at any time during treatment and requires continuous monitoring.

Existing formulations of trastuzumab deruxtecan are administered intravenously at a fixed dose of 5.4 milligrams per kilogram of body weight every 3 weeks. This dosing regimen does not account for individual patient variability in drug metabolism, tumor burden, HER2 expression levels, or pharmacokinetic parameters. Exposure-adjusted incidence rates for grade 3 or higher treatment-emergent adverse events were 0.64 per patient-year for trastuzumab deruxtecan, indicating that toxicity accumulates with prolonged exposure. The median treatment duration in the DESTINY-Breast04 trial was 8.2 months, with a range extending to 39.1 months in some patients, demonstrating that a subset of patients can tolerate extended therapy. However, the lack of personalized dosing strategies limits the ability to optimize the therapeutic index for individual patients.

The development of resistance to antibody-drug conjugates represents another significant clinical challenge. In the DESTINY-Breast04 trial, the median progression-free survival from randomization to progression on the next line of therapy was 15.4 months for trastuzumab deruxtecan compared with 9.7 months for chemotherapy in the overall cohort, suggesting that trastuzumab deruxtecan does not induce cross-resistance to subsequent therapies. However, the mechanisms underlying primary and acquired resistance to antibody-drug conjugates remain incompletely understood. Potential resistance mechanisms include downregulation of HER2 expression, alterations in antibody internalization pathways, increased expression of drug efflux transporters, and activation of alternative survival signaling pathways. Addressing these resistance mechanisms through combination therapies or novel formulation strategies represents a critical area for therapeutic innovation.

The tumor microenvironment plays a crucial role in determining antibody-drug conjugate efficacy and toxicity. Factors such as tumor vascularity, interstitial fluid pressure, extracellular matrix composition, and immune cell infiltration influence antibody penetration and distribution within solid tumors. Heterogeneous HER2 expression within individual tumors, as well as between primary and metastatic lesions, further complicates therapeutic targeting. The DAISY trial demonstrated antitumor activity of trastuzumab deruxtecan across all HER2 expression levels, including HER2 immunohistochemistry 0, suggesting that HER2-independent mechanisms such as immune activation and vascular disruption may contribute to therapeutic efficacy. However, current formulations do not specifically exploit these mechanisms to enhance tumor selectivity and reduce systemic toxicity.

Pharmacokinetic modeling of antibody-drug conjugates reveals complex disposition characteristics including target-mediated drug disposition, where binding to HER2-expressing cells influences systemic clearance, and payload release kinetics, which determine the duration and magnitude of cytotoxic exposure. The topoisomerase I inhibitor payload of trastuzumab deruxtecan exhibits potent cytotoxicity with a mechanism of action involving DNA damage and cell cycle arrest. However, the released payload has limited plasma stability and is subject to rapid metabolism and elimination, resulting in a narrow therapeutic window. Optimizing the linker chemistry, payload potency, and antibody affinity represents a potential strategy to enhance the therapeutic index, but such modifications require extensive preclinical and clinical validation.

The integration of biomarkers to predict response and toxicity to antibody-drug conjugates remains an area of active investigation. While HER2 expression level is the primary determinant of trastuzumab deruxtecan eligibility, additional biomarkers such as tumor mutational burden, immune checkpoint expression, circulating tumor DNA, and metabolic imaging parameters may provide complementary information to guide treatment selection and monitoring. The DESTINY-Breast04 trial demonstrated consistent efficacy across demographic subgroups including age, race, geographic region, and prior treatment history, suggesting that HER2 expression is the dominant predictive biomarker. However, the identification of patients at highest risk for interstitial lung disease or left ventricular dysfunction would enable more personalized risk-benefit assessments and proactive monitoring strategies.

The economic burden of metastatic breast cancer treatment is substantial, with costs associated with drug acquisition, administration, supportive care, and management of adverse events. The median overall survival benefit of 6.1 months observed with trastuzumab deruxtecan compared with chemotherapy in the DESTINY-Breast04 trial represents a clinically meaningful improvement, but

the cost-effectiveness of antibody-drug conjugates relative to conventional chemotherapy and other targeted therapies requires careful evaluation. Strategies to reduce treatment-related toxicities and improve response rates would enhance both clinical outcomes and economic value.

Therefore, there exists a significant unmet medical need for an improved antibody-drug conjugate formulation and treatment method that maximizes therapeutic efficacy in patients with HER2-low and HER2-ultralow metastatic breast cancer while minimizing the incidence and severity of adverse events, particularly interstitial lung disease, left ventricular dysfunction, and gastrointestinal toxicities. Such an innovation would enable more patients to achieve complete or partial responses, extend progression-free survival and overall survival, reduce treatment discontinuation rates, and improve quality of life. The present invention addresses these needs through a novel pharmaceutical composition and dosing regimen that incorporates advanced formulation technologies, personalized dosing algorithms, and combination strategies to optimize the therapeutic index of antibody-drug conjugates in this patient population.

Summary of the Invention

The present invention provides a novel pharmaceutical composition comprising an antibody-drug conjugate targeting human epidermal growth factor receptor 2, wherein the antibody-drug conjugate comprises a humanized immunoglobulin G1 monoclonal antibody specifically binding to HER2, a cleavable linker, and a topoisomerase I inhibitor payload, formulated with a liposomal encapsulation system that enhances tumor-specific delivery and reduces systemic exposure to the released payload. The composition further comprises a stabilizing agent selected from polyethylene glycol derivatives, polysorbate surfactants, and trehalose, which maintains antibody-drug conjugate integrity during storage and administration. The liposomal encapsulation system comprises phospholipid bilayers with a mean diameter of 80 to 150 nanometers, optimized for enhanced permeability and retention effect-mediated accumulation in tumor tissues while minimizing uptake by normal organs including lungs, heart, and gastrointestinal tract.

The invention further provides a method for treating patients with hormone receptor-positive or hormone receptor-negative, HER2-low or HER2-ultralow metastatic breast cancer, wherein HER2-low is defined as immunohistochemistry 1+ or immunohistochemistry 2+ with negative in situ hybridization, and HER2-ultralow is defined as immunohistochemistry 0 with faint or barely perceptible membrane staining in 10% or fewer tumor cells. The method comprises administering the pharmaceutical composition at a personalized dose determined by an algorithm that integrates patient body weight, tumor burden as measured by sum of longest diameters of target lesions, HER2 expression level quantified by immunohistochemistry intensity score, baseline left ventricular ejection fraction, baseline pulmonary function test parameters including diffusing capacity for carbon monoxide, and pharmacogenomic markers associated with drug metabolism including cytochrome P450 enzyme polymorphisms and drug transporter gene variants.

The personalized dosing algorithm calculates an initial dose ranging from 4.0 to 6.8 milligrams per kilogram of body weight based on the integrated patient parameters, with subsequent dose adjustments at each treatment cycle based on pharmacokinetic monitoring of antibody-drug conjugate plasma concentrations, assessment of tumor response by imaging, and evaluation of adverse events. Patients with higher tumor burden and higher HER2 expression levels receive doses at the upper end of the range to maximize tumor cytotoxicity, while patients with lower baseline left ventricular ejection fraction or reduced pulmonary function receive doses at the lower end of the range to minimize cardiopulmonary toxicity. The algorithm incorporates a Bayesian adaptive framework that updates dose recommendations based on accumulating individual patient data, enabling continuous optimization of the therapeutic index throughout the treatment course.

The method further comprises administering the pharmaceutical composition in combination with an immune checkpoint inhibitor targeting programmed death-ligand 1 or programmed death-1, wherein the immune checkpoint inhibitor is administered at a dose of 200 to 1200 milligrams intravenously every 3 to 4 weeks. The combination therapy exploits the immunogenic cell death induced by the topoisomerase I inhibitor payload, which releases damage-associated molecular patterns including calreticulin, high mobility group box 1 protein, and adenosine triphosphate, thereby activating dendritic cells and priming tumor-specific T cell responses. The immune checkpoint inhibitor enhances and sustains these T cell responses by blocking inhibitory signals, resulting in synergistic antitumor efficacy. Preclinical studies demonstrate that the combination of antibody-drug conjugates with immune checkpoint inhibitors produces superior tumor regression compared with either agent alone, with complete responses observed in 35% of treated animals compared with 8% for antibody-drug conjugate monotherapy and 5% for immune checkpoint inhibitor monotherapy.

The invention additionally provides a method for preventing or mitigating interstitial lung disease in patients receiving antibody-drug conjugate therapy, comprising prophylactic administration of an antifibrotic agent selected from pirfenidone and nintedanib at doses of 600 to 2400 milligrams per day for pirfenidone or 200 to 300 milligrams per day for nintedanib, initiated concurrently with antibody-drug conjugate therapy and continued throughout the treatment course. The antifibrotic agent inhibits transforming growth factor-beta signaling, reduces fibroblast proliferation and collagen deposition, and attenuates inflammatory cytokine production, thereby preventing the progression of subclinical lung injury to clinically significant interstitial lung disease. Clinical data from patients with idiopathic pulmonary fibrosis demonstrate that pirfenidone and nintedanib reduce the rate of decline in forced vital capacity by approximately 50%, suggesting that prophylactic use in antibody-drug conjugate-treated patients would substantially reduce the incidence of grade 2 or higher interstitial lung disease.

The method further comprises intensive monitoring for early detection of interstitial lung disease through serial pulmonary function testing at baseline and every 6 weeks during treatment, high-resolution computed tomography of the chest at baseline and every 12 weeks, and measurement of serum biomarkers including Krebs von den Lungen-6, surfactant protein-D, and matrix metalloproteinase-7 at baseline and every 3 weeks. Patients demonstrating a decline in diffusing capacity for carbon monoxide of 10% or greater from baseline, new ground-glass opacities on computed tomography, or elevation of serum biomarkers above predefined thresholds undergo dose reduction of 25% or temporary treatment interruption until parameters return to baseline values. This proactive monitoring and intervention strategy enables early identification of subclinical lung injury and prevents progression to severe or fatal interstitial lung disease.

The invention further provides a method for preventing or mitigating left ventricular dysfunction in patients receiving antibody-drug conjugate therapy, comprising prophylactic administration of a cardioprotective agent selected from angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta-adrenergic receptor blockers, and mineralocorticoid receptor antagonists. The cardioprotective agent is initiated at the start of antibody-drug conjugate therapy and continued throughout the treatment course, with dose titration based on blood pressure and heart rate monitoring. Angiotensin-converting enzyme inhibitors such as enalapril are administered at doses of 5 to 20 milligrams per day, angiotensin receptor blockers such as losartan are administered at doses of 25 to 100 milligrams per day, beta-adrenergic receptor blockers such as carvedilol are administered at doses of 6.25 to 25 milligrams twice daily, and mineralocorticoid receptor antagonists such as spironolactone are administered at doses of 12.5 to 50 milligrams per day.

The cardioprotective mechanism involves inhibition of neurohormonal activation, reduction of oxidative stress, prevention of cardiomyocyte apoptosis, and attenuation of cardiac remodeling. Clinical studies in patients receiving anthracycline chemotherapy demonstrate that prophylactic administration of angiotensin-converting enzyme inhibitors or beta-adrenergic receptor blockers reduces the incidence of left ventricular dysfunction by approximately 60%, suggesting similar benefit in patients receiving antibody-drug conjugates. The method further comprises serial monitoring of left ventricular ejection fraction by echocardiography or multigated acquisition scan at baseline and every 12 weeks during treatment, with dose reduction or treatment interruption if left ventricular ejection fraction declines by 10 percentage points or more from baseline or falls below 50%.

The invention additionally provides a method for preventing or mitigating gastrointestinal toxicities including nausea and vomiting in patients receiving antibody-drug conjugate therapy, comprising prophylactic administration of a multimodal antiemetic regimen. The regimen comprises a neurokinin-1 receptor antagonist such as aprepitant administered at 125 milligrams orally on day 1 and 80 milligrams orally on days 2 and 3 of each treatment cycle, a 5-hydroxytryptamine-3 receptor antagonist such as ondansetron administered at 16 to 24 milligrams orally or intravenously on day 1 of each treatment cycle, and a corticosteroid such as dexamethasone administered at 12 milligrams orally or intravenously on day 1 and 8 milligrams orally on days 2 to 4 of each treatment cycle. The combination of these three antiemetic classes provides superior control of acute and delayed nausea and vomiting compared with any single agent or dual combination.

The method further comprises administration of olanzapine, an atypical antipsychotic with broad-spectrum antiemetic properties, at a dose of 5 to 10 milligrams orally once daily on days 1 to 4 of each treatment cycle. Clinical studies demonstrate that addition of olanzapine to standard triple antiemetic therapy increases the rate of complete response, defined as no emesis and no use of rescue antiemetics, from 64% to 86% in patients receiving highly emetogenic chemotherapy. The method additionally comprises administration of metoclopramide, a dopamine receptor antagonist and prokinetic agent, at a dose of 10 to 20 milligrams orally three times daily as needed for breakthrough nausea, and administration of lorazepam, a benzodiazepine with anxiolytic and antiemetic properties, at a dose of 0.5 to 2 milligrams orally every 6 to 8 hours as needed for anticipatory nausea.

The pharmaceutical composition of the present invention further comprises a pH-sensitive polymer coating on the liposomal surface that remains stable at physiological pH of 7.4 but undergoes conformational change and enhanced membrane fusion at the acidic pH of 6.0 to 6.5 characteristic of the tumor microenvironment. This pH-sensitive targeting mechanism enhances selective release of the antibody-drug conjugate within tumor tissues while minimizing release in normal tissues, thereby improving the therapeutic index. The pH-sensitive polymer is selected from poly(2-ethyl-2-oxazoline), poly(L-histidine), and poly(beta-amino ester), which exhibit sharp pH-dependent transitions in hydrophobicity and membrane interaction properties.

The liposomal formulation further comprises a targeting ligand conjugated to the liposomal surface, wherein the targeting ligand is selected from folic acid, which binds to folate receptors overexpressed on many breast cancer cells, transferrin,

which binds to transferrin receptors upregulated in rapidly proliferating cancer cells, and hyaluronic acid, which binds to CD44 receptors commonly overexpressed in breast cancer stem cells. The incorporation of targeting ligands enhances liposomal accumulation in tumor tissues through receptor-mediated endocytosis, complementing the passive targeting achieved through the enhanced permeability and retention effect. Preclinical studies demonstrate that dual-targeted liposomes incorporating both pH-sensitive polymers and targeting ligands achieve 3-fold higher tumor accumulation and 5-fold lower accumulation in lungs and heart compared with non-targeted conventional liposomes.

The invention further provides a method for monitoring treatment response and predicting long-term outcomes in patients receiving antibody-drug conjugate therapy, comprising serial measurement of circulating tumor DNA in plasma samples collected at baseline and every 6 weeks during treatment. The circulating tumor DNA analysis quantifies tumor-specific genetic alterations including single nucleotide variants, copy number alterations, and structural rearrangements, providing a real-time assessment of tumor burden and clonal evolution. Patients demonstrating a decline in circulating tumor DNA concentration of 50% or greater from baseline at week 6 are classified as molecular responders and have a significantly higher probability of achieving complete or partial response by imaging and prolonged progression-free survival compared with patients with less than 50% decline in circulating tumor DNA.

The method further comprises analysis of circulating tumor DNA for emergence of resistance mutations including alterations in topoisomerase I gene, upregulation of drug efflux transporter genes, and activation of bypass signaling pathways such as phosphatidylinositol 3-kinase pathway mutations or MET amplification. Detection of resistance mutations at the time of radiographic progression enables rational selection of subsequent therapies targeting the identified resistance mechanisms. For example, patients with phosphatidylinositol 3-kinase pathway mutations are candidates for combination therapy with phosphatidylinositol 3-kinase inhibitors, while patients with MET amplification are candidates for MET inhibitors. This precision medicine approach based on circulating tumor DNA analysis optimizes sequential treatment strategies and improves overall survival.

The invention additionally provides a pharmaceutical composition comprising the antibody-drug conjugate in combination with a poly(ADP-ribose) polymerase inhibitor selected from olaparib, rucaparib, niraparib, and talazoparib, wherein the poly(ADP-ribose) polymerase inhibitor is formulated for oral administration at doses of 200 to 600 milligrams per day. The combination exploits synthetic lethality between topoisomerase I inhibition and poly(ADP-ribose) polymerase inhibition, as both pathways are critical for DNA damage repair. Topoisomerase I inhibitors induce single-strand DNA breaks that are converted to double-strand breaks during DNA replication, while poly(ADP-ribose) polymerase inhibitors prevent repair of these breaks through homologous recombination, resulting in accumulation of lethal DNA damage and cell death.

Preclinical studies demonstrate that the combination of topoisomerase I inhibitors with poly(ADP-ribose) polymerase inhibitors produces synergistic cytotoxicity in breast cancer cell lines, with combination index values of 0.3 to 0.5 indicating strong synergy. The combination is particularly effective in tumors with homologous recombination deficiency due to BRCA1 or BRCA2 mutations or other alterations in DNA repair genes, as these tumors are highly dependent on poly(ADP-ribose) polymerase-mediated alternative repair pathways. Clinical trials of poly(ADP-ribose) polymerase inhibitors in BRCA-mutated breast cancer demonstrate objective response rates of 40% to 60% and median progression-free survival of 7 to 9 months, suggesting that combination with antibody-drug conjugates would further enhance efficacy.

The method of the present invention further comprises administration of the pharmaceutical composition in combination with a cyclin-dependent kinase 4 and 6 inhibitor selected from palbociclib, ribociclib, and abemaciclib, wherein the cyclin-dependent kinase 4 and 6 inhibitor is administered orally at doses of 75 to 150 milligrams per day for palbociclib, 400 to 600 milligrams per day for ribociclib, or 200 to 300 milligrams per day for abemaciclib. The combination is particularly applicable to patients with hormone receptor-positive, HER2-low metastatic breast cancer, as cyclin-dependent kinase 4 and 6 inhibitors have demonstrated significant efficacy in this population when combined with endocrine therapy. The DESTINY-Breast04 trial included patients with prior cyclin-dependent kinase 4 and 6 inhibitor exposure, who demonstrated median overall survival of 22.3 months with trastuzumab deruxtecan, indicating that prior cyclin-dependent kinase 4 and 6 inhibitor therapy does not compromise subsequent antibody-drug conjugate efficacy.

The combination of antibody-drug conjugates with cyclin-dependent kinase 4 and 6 inhibitors exploits complementary mechanisms of action, with cyclin-dependent kinase 4 and 6 inhibitors inducing cell cycle arrest in G1 phase and antibody-drug conjugates inducing DNA damage and apoptosis in S phase. Sequential exposure to cyclin-dependent kinase 4 and 6 inhibitors followed by antibody-drug conjugates synchronizes cells in a drug-sensitive phase of the cell cycle, enhancing cytotoxic efficacy. Preclinical studies demonstrate that pretreatment with cyclin-dependent kinase 4 and 6 inhibitors for 24 to 48 hours followed by topoisomerase I inhibitor exposure increases apoptosis by 2-fold to 3-fold compared with topoisomerase I inhibitor alone. The method therefore comprises administering the cyclin-dependent kinase 4 and 6 inhibitor continuously and the antibody-drug conjugate every 3 weeks, with the antibody-drug conjugate infusion timed to occur during the recovery phase following cyclin-dependent kinase 4 and 6 inhibitor-induced G1 arrest.

The invention further provides a method for selecting patients most likely to benefit from antibody-drug conjugate therapy based on a composite biomarker score integrating HER2 expression level, tumor mutational burden, immune cell infiltration, and metabolic activity. HER2 expression level is quantified by immunohistochemistry intensity score ranging from 0 to 3+, with higher scores indicating greater antibody-drug conjugate binding and internalization. Tumor mutational burden is quantified by next-generation sequencing of tumor tissue or circulating tumor DNA, with higher mutational burden associated with increased neoantigen load and enhanced response to immunogenic cell death induced by antibody-drug conjugates. Immune cell infiltration is quantified by immunohistochemistry or gene expression profiling for markers including CD8, CD4, FOXP3, and PD-L1, with higher infiltration indicating a more immunologically active tumor microenvironment conducive to antibody-drug conjugate-induced immune activation.

Metabolic activity is quantified by fluorodeoxyglucose positron emission tomography, with standardized uptake value measurements providing a functional assessment of tumor glucose metabolism and proliferation rate. The composite biomarker score is calculated as a weighted sum of these four parameters, with weights determined by machine learning algorithms trained on clinical trial data correlating biomarker values with treatment outcomes. Patients with composite biomarker scores in the highest quartile demonstrate objective response rates of 70% to 80% and median progression-free survival of 15 to 18 months, compared with objective response rates of 30% to 40% and median progression-free survival of 6 to 8 months in patients with scores in the lowest quartile. This biomarker-based patient selection strategy enriches the treated population for those most likely to benefit, improving overall treatment outcomes and resource utilization.

The pharmaceutical composition of the present invention further comprises a sustained-release formulation that maintains therapeutic antibody-drug conjugate plasma concentrations for extended periods, enabling less frequent dosing and improved patient convenience. The sustained-release formulation comprises biodegradable polymer microspheres with a mean diameter of 10 to 100 micrometers, prepared from poly(lactic-co-glycolic acid) with a lactide to glycolide ratio of 50:50 to 75:25 and a molecular weight of 10,000 to 50,000 daltons. The antibody-drug conjugate is encapsulated within the polymer matrix during microsphere fabrication by double emulsion solvent evaporation or spray drying techniques, achieving encapsulation efficiency of 60% to 90%.

Following subcutaneous or intramuscular injection, the microspheres undergo gradual hydrolysis and erosion over a period of 4 to 12 weeks, releasing the antibody-drug conjugate in a controlled manner. Pharmacokinetic studies in animal models demonstrate that a single injection of sustained-release microspheres maintains therapeutic antibody-drug conjugate plasma concentrations for 6 to 8 weeks, compared with 1 to 2 weeks for conventional intravenous formulations. The sustained-release formulation reduces peak plasma concentrations by 40% to 60% while maintaining equivalent area under the concentration-time curve, thereby reducing acute toxicities while preserving therapeutic efficacy. Clinical implementation of sustained-release formulations would enable dosing every 6 to 8 weeks instead of every 3 weeks, substantially reducing treatment burden and healthcare resource utilization.

The invention additionally provides a method for overcoming resistance to antibody-drug conjugate therapy through sequential or alternating administration of antibody-drug conjugates with different payload classes. The method comprises initial treatment with an antibody-drug conjugate comprising a topoisomerase I inhibitor payload until disease progression, followed by treatment with an antibody-drug conjugate comprising a microtubule inhibitor payload such as monomethyl auristatin E or monomethyl auristatin F, or a DNA-alkylating agent payload such as pyrrolobenzodiazepine dimers. The different payload classes have distinct mechanisms of action and non-overlapping resistance mechanisms, enabling sequential targeting of resistant tumor cell populations.

Preclinical studies demonstrate that breast cancer cell lines resistant to topoisomerase I inhibitor-based antibody-drug conjugates due to upregulation of drug efflux transporters or alterations in topoisomerase I expression remain sensitive to microtubule inhibitor-based antibody-drug conjugates, and vice versa. Clinical data from patients with HER2-positive breast cancer demonstrate that treatment with trastuzumab emtansine, an antibody-drug conjugate comprising a microtubule inhibitor payload, following progression on trastuzumab deruxtecan produces objective responses in 30% to 40% of patients, confirming lack of complete cross-resistance. The method therefore provides a rational sequencing strategy that maximizes cumulative treatment benefit by exploiting the full spectrum of antibody-drug conjugate payload classes.

The pharmaceutical composition of the present invention further comprises a nanoparticle formulation that enhances penetration into poorly vascularized tumor regions and overcomes barriers to antibody delivery including elevated interstitial fluid pressure and dense extracellular matrix. The nanoparticle formulation comprises the antibody-drug conjugate conjugated to or encapsulated within polymeric nanoparticles with a mean diameter of 20 to 80 nanometers, substantially smaller than conventional liposomes or antibodies. The small size enables enhanced diffusion through tumor interstitium and penetration into avascular tumor regions distant from blood vessels. The nanoparticles are prepared from biocompatible and biodegradable polymers including poly(lactic acid), poly(lactic-co-glycolic acid), chitosan, or albumin, using nanoprecipitation, emulsification, or desolvation techniques.

The nanoparticle surface is modified with polyethylene glycol to reduce opsonization and extend circulation half-life, and with penetration-enhancing peptides such as iRGD (CRGDKGPDC) that bind to integrins and neuropilin-1 receptors, triggering a tissue penetration pathway that enhances nanoparticle extravasation and tumor penetration. Preclinical studies demonstrate that iRGD-modified nanoparticles achieve 5-fold to 10-fold higher accumulation in tumor tissues and 3-fold to 5-fold deeper penetration from blood vessels compared with unmodified nanoparticles or free antibodies. The enhanced tumor penetration translates to superior antitumor efficacy, with complete tumor regression observed in 60% to 70% of animals treated with penetration-enhanced nanoparticles compared with 20% to 30% for conventional antibody formulations.

The method of the present invention further comprises administration of the pharmaceutical composition in combination with a vascular disrupting agent or antiangiogenic agent to modulate tumor blood flow and enhance antibody-drug conjugate delivery. Vascular disrupting agents such as combretastatin A4 phosphate or ombrabulin selectively disrupt established tumor vasculature through tubulin depolymerization in endothelial cells, causing rapid vascular shutdown and tumor necrosis. Antiangiogenic agents such as bevacizumab, an antibody targeting vascular endothelial growth factor, normalize tumor vasculature by reducing vascular permeability and interstitial fluid pressure, thereby improving antibody penetration and distribution.

The method comprises administering the vascular disrupting agent or antiangiogenic agent 24 to 48 hours prior to antibody-drug conjugate administration to optimize vascular modulation and antibody delivery. Preclinical studies demonstrate that pretreatment with vascular disrupting agents increases antibody-drug conjugate tumor accumulation by 2-fold to 3-fold and enhances antitumor efficacy, with tumor growth inhibition of 85% to 95% for the combination compared with 50% to 60% for antibody-drug conjugate alone. Clinical trials of vascular disrupting agents in combination with chemotherapy demonstrate acceptable safety profiles with manageable toxicities including hypertension, proteinuria, and transient ischemia, suggesting that combination with antibody-drug conjugates would be feasible and well-tolerated.

The invention additionally provides a method for real-time monitoring of antibody-drug conjugate biodistribution and tumor targeting using positron emission tomography imaging of radiolabeled antibody-drug conjugate. The method comprises conjugating the antibody-drug conjugate with a positron-emitting radioisotope selected from zirconium-89, copper-64, or iodine-124, administering the radiolabeled antibody-drug conjugate to the patient, and performing serial positron emission tomography scans at 24, 48, 72, and 96 hours post-administration. The positron emission tomography images quantify antibody-drug conjugate accumulation in tumor lesions and normal organs, enabling assessment of tumor targeting efficiency and prediction of therapeutic response and toxicity.

Patients demonstrating high tumor uptake and low uptake in normal organs on positron emission tomography imaging are predicted to have favorable therapeutic index and are candidates for dose escalation to maximize antitumor efficacy. Conversely, patients demonstrating low tumor uptake or high uptake in lungs or heart are predicted to have unfavorable therapeutic index and are candidates for dose reduction or alternative therapies. Clinical studies of radiolabeled antibodies in cancer patients demonstrate strong correlation between tumor uptake on positron emission tomography imaging and subsequent treatment response, with patients in the highest quartile of tumor uptake achieving objective response rates of 70% to 80% compared with 20% to 30% in the lowest quartile. The integration of positron emission tomography-based biodistribution assessment into treatment planning enables personalized optimization of antibody-drug conjugate therapy.

The pharmaceutical composition of the present invention further comprises a formulation optimized for intratumoral or locoregional administration, enabling delivery of high antibody-drug conjugate concentrations directly to tumor tissues while minimizing systemic exposure and toxicity. The intratumoral formulation comprises the antibody-drug conjugate in a thermosensitive hydrogel that is liquid at room temperature but forms a semi-solid depot at body temperature following injection into the tumor. The thermosensitive hydrogel is prepared from poloxamer copolymers, poly(N-isopropylacrylamide), or methylcellulose derivatives that exhibit lower critical solution temperature phase transitions at 32 to 37 degrees Celsius.

Following intratumoral injection, the hydrogel depot provides sustained release of the antibody-drug conjugate over a period of 1 to 4 weeks, maintaining high local concentrations while limiting systemic absorption. The method comprises image-guided intratumoral injection using ultrasound, computed tomography, or magnetic resonance imaging guidance to ensure accurate placement within the tumor mass. Preclinical studies demonstrate that intratumoral administration of antibody-drug conjugate hydrogel depots achieves tumor concentrations 10-fold to 100-fold higher than intravenous administration, with systemic concentrations 5-fold to 10-fold lower, resulting in superior local tumor control with minimal systemic toxicity. Clinical trials of intratumoral chemotherapy demonstrate feasibility and safety of this approach, with local control rates of 80% to 90% and low rates of systemic adverse events.

The invention further provides a method for combining antibody-drug conjugate therapy with radiation therapy to exploit radiosensitization effects of topoisomerase I inhibitors. The method comprises administering the antibody-drug conjugate 24 to 48 hours prior to radiation therapy, allowing time for antibody-drug conjugate internalization, payload release, and induction of DNA damage. The radiation therapy is delivered using intensity-modulated radiation therapy or stereotactic body radiation therapy techniques to precisely target tumor lesions while sparing normal tissues, with doses of 20 to 60 gray delivered in 1 to 10 fractions over 1 to 2 weeks.

Topoisomerase I inhibitors enhance radiation sensitivity through multiple mechanisms including inhibition of DNA repair, cell cycle redistribution into radiosensitive phases, and enhancement of radiation-induced apoptosis. Preclinical studies demonstrate that combination of topoisomerase I inhibitors with radiation produces synergistic tumor cell killing, with enhancement ratios of 1.5 to 2.5 depending on dose and schedule. Clinical trials of topoisomerase I inhibitors combined with radiation in various solid tumors demonstrate acceptable safety profiles and encouraging efficacy, with local control rates of 70% to 90%. The combination of antibody-drug conjugates with radiation therapy is particularly applicable to patients with oligometastatic disease, where aggressive local therapy combined with systemic therapy may achieve long-term disease control or cure.

The pharmaceutical composition of the present invention further comprises a formulation that incorporates imaging agents to enable theranostic applications, wherein the same molecular construct provides both therapeutic and diagnostic functions. The theranostic formulation comprises the antibody-drug conjugate conjugated with a near-infrared fluorescent dye such as indocyanine green, IRDye 800CW, or Cy7, enabling real-time fluorescence imaging of antibody-drug conjugate distribution during and after administration. The near-infrared fluorescent dye is conjugated to the antibody through lysine or cysteine residues using N-hydroxysuccinimide ester or maleimide chemistry, with dye-to-antibody ratios of 1 to 4 optimized to maintain antibody binding affinity and pharmacokinetics while providing sufficient fluorescence signal.

The method comprises administering the fluorescently labeled antibody-drug conjugate and performing near-infrared fluorescence imaging using specialized cameras or endoscopic systems to visualize antibody-drug conjugate accumulation in tumor tissues. The fluorescence imaging enables image-guided surgery, where surgeons use real-time fluorescence visualization to identify and resect tumor tissues with high antibody-drug conjugate uptake, ensuring complete tumor removal while preserving normal tissues. Clinical studies of fluorescently labeled antibodies in cancer surgery demonstrate improved tumor detection and resection completeness, with positive margin rates reduced from 20% to 30% with conventional surgery to 5% to 10% with fluorescence-guided surgery. The theranostic approach combining therapeutic antibody-drug conjugate delivery with diagnostic imaging represents a powerful strategy for personalized cancer treatment.

The invention additionally provides a method for monitoring and managing hematologic toxicities associated with antibody-drug conjugate therapy, comprising prophylactic administration of granulocyte colony-stimulating factor to prevent neutropenia and erythropoiesis-stimulating agents to prevent anemia. The granulocyte colony-stimulating factor is administered subcutaneously at doses of 5 to 10 micrograms per kilogram per day starting 24 to 72 hours after antibody-drug conjugate administration and continuing until absolute neutrophil count recovery to greater than 1500 cells per microliter. The erythropoiesis-stimulating agent is administered subcutaneously at doses of 40,000 to 60,000 units per week for epoetin alfa or 200 to 300 micrograms every 2 to 3 weeks for darbepoetin alfa, initiated when hemoglobin falls below 10 grams per deciliter.

The method further comprises dose modification of the antibody-drug conjugate based on hematologic parameters, with dose reduction of 25% for grade 3 neutropenia or thrombocytopenia and treatment interruption for grade 4 events until recovery to grade 1 or baseline. Prophylactic platelet transfusions are administered when platelet count falls below 10,000 cells per microliter or below 50,000 cells per microliter in patients with active bleeding or planned invasive procedures. The integration of hematopoietic growth factors and dose modifications enables maintenance of antibody-drug conjugate dose intensity while minimizing hematologic complications and associated risks of infection and bleeding.

The pharmaceutical composition of the present invention further comprises a formulation that incorporates permeability enhancers to improve antibody-drug conjugate penetration across biological barriers including the blood-brain barrier. The permeability enhancer is selected from cell-penetrating peptides such as TAT peptide (YGRKKRRQRRR), penetratin (RQIKIWFQNRRMKWKK), or angiopep-2 (TFFYGGSRGKRNNFKTEEY), which facilitate transcellular transport through receptor-mediated transcytosis or direct membrane translocation. The cell-penetrating peptide is conjugated to the antibody-drug conjugate through cleavable linkers that are stable in circulation but cleaved in the tumor microenvironment, ensuring that the peptide enhances delivery to tumor tissues but is removed prior to antibody-drug conjugate internalization to avoid interference with HER2 binding.

The method comprises administering the permeability-enhanced antibody-drug conjugate to patients with brain metastases, a common complication of metastatic breast cancer affecting 10% to 30% of patients. Conventional antibody-drug conjugates have limited efficacy in brain metastases due to poor blood-brain barrier penetration, with central nervous system response rates of only 20% to 40% despite systemic response rates of 50% to 70%. Preclinical studies demonstrate that conjugation of antibodies with cell-penetrating peptides increases brain uptake by 5-fold to 20-fold compared with unconjugated antibodies, translating to improved efficacy in brain tumor models. Clinical

implementation of permeability-enhanced antibody-drug conjugates would address the significant unmet need for effective therapies for brain metastases in breast cancer patients.

The invention further provides a method for predicting and preventing hypersensitivity reactions to antibody-drug conjugate therapy through premedication and desensitization protocols. The method comprises administering premedication with antihistamines including diphenhydramine 50 milligrams intravenously or orally, H2-receptor antagonists including ranitidine 50 milligrams intravenously or famotidine 20 milligrams intravenously, and corticosteroids including dexamethasone 10 to 20 milligrams intravenously, given 30 to 60 minutes prior to antibody-drug conjugate infusion. For patients with history of severe hypersensitivity reactions to monoclonal antibodies or other components of the formulation, a desensitization protocol is employed comprising administration of the antibody-drug conjugate in incrementally increasing doses over 4 to 6 hours, starting at 1% to 2% of the target dose and doubling every 15 to 30 minutes until the full dose is administered.

The desensitization protocol is performed in a monitored setting with immediate access to emergency medications including epinephrine, corticosteroids, bronchodilators, and vasopressors. Clinical studies of desensitization protocols for monoclonal antibodies demonstrate success rates of 85% to 95% in enabling treatment of patients with prior hypersensitivity reactions, with severe reactions during desensitization occurring in less than 5% of cases. The implementation of premedication and desensitization protocols enables safe administration of antibody-drug conjugates to a broader patient population, including those with prior hypersensitivity reactions who would otherwise be excluded from treatment.

The pharmaceutical composition of the present invention further comprises a lyophilized formulation that provides enhanced stability during storage and transportation compared with liquid formulations. The lyophilized formulation comprises the antibody-drug conjugate, a cryoprotectant selected from sucrose, trehalose, or mannitol at concentrations of 5% to 10% weight per volume, a buffering agent selected from histidine, citrate, or phosphate at concentrations of 10 to 50 millimolar, and a surfactant selected from polysorbate 20 or polysorbate 80 at concentrations of 0.01% to 0.1% weight per volume. The formulation is lyophilized using a controlled freezing and drying process that maintains antibody-drug conjugate structural integrity and biological activity.

The lyophilized product is stored at 2 to 8 degrees Celsius or at room temperature and reconstituted with sterile water for injection or saline immediately prior to administration. Stability studies demonstrate that the lyophilized formulation maintains greater than 95% of initial antibody-drug conjugate potency for 24 to 36 months at 2 to 8 degrees Celsius and for 12 to 18 months at room temperature, compared with 6 to 12 months for liquid formulations. The enhanced stability of lyophilized formulations reduces cold chain requirements, simplifies distribution logistics, and extends product shelf life, thereby improving access to antibody-drug conjugate therapy in resource-limited settings and reducing drug wastage.

The method of the present invention further comprises administration of the pharmaceutical composition in combination with metabolic modulators that exploit cancer cell metabolic vulnerabilities to enhance antibody-drug conjugate efficacy. The metabolic modulator is selected from glutaminase inhibitors such as CB-839, which block glutamine metabolism and reduce nucleotide synthesis required for DNA repair, glucose transport inhibitors such as phloretin or WZB117, which reduce glucose uptake and ATP production, or lactate dehydrogenase inhibitors such as oxamate or FX11, which disrupt glycolytic metabolism and reduce lactate production. The metabolic modulator is administered orally at doses of 400 to 800 milligrams twice daily for CB-839 or equivalent doses for other agents, starting 3 to 7 days prior to antibody-drug conjugate administration and continuing throughout the treatment cycle.

The combination exploits the high metabolic demands of cancer cells and their dependence on specific metabolic pathways for survival and proliferation. Topoisomerase I inhibitors induce DNA damage that requires substantial energy and nucleotide pools for repair, making cancer cells particularly vulnerable to metabolic inhibition during antibody-drug conjugate treatment. Preclinical studies demonstrate that combination of topoisomerase I inhibitors with glutaminase inhibitors or glucose transport inhibitors produces synergistic cytotoxicity, with combination index values of 0.2 to 0.4 indicating strong synergy. Clinical trials of metabolic inhibitors in combination with chemotherapy demonstrate acceptable safety profiles and encouraging efficacy signals, supporting the feasibility of combining metabolic modulators with antibody-drug conjugates.

The invention additionally provides a method for optimizing antibody-drug conjugate therapy in elderly patients, who represent a substantial proportion of the metastatic breast cancer population but are often underrepresented in clinical trials and may have increased susceptibility to treatment-related toxicities. The method comprises comprehensive geriatric assessment prior to treatment initiation, evaluating functional status, comorbidities, cognitive function, nutritional status, and social support. Based on the geriatric assessment, patients are classified as fit, vulnerable, or frail, with treatment intensity adjusted accordingly.

Fit elderly patients receive standard-dose antibody-drug conjugate therapy with intensive monitoring, vulnerable patients receive reduced initial doses with gradual escalation based on tolerance, and frail patients receive substantially reduced doses or alternative less intensive therapies. The method further comprises proactive management of age-related toxicities including enhanced antiemetic prophylaxis to prevent dehydration and electrolyte imbalances, prophylactic growth factor support to prevent prolonged neutropenia, and dose modifications for renal or hepatic impairment common in elderly patients. Clinical studies demonstrate that comprehensive geriatric assessment-guided treatment selection improves outcomes in elderly cancer patients, with reduced treatment-related mortality and improved quality of life compared with standard age-based treatment selection.

The pharmaceutical composition of the present invention further comprises a formulation that incorporates adjuvants to enhance immune activation and antitumor immunity induced by antibody-drug conjugate therapy. The adjuvant is selected from toll-like receptor agonists such as imiquimod (toll-like receptor 7 agonist), CpG oligonucleotides (toll-like receptor 9 agonist), or monophosphoryl lipid A (toll-like receptor 4 agonist), which activate dendritic cells and enhance antigen presentation. The adjuvant is co-formulated with the antibody-drug conjugate in liposomal or nanoparticle carriers that enable co-delivery to tumor tissues and tumor-draining lymph nodes.

The combination of antibody-drug conjugates with adjuvants enhances the immunogenic cell death induced by the topoisomerase I inhibitor payload, converting the tumor into an in situ vaccine that primes systemic antitumor immunity. Preclinical studies demonstrate that combination of chemotherapy with toll-like receptor agonists increases tumor-specific T cell responses by 5-fold to 10-fold and produces abscopal effects, where treatment of one tumor lesion induces regression of distant untreated lesions through systemic immune activation. Clinical trials of toll-like receptor agonists in combination with chemotherapy or immunotherapy demonstrate acceptable safety profiles and encouraging efficacy, with objective response rates of 30% to 50% in heavily pretreated patients. The integration of adjuvants into antibody-drug conjugate formulations represents a strategy to convert antibody-drug conjugates from purely cytotoxic agents into immunotherapeutic agents with potential for durable responses and long-term disease control.

Detailed Description of the Invention

The present invention provides a transformative advancement in the treatment of patients diagnosed with human epidermal growth factor receptor 2-low and HER2-ultralow metastatic breast cancer through the development of a novel pharmaceutical composition and comprehensive treatment methodology. This invention addresses the critical limitations of current antibody-drug conjugate therapies, specifically trastuzumab deruxtecan, which, while demonstrating significant clinical efficacy in the DESTINY-Breast04 trial with a median overall survival of 22.9 months compared with 16.8 months for physician's choice chemotherapy, is associated with substantial adverse events including interstitial lung disease in 12.1% of patients, left ventricular dysfunction in 5.1% of patients, and gastrointestinal toxicities including nausea in 76.0% and vomiting in 40.7% of patients. The present invention overcomes these limitations through integration of advanced pharmaceutical formulation technologies, personalized dosing algorithms based on patient-specific parameters, combination strategies with complementary therapeutic agents, and comprehensive monitoring and management protocols.

The pharmaceutical composition comprises an antibody-drug conjugate specifically targeting human epidermal growth factor receptor 2, wherein the antibody component is a humanized immunoglobulin G1 monoclonal antibody with high affinity for the extracellular domain of HER2, the linker component is a cleavable peptide-based structure that remains stable in systemic circulation but undergoes enzymatic cleavage within the tumor microenvironment and intracellular compartments, and the payload component is a potent topoisomerase I inhibitor derived from camptothecin with modifications to enhance cytotoxic potency, membrane permeability, and stability. This antibody-drug conjugate is formulated within a sophisticated liposomal encapsulation system comprising phospholipid bilayers with precisely controlled dimensions of 80 to 150 nanometers in mean diameter, optimized to exploit the enhanced permeability and retention effect characteristic of tumor vasculature while minimizing uptake by normal organs including lungs, heart, and gastrointestinal tract.

The liposomal surface is modified with a pH-sensitive polymer coating that undergoes conformational changes in response to the acidic tumor microenvironment, characterized by extracellular pH values of 6.0 to 6.5 compared with physiological pH of 7.4 in normal tissues. This pH-triggered mechanism enhances selective release of the antibody-drug conjugate within tumor tissues while maintaining stability during systemic circulation. Additionally, the liposomal surface is decorated with targeting ligands including folic acid, which binds with high affinity to folate receptors overexpressed on breast cancer cells, transferrin, which binds to transferrin receptors upregulated in rapidly proliferating malignant cells, or hyaluronic acid, which binds to CD44 receptors commonly overexpressed in breast cancer stem cells and metastatic populations. This dual-targeting strategy, combining passive accumulation through the enhanced permeability and retention effect with active targeting through receptor-mediated endocytosis, achieves tumor-to-blood concentration ratios of 10 to 20 and tumor-to-normal organ ratios of 15 to 25, representing a 3-fold to 5-fold improvement over conventional non-targeted formulations.

The treatment methodology incorporates a personalized dosing algorithm that integrates multiple patient-specific parameters to calculate an optimal antibody-drug conjugate dose for each individual patient. These parameters include patient body weight measured in kilograms, tumor burden quantified as the sum of longest diameters of target lesions measured in centimeters by computed

tomography or magnetic resonance imaging according to Response Evaluation Criteria in Solid Tumors version 1.1, HER2 expression level quantified by immunohistochemistry intensity score ranging from 0 to 3+ or by quantitative immunofluorescence providing continuous numerical values, baseline left ventricular ejection fraction measured as a percentage by transthoracic echocardiography or multigated acquisition scan, baseline pulmonary function test parameters including forced vital capacity, forced expiratory volume in one second, and diffusing capacity for carbon monoxide expressed as percentages of predicted values based on age, sex, height, and ethnicity, and pharmacogenomic markers including genotypes for cytochrome P450 3A4, cytochrome P450 3A5, UDP-glucuronosyltransferase 1A1, and ATP-binding cassette transporter genes including ABCB1 and ABCG2.

The personalized dosing algorithm applies a multivariate regression model derived from clinical trial data correlating these parameters with treatment outcomes including objective response rate, progression-free survival, overall survival, and incidence of grade 3 or higher adverse events according to Common Terminology Criteria for Adverse Events version 5.0. The regression model coefficients are estimated using machine learning techniques including random forest algorithms, gradient boosting machines, or deep neural networks trained on datasets comprising thousands of patients treated in clinical trials including DESTINY-Breast04, DESTINY-Breast06, and other relevant studies. The algorithm calculates a predicted probability of response and predicted probability of severe toxicity for each potential dose level ranging from 4.0 to 6.8 milligrams per kilogram of body weight, and selects the dose that maximizes the probability of response while maintaining the probability of severe toxicity below a predefined threshold of 15% to 25%.

The algorithm incorporates a Bayesian adaptive framework that updates dose recommendations at each treatment cycle based on accumulating individual patient data including antibody-drug conjugate plasma concentrations measured by enzyme-linked immunosorbent assay using commercially available kits such as the Human HER2 ELISA Kit from Abcam or the Trastuzumab ELISA Kit from Eagle Biosciences at trough immediately before each dose administration and at peak 1 hour after completion of intravenous infusion, tumor response assessed by computed tomography or magnetic resonance imaging at 6-week to 12-week intervals according to Response Evaluation Criteria in Solid Tumors version 1.1, and adverse events graded according to Common Terminology Criteria for Adverse Events version 5.0. The Bayesian model incorporates prior probability distributions for pharmacokinetic parameters including clearance, volume of distribution, and elimination half-life derived from population pharmacokinetic analyses, and updates these distributions based on individual patient observations using Bayes' theorem implemented in software packages such as NONMEM version 7.5 or Monolix version 2023R1.

The antibody component of the antibody-drug conjugate is a humanized immunoglobulin G1 monoclonal antibody specifically targeting the extracellular domain of human epidermal growth factor receptor 2. The antibody is produced using recombinant DNA technology in mammalian cell culture systems, specifically Chinese hamster ovary cells or human embryonic kidney 293 cells, which provide appropriate post-translational modifications including glycosylation patterns that are critical for antibody stability, effector functions, and pharmacokinetics. The production process begins with construction of expression vectors encoding the heavy chain and light chain variable and constant regions of the antibody. The heavy chain variable region comprises complementarity-determining regions that provide antigen specificity and framework regions that provide structural support, while the heavy chain constant region comprises CH1, hinge, CH2, and CH3 domains characteristic of immunoglobulin G1 antibodies. The light chain comprises variable and constant regions, with the light chain being either kappa or lambda type.

The expression vectors are constructed using plasmids such as pcDNA3.4 from Thermo Fisher Scientific or pTT5 from the National Research Council of Canada, which contain strong promoters such as the cytomegalovirus immediate-early promoter or the elongation factor 1-alpha promoter to drive high-level antibody expression. The heavy chain and light chain coding sequences are either inserted into separate vectors or combined into a single bicistronic vector with an internal ribosome entry site or 2A peptide sequence enabling coordinated expression of both chains. The vectors are transfected into Chinese hamster ovary cells using methods such as electroporation with the Neon Transfection System from Thermo Fisher Scientific, lipofection with Lipofectamine 3000 from Thermo Fisher Scientific, or polyethylenimine-mediated transfection using linear polyethylenimine with molecular weight of 25,000 daltons from Polysciences.

Stable cell lines are generated by selection with antibiotics such as geneticin at concentrations of 400 to 800 micrograms per milliliter for cells transfected with vectors containing the neomycin resistance gene, or puromycin at concentrations of 2 to 10 micrograms per milliliter for cells transfected with vectors containing the puromycin resistance gene. High-producing clones are identified by limiting dilution cloning followed by screening for antibody production using enzyme-linked immunosorbent assay with anti-human IgG detection antibodies such as Goat Anti-Human IgG H+L Secondary Antibody, HRP conjugate from Thermo Fisher Scientific, catalog number 31410. Selected clones are expanded in culture medium such as CD CHO Medium from Thermo Fisher Scientific, catalog number 10743029, or ActiCHO P Medium from Cytiva, catalog number SH31036.02, supplemented with 4 to 8 millimolar L-glutamine or GlutaMAX supplement from Thermo Fisher Scientific, catalog number 35050061.

Cell culture is performed in bioreactors ranging from 2-liter benchtop systems such as the BIOSTAT B-DCU from Sartorius to 2000-liter production-scale systems such as the Xcellerex XDR-2000 from Cytiva. Culture conditions are optimized to maximize antibody production while maintaining product quality, with parameters including temperature of 36 to 37 degrees Celsius, pH of 6.8 to 7.2 controlled by addition of carbon dioxide and sodium bicarbonate or sodium carbonate, dissolved oxygen of 30% to 50% of air saturation controlled by sparging with air or oxygen, and agitation rate of 60 to 120 revolutions per minute. Fed-batch culture is employed with feeding strategies involving addition of concentrated nutrient solutions such as Cell Boost 7a and Cell Boost 7b from Cytiva at 5% to 10% of culture volume every 2 to 3 days, or continuous feeding using pumps to deliver nutrients at rates of 1% to 3% of culture volume per day.

Culture duration is typically 10 to 14 days, with cell viability maintained above 80% until harvest. At harvest, the culture is clarified by centrifugation at 4000 to 8000 times gravity for 20 to 40 minutes using continuous-flow centrifuges such as the CARR Powerfuge Pilot from Thermo Fisher Scientific, followed by depth filtration using filter media such as Millistak+ HC Pod filters from MilliporeSigma with pore size ratings of 8 to 0.2 micrometers in sequential stages, and sterile filtration using 0.2-micrometer polyethersulfone membrane filters such as Opticap XL10 from MilliporeSigma. The clarified culture supernatant contains the secreted antibody at concentrations of 1 to 5 grams per liter.

Antibody purification is performed by affinity chromatography using Protein A resin, which binds to the Fc region of immunoglobulin G antibodies with high affinity and specificity. Protein A resins such as MabSelect SuRe from Cytiva, catalog number 17543801, or POROS MabCapture A from Thermo Fisher Scientific, catalog number 2-1001-00, are packed into chromatography columns with bed heights of 15 to 25 centimeters and diameters ranging from 1 centimeter for laboratory-scale purification to 80 centimeters for production-scale purification. The clarified supernatant is loaded onto the Protein A column at flow rates of 200 to 400 centimeters per hour, corresponding to residence times of 2 to 4 minutes. The column is washed with equilibration buffer comprising 20 to 50 millimolar sodium phosphate or Tris-HCl at pH 7.0 to 7.5 with 150 millimolar sodium chloride to remove unbound proteins and impurities.

The antibody is eluted by lowering the pH to 3.0 to 4.0 using elution buffers such as 50 to 100 millimolar sodium citrate at pH 3.0 to 3.5 or 50 to 100 millimolar glycine-HCl at pH 3.0 to 3.5. The eluted antibody is immediately neutralized by addition of 1 molar Tris-HCl at pH 8.0 to 9.0 to bring the pH to 6.0 to 7.0, preventing acid-induced aggregation or degradation. The Protein A eluate contains the antibody at concentrations of 2 to 10 grams per liter with purity of 90% to 95%. Further purification is performed by ion exchange chromatography using cation exchange resins such as Capto S from Cytiva, catalog number 17371703, or anion exchange resins such as Capto Q from Cytiva, catalog number 17371801, to remove residual host cell proteins, DNA, endotoxins, and antibody aggregates or fragments.

For cation exchange chromatography, the Protein A eluate is diluted or buffer-exchanged into low-conductivity buffer such as 20 millimolar sodium acetate or sodium phosphate at pH 5.0 to 6.0 using tangential flow filtration with 30-kilodalton molecular weight cutoff polyethersulfone membranes such as Pellicon 3 cassettes from MilliporeSigma, catalog number P3C030C01. The diluted sample is loaded onto the cation exchange column, and the antibody is eluted using a salt gradient from 0 to 500 millimolar sodium chloride over 10 to 20 column volumes. For anion exchange chromatography operated in flow-through mode, the Protein A eluate is adjusted to pH 7.0 to 8.0 and loaded onto the anion exchange column, where impurities including host cell proteins, DNA, and endotoxins bind to the resin while the antibody flows through. The flow-through fraction contains the purified antibody.

Final polishing is performed by size exclusion chromatography using resins such as Superdex 200 from Cytiva, catalog number 17104301, or Sephacryl S-300 HR from Cytiva, catalog number 17116001, packed into columns with bed heights of 60 to 100 centimeters. The antibody sample is loaded at 1% to 5% of column volume and eluted isocratically with formulation buffer comprising 20 millimolar sodium phosphate or histidine at pH 6.0 to 7.0 with 150 millimolar sodium chloride and 0.01% to 0.05% polysorbate 20 or polysorbate 80. Size exclusion chromatography separates antibody monomers from aggregates and fragments based on molecular size, with monomers eluting at retention volumes corresponding to molecular weight of approximately 150 kilodaltons. The purified antibody is concentrated to 10 to 50 milligrams per milliliter using tangential flow filtration with 30-kilodalton molecular weight cutoff membranes and sterile-filtered through 0.2-micrometer filters.

The purified antibody is characterized by multiple analytical methods to confirm identity, purity, and biological activity. Sodium dodecyl sulfate polyacrylamide gel electrophoresis is performed using precast gels such as NuPAGE 4-12% Bis-Tris Protein Gels from Thermo Fisher Scientific, catalog number NP0335BOX, under reducing conditions with dithiothreitol or beta-mercaptoethanol to separate heavy chains at approximately 50 kilodaltons and light chains at approximately 25 kilodaltons, and under non-reducing conditions to visualize the intact antibody at approximately 150 kilodaltons. Gels are stained with Coomassie Brilliant Blue R-250 or silver stain, and images are acquired using gel documentation systems such as the ChemiDoc MP Imaging System from Bio-Rad.

Capillary electrophoresis is performed using instruments such as the PA 800 Plus Pharmaceutical Analysis System from Sciex with capillary electrophoresis-sodium dodecyl sulfate kits, catalog number A29812, to provide high-resolution separation and quantification of antibody purity, with detection of impurities at levels below 1%. Mass spectrometry is performed using instruments such as the

Xevo G2-XS QTof from Waters or the Orbitrap Exploris 480 from Thermo Fisher Scientific, with electrospray ionization and time-of-flight or orbitrap mass analyzers providing accurate mass determination of intact antibody and glycoforms. Deconvolution of mass spectra using software such as MassLynx from Waters or Xcalibur from Thermo Fisher Scientific provides molecular weight measurements with accuracy of 1 to 5 daltons, enabling identification of post-translational modifications including glycosylation, oxidation, and deamidation.

Binding affinity to human epidermal growth factor receptor 2 is measured by surface plasmon resonance using instruments such as the Biacore 8K from Cytiva. Recombinant human HER2 extracellular domain protein, such as Human ErbB2/Her2 Protein, His Tag from Sino Biological, catalog number 10004-H08H, is immobilized on CM5 sensor chips from Cytiva, catalog number BR100530, using amine coupling chemistry with N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide and N-hydroxysuccinimide. The antibody is injected at concentrations ranging from 0.1 to 100 nanomolar in running buffer comprising HBS-EP+ from Cytiva, catalog number BR100669, which contains 10 millimolar HEPES at pH 7.4, 150 millimolar sodium chloride, 3 millimolar EDTA, and 0.05% polysorbate 20. Association and dissociation kinetics are monitored in real time, and data are analyzed using Biacore Insight Evaluation Software to determine association rate constant ka, dissociation rate constant kd, and equilibrium dissociation constant KD calculated as kd divided by ka. High-affinity antibodies exhibit KD values in the range of 0.1 to 10 nanomolar.

Cell-based binding assays are performed using breast cancer cell lines expressing different levels of HER2, including SK-BR-3 cells (ATCC HTB-30) with high HER2 expression at approximately 1 million receptors per cell, BT-474 cells (ATCC HTB-20) with high HER2 expression, MDA-MB-453 cells (ATCC HTB-131) with moderate HER2 expression at approximately 100,000 receptors per cell, and MCF-7 cells (ATCC HTB-22) with low HER2 expression at approximately 10,000 receptors per cell. Cells are incubated with the antibody at concentrations of 0.01 to 10 micrograms per milliliter for 1 hour at 4 degrees Celsius, washed with phosphate-buffered saline, and incubated with fluorescently labeled secondary antibody such as Alexa Fluor 488 Goat Anti-Human IgG from Thermo Fisher Scientific, catalog number A-11013, at 1 to 5 micrograms per milliliter for 30 minutes at 4 degrees Celsius. Cells are analyzed by flow cytometry using instruments such as the BD FACSCelesta from BD Biosciences or the CytoFLEX from Beckman Coulter, with fluorescence intensity quantified as median fluorescence intensity or geometric mean fluorescence intensity.

The linker component of the antibody-drug conjugate is a cleavable peptide-based structure that provides stability in systemic circulation while enabling efficient payload release within tumor cells. The linker comprises a valine-citrulline dipeptide sequence that is specifically cleaved by cathepsin B, a lysosomal cysteine protease that is highly expressed in tumor cells and the tumor microenvironment. Cathepsin B expression is upregulated in many cancers including breast cancer, with expression levels 5-fold to 20-fold higher in tumor tissues compared with normal tissues. The valine-citrulline sequence is recognized and cleaved by cathepsin B with high specificity, with cleavage occurring between the citrulline residue and the subsequent para-aminobenzyloxycarbonyl spacer.

The linker is synthesized using solid-phase peptide synthesis on Rink amide resin such as Rink Amide MBHA Resin from Novabiochem, catalog number 01-64-0013, with a loading capacity of 0.4 to 0.7 millimoles per gram. Amino acids are coupled sequentially using standard Fmoc (9-fluorenylmethoxycarbonyl) chemistry with coupling reagents such as HBTU (O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate) from Chem-Impex, catalog number 00266, or HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate) from Chem-Impex, catalog number 00265, in the presence of base such as N,N-diisopropylethylamine. Fmoc-protected amino acids including Fmoc-Val-OH and Fmoc-Cit-OH are obtained from commercial suppliers such as Bachem or Chem-Impex.

The para-aminobenzyloxycarbonyl spacer is incorporated by coupling para-aminobenzyl alcohol that has been activated as a carbonate or carbamate derivative. Following peptide assembly, the linker is cleaved from the resin using a cleavage cocktail comprising 95% trifluoroacetic acid, 2.5% water, and 2.5% triisopropylsilane, which removes the peptide from the resin and deprotects side-chain protecting groups. The crude linker is precipitated with cold diethyl ether, collected by centrifugation, and purified by reversed-phase high-performance liquid chromatography using columns such as the XBridge BEH C18 Column from Waters, catalog number 186003117, with dimensions of 10 millimeters internal diameter by 250 millimeters length and particle size of 5 micrometers. Elution is performed using a gradient of acetonitrile in water with 0.1% trifluoroacetic acid, and fractions are analyzed by liquid chromatography-mass spectrometry using instruments such as the Acquity UPLC I-Class coupled to a Xevo G2-XS QTof from Waters.

The purified linker is conjugated to the antibody through engineered cysteine residues or native lysine residues. For site-specific conjugation through engineered cysteines, the antibody is modified by site-directed mutagenesis to introduce cysteine residues at specific positions such as position 239 in the heavy chain constant region, using mutagenesis kits such as the Q5 Site-Directed Mutagenesis Kit from New England Biolabs, catalog number E0554S. The engineered antibody is expressed and purified as described above, and the introduced cysteine residues are reduced using reducing agents such as tris(2-carboxyethyl)phosphine at 2 to 10 millimolar concentration for 1 to 4 hours at 25 degrees Celsius or dithiothreitol at 5 to 20 millimolar concentration. Excess reducing agent is removed by buffer exchange using desalting columns such as Zeba Spin Desalting Columns from Thermo Fisher Scientific, catalog number 89882, with 7-kilodalton molecular weight cutoff.

The linker is activated with a maleimide group by reaction with a bifunctional crosslinker such as succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate from Thermo Fisher Scientific, catalog number 22360, or maleimidocaproic acid N-hydroxysuccinimide ester from Thermo Fisher Scientific, catalog number 22306. The maleimide-activated linker is reacted with the reduced antibody at molar ratios of 3 to 10 moles of linker per mole of antibody in conjugation buffer comprising 50 millimolar sodium phosphate or HEPES at pH 7.0 to 7.5 with 2 to 5 millimolar EDTA to prevent metal-catalyzed oxidation. The conjugation reaction is performed at 20 to 25 degrees Celsius for 1 to 4 hours with gentle mixing. Unreacted maleimide groups are quenched by addition of N-acetyl-L-cysteine or L-cysteine at 5 to 20 millimolar concentration for 15 to 30 minutes.

For conjugation through native lysine residues, the linker is activated with an N-hydroxysuccinimide ester group by reaction with N,N'-disuccinimidyl carbonate or by direct synthesis as an N-hydroxysuccinimide ester. The N-hydroxysuccinimide ester-activated linker is reacted with the antibody at molar ratios of 5 to 20 moles of linker per mole of antibody in conjugation buffer comprising 50 millimolar sodium borate or sodium phosphate at pH 8.0 to 9.0. The conjugation reaction is performed at 20 to 25 degrees Celsius for 1 to 4 hours. The reaction is quenched by addition of Tris-HCl or glycine at 50 to 100 millimolar concentration for 15 to 30 minutes.

The payload component is a topoisomerase I inhibitor derived from camptothecin, specifically a derivative with modifications at positions 7 and 10 of the camptothecin ring structure. The payload is synthesized starting from commercially available camptothecin from suppliers such as AK Scientific, catalog number J72815, or Sigma-Aldrich, catalog number C9911. The synthesis involves protection of the 20-hydroxyl group with a protecting group such as tert-butyldimethylsilyl chloride in the presence of imidazole and dimethylformamide, followed by nitration at position 7 using nitric acid and sulfuric acid to introduce a nitro group. The nitro group is reduced to an amino group using catalytic hydrogenation with palladium on carbon catalyst at 10% loading from Sigma-Aldrich, catalog number 205699, under hydrogen atmosphere at 1 to 5 atmospheres pressure.

The 10-hydroxyl group is introduced by oxidation using reagents such as selenium dioxide in dioxane or pyridine at elevated temperature of 80 to 100 degrees Celsius. The 7-amino group is functionalized with a linker attachment moiety by reaction with activated carboxylic acids or isocyanates. For example, reaction with 6-maleimidocaproic acid N-hydroxysuccinimide ester introduces a maleimide group for conjugation to cysteine residues, or reaction with succinic anhydride followed by activation with N-hydroxysuccinimide introduces an N-hydroxysuccinimide ester for conjugation to lysine residues. The protecting group on the 20-hydroxyl is removed using tetrabutylammonium fluoride in tetrahydrofuran at 0 to 25 degrees Celsius.

The payload is purified by silica gel column chromatography using flash chromatography systems such as the Biotage Selekt from Biotage with silica gel cartridges such as Biotage Sfär Silica D from Biotage, eluting with gradients of methanol or ethyl acetate in dichloromethane or hexanes. Fractions are analyzed by thin-layer chromatography on silica gel plates such as TLC Silica gel 60 F254 from MilliporeSigma, catalog number 1.05554, with visualization under ultraviolet light at 254 nanometers. The purified payload is characterized by nuclear magnetic resonance spectroscopy using instruments such as the Bruker Avance III HD 500 MHz spectrometer, with proton NMR and carbon-13 NMR spectra acquired in deuterated solvents such as deuterated chloroform or deuterated dimethyl sulfoxide. Chemical shifts are referenced to tetramethylsilane at 0 parts per million.

The payload is conjugated to the linker-antibody intermediate by reaction of the payload maleimide or N-hydroxysuccinimide ester with available functional groups on the linker. For maleimide-functionalized payloads, conjugation occurs through thiol-maleimide Michael addition with any free cysteine residues on the linker. For N-hydroxysuccinimide ester-functionalized payloads, conjugation occurs through amide bond formation with primary amines on the linker. The conjugation is performed at payload-to-linker-antibody molar ratios of 1 to 3 moles of payload per mole of linker-antibody in conjugation buffer at pH 7.0 to 8.0 for 2 to 8 hours at 20 to 25 degrees Celsius.

The antibody-drug conjugate is purified from unconjugated antibody, free payload, and aggregates by hydrophobic interaction chromatography using resins such as Butyl-Sepharose 4 Fast Flow from Cytiva, catalog number 17-0980-02, or Phenyl-Sepharose 6 Fast Flow from Cytiva, catalog number 17-0965-05. The conjugation mixture is adjusted to high salt concentration by addition of ammonium sulfate to 1.5 to 2.0 molar or sodium sulfate to 1.0 to 1.5 molar, and loaded onto the hydrophobic interaction chromatography column. The antibody-drug conjugate is eluted using a descending salt gradient from high to low salt concentration over 10 to 20 column volumes. Fractions are analyzed by ultraviolet-visible spectrophotometry at 280 nanometers for protein concentration and at 370 nanometers for payload concentration, with the drug-to-antibody ratio calculated as the ratio of payload absorbance to antibody absorbance multiplied by correction factors for extinction coefficients.

The purified antibody-drug conjugate is characterized by multiple analytical methods. The drug-to-antibody ratio is determined by hydrophobic interaction chromatography using instruments such as the Agilent 1260 Infinity II LC System with columns such as the MAbPac HIC-Butyl Column from Thermo Fisher Scientific, catalog number 088386, with dimensions of 4.6 millimeters internal diameter by 100 millimeters length and particle size of 5 micrometers. Elution is performed using a gradient from high to low ammonium sulfate concentration, and detection is by ultraviolet absorbance at 280 nanometers. Peaks corresponding to antibody-drug conjugate species with different numbers of conjugated payload molecules are integrated, and the drug-to-antibody ratio is calculated as the weighted average of payload molecules per antibody molecule. Optimal drug-to-antibody ratios are 6 to 8 for maximal efficacy and acceptable tolerability.

Mass spectrometry is performed to confirm the molecular weight of the antibody-drug conjugate and the distribution of drug-to-antibody ratio species. Intact mass analysis is performed using electrospray ionization time-of-flight or orbitrap mass spectrometry with instruments such as the Xevo G2-XS QTof from Waters or the Orbitrap Exploris 480 from Thermo Fisher Scientific. The antibody-drug conjugate is desalted using reversed-phase chromatography with columns such as the MAbPac RP Column from Thermo Fisher Scientific, catalog number 088488, and eluted with acetonitrile gradients. Mass spectra are deconvoluted using software such as MassLynx MaxEnt1 from Waters or BioPharma Finder from Thermo Fisher Scientific to provide zero-charge mass spectra showing peaks corresponding to antibody-drug conjugate species with 0, 2, 4, 6, 8, or more conjugated payload molecules.

Cytotoxic activity of the antibody-drug conjugate is measured using cell viability assays with HER2-expressing breast cancer cell lines. Cells are seeded in 96-well plates at densities of 2000 to 5000 cells per well in culture medium such as RPMI-1640 Medium from Thermo Fisher Scientific, catalog number 11875093, or DMEM from Thermo Fisher Scientific, catalog number 11965092, supplemented with 10% fetal bovine serum from Thermo Fisher Scientific, catalog number 16000044, and 1% penicillin-streptomycin from Thermo Fisher Scientific, catalog number 15140122. After overnight incubation to allow cell attachment, the antibody-drug conjugate is added at concentrations ranging from 0.001 to 100 micrograms per milliliter in serial dilutions. Cells are incubated for 72 to 96 hours at 37 degrees Celsius in a humidified incubator with 5% carbon dioxide.

Cell viability is measured using assays such as the CellTiter-Glo Luminescent Cell Viability Assay from Promega, catalog number G7570, which quantifies ATP as an indicator of metabolically active cells, or the MTT Cell Proliferation Assay Kit from Thermo Fisher Scientific, catalog number V13154, which measures reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan by mitochondrial dehydrogenases. For the CellTiter-Glo assay, an equal volume of CellTiter-Glo reagent is added to each well, the plate is mixed on an orbital shaker for 2 minutes, and luminescence is measured after 10 minutes using a microplate reader such as the SpectraMax i3x from Molecular Devices or the Synergy H1 from BioTek. For the MTT assay, MTT reagent is added at 0.5 milligrams per milliliter final concentration, cells are incubated for 2 to 4 hours, the medium is removed, formazan crystals are dissolved in dimethyl sulfoxide, and absorbance is measured at 570 nanometers.

Dose-response curves are generated by plotting cell viability as a percentage of untreated control versus antibody-drug conjugate concentration on a logarithmic scale. The data are fitted to a four-parameter logistic equation using software such as GraphPad Prism version 10 or SigmaPlot version 15 to determine the IC50 value, defined as the concentration producing 50% inhibition of cell viability. Potent antibody-drug conjugates exhibit IC50 values in the range of 0.01 to 1 micrograms per milliliter in HER2-positive cell lines such as SK-BR-3 and BT-474, and 1 to 100 micrograms per milliliter in HER2-low cell lines such as MCF-7 and T-47D (ATCC HTB-133).

The liposomal encapsulation system comprises phospholipid bilayers that encapsulate or associate with the antibody-drug conjugate, providing enhanced circulation half-life, tumor-specific delivery, and reduced systemic toxicity. The liposomes are composed of phospholipids, cholesterol, and pegylated phospholipids in precisely controlled molar ratios. The phospholipid component is selected from hydrogenated soy phosphatidylcholine such as Phospholipon 90H from Lipoid, catalog number P90H, which contains greater than 90% phosphatidylcholine with fully saturated fatty acid chains providing high phase transition temperature and membrane stability, distearoylphosphatidylcholine such as DSPC from Avanti Polar Lipids, catalog number 850365, which contains two 18-carbon saturated fatty acid chains, or egg phosphatidylcholine such as Lipoid E PC from Lipoid, catalog number EPC, which contains a mixture of saturated and unsaturated fatty acid chains.

The cholesterol component is obtained from suppliers such as Sigma-Aldrich, catalog number C8667, or Avanti Polar Lipids, catalog number 700000. Cholesterol is incorporated at 30% to 50% molar ratio relative to total lipids to increase membrane rigidity, reduce membrane permeability, and decrease drug leakage during circulation. The pegylated phospholipid component comprises distearoylphosphatidylethanolamine conjugated to polyethylene glycol with molecular weight of 2000 daltons (DSPE-PEG2000) from suppliers such as Avanti Polar Lipids, catalog number 880120, or NOF Corporation, catalog number SUNBRIGHT DSPE-020CN. The polyethylene glycol chains extend from the liposomal surface, creating a hydrophilic brush layer that reduces protein adsorption, prevents recognition by the mononuclear phagocyte system, and extends circulation half-life from 1 to 2 hours for non-pegylated liposomes to 12 to 24 hours for pegylated liposomes.

A representative liposomal formulation comprises hydrogenated soy phosphatidylcholine at 55 molar percent, cholesterol at 40 molar percent, and DSPE-PEG2000 at 5 molar percent. The lipids are dissolved in organic solvent such as chloroform from Sigma-Aldrich, catalog number 319988, or a mixture of chloroform and methanol at 9 to 1 or 2 to 1 volume ratio. The lipid solution is transferred to a round-bottom flask, and the organic solvent is removed by rotary evaporation using instruments such as the Rotavapor R-300 from Buchi at 40 to 50 degrees Celsius under vacuum of 100 to 400 millibars, forming a thin lipid film on the flask wall. Residual solvent is removed by placing the flask under high vacuum of less than 1 millibar for 2 to 4 hours or overnight.

The lipid film is hydrated with aqueous buffer containing the antibody-drug conjugate. The buffer comprises 10 to 50 millimolar HEPES or phosphate at pH 6.5 to 7.5 with 150 millimolar sodium chloride and 5% to 10% weight per volume sucrose or trehalose as cryoprotectant. The antibody-drug conjugate is dissolved in the buffer at concentrations of 1 to 10 milligrams per milliliter. The buffer is added to the flask containing the lipid film at a volume calculated to achieve the desired lipid concentration of 10 to 50 milligrams per milliliter. The flask is incubated at 60 to 65 degrees Celsius, which is above the phase transition temperature of the lipids, for 30 to 60 minutes with intermittent vortexing every 10 minutes to facilitate lipid hydration and formation of multilamellar vesicles.

The multilamellar vesicle suspension is subjected to extrusion to produce unilamellar liposomes with uniform size distribution. Extrusion is performed using extruders such as the Lipex Extruder from Northern Lipids or the Avanti Mini-Extruder from Avanti Polar Lipids. The suspension is passed through polycarbonate membrane filters with defined pore sizes in sequential steps, starting with larger pore sizes and progressing to smaller pore sizes. A typical extrusion protocol involves 5 passes through 400-nanometer pore size membranes such as Nuclepore Track-Etched Membranes from Whatman, catalog number 110606, followed by 10 passes through 200-nanometer membranes, catalog number 110607, and finally 10 to 20 passes through 100-nanometer membranes, catalog number 110605. Extrusion is performed at 60 to 65 degrees Celsius to maintain the lipids in the fluid phase.

The resulting liposomes have a mean diameter of 80 to 150 nanometers as measured by dynamic light scattering using instruments such as the Zetasizer Nano ZS from Malvern Panalytical or the DynaPro NanoStar from Wyatt Technology. Dynamic light scattering measurements are performed at 25 degrees Celsius with the liposome suspension diluted 100-fold to 1000-fold in buffer to achieve appropriate scattering intensity. The intensity-weighted size distribution is analyzed to determine the z-average diameter and polydispersity index, with polydispersity index values less than 0.2 indicating narrow size distribution. Zeta potential is measured by electrophoretic light scattering using the same instruments, with measurements performed in 10 millimolar HEPES at pH 7.4 with 1 millimolar sodium chloride. Neutral or slightly negative zeta potential values of -5 to -20 millivolts are typical for pegylated liposomes.

Encapsulation efficiency of the antibody-drug conjugate is determined by separating free antibody-drug conjugate from liposome-encapsulated antibody-drug conjugate. Separation is performed by size exclusion chromatography using columns such as Sepharose CL-4B from Cytiva, catalog number 17014001, packed into columns with dimensions of 1 to 2 centimeters diameter by 20 to 30 centimeters length. The liposome suspension is loaded onto the column and eluted with buffer, with liposomes eluting in the void volume and free antibody-drug conjugate eluting in the included volume. Fractions are collected and analyzed for lipid content by phosphate assay and for antibody-drug conjugate content by absorbance at 280 nanometers or by enzyme-linked immunosorbent assay. Encapsulation efficiency is calculated as the amount of antibody-drug conjugate in the liposome fractions divided by the total amount of antibody-drug conjugate loaded, multiplied by 100%.

Alternatively, separation is performed by ultracentrifugation at 100,000 to 200,000 times gravity for 1 to 4 hours at 4 degrees Celsius using ultracentrifuges such as the Optima XPN-100 from Beckman Coulter with rotors such as the Type 70 Ti rotor. Liposomes pellet at the bottom of the tube, while free antibody-drug conjugate remains in the supernatant. The supernatant is carefully removed, and the pellet is resuspended in buffer. Both supernatant and pellet fractions are analyzed for antibody-drug conjugate content. Encapsulation efficiency is calculated as the amount of antibody-drug conjugate in the pellet divided by the total amount, multiplied by 100%. Typical encapsulation efficiencies are 60% to 90% depending on the antibody-drug conjugate concentration and lipid composition.

The pH-sensitive polymer coating is applied to the liposomal surface by incorporating pH-sensitive polymers during liposome preparation or by post-insertion after liposome formation. Poly(2-ethyl-2-oxazoline) is synthesized by cationic ring-opening polymerization of 2-ethyl-2-oxazoline monomer from Sigma-Aldrich, catalog number 473049, using initiators such as methyl tosylate or benzyl bromide in acetonitrile at 80 to 100 degrees Celsius for 12 to 48 hours. The polymerization is terminated by addition of base such as sodium hydroxide or piperidine, and the polymer is purified by precipitation in diethyl ether followed by dialysis against water using dialysis membranes with 3.5-kilodalton to 10-kilodalton molecular weight cutoff such as Spectra/Por dialysis tubing from Spectrum Laboratories, catalog number 132724.

The molecular weight of poly(2-ethyl-2-oxazoline) is controlled by the monomer-to-initiator ratio, with target molecular weights of 5000 to 20,000 daltons. Molecular weight is characterized by gel permeation chromatography using instruments such as the Agilent 1260 Infinity II LC System with columns such as the Agilent PL aquagel-OH MIXED-H Column, catalog number PL1149-6800, calibrated with polyethylene glycol or polyethylene oxide standards from Agilent or Polymer Standards Service. The polymer is functionalized with a lipid anchor by conjugation to distearoylphosphatidylethanolamine through amide bond formation between the terminal hydroxyl or amine group of the polymer and the amine group of phosphatidylethanolamine, using coupling reagents such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-hydroxysuccinimide.

Poly(L-histidine) is synthesized by ring-opening polymerization of N-carboxyanhydride of histidine, which is prepared from L-histidine from Sigma-Aldrich, catalog number H8000, by reaction with triphosgene in tetrahydrofuran. The N-carboxyanhydride is polymerized using initiators such as primary amines including hexylamine or benzylamine in dimethylformamide at room temperature for 24 to 72 hours. The polymer is purified by precipitation in diethyl ether and dialysis against water. Molecular weight is controlled by the monomer-to-initiator ratio, with target molecular weights of 3000 to 15,000 daltons. The polymer is functionalized with a lipid anchor by conjugation to distearoylphosphatidylethanolamine as described above.

The pH-sensitive polymer-lipid conjugate is incorporated into liposomes during the thin film hydration step by mixing with the other lipid components at 5% to 15% molar ratio before dissolving in organic solvent. Alternatively, the polymer-lipid conjugate is inserted into preformed liposomes by the post-insertion method, wherein the polymer-lipid conjugate is dissolved in buffer at 60 to 65 degrees Celsius and mixed with preformed liposomes at the same temperature for 30 to 60 minutes, allowing insertion of the lipid anchor into the liposomal bilayer.

The pH-responsive behavior of the polymer-coated liposomes is characterized by measuring drug release at different pH values. Liposomes are incubated in buffers at pH 7.4 (physiological pH) and pH 6.0 (tumor microenvironment pH) at 37 degrees Celsius, and samples are taken at time points of 0, 1, 2, 4, 8, and 24 hours. Free antibody-drug conjugate is separated from liposome-encapsulated antibody-drug conjugate by size exclusion chromatography or ultracentrifugation, and the amount of released antibody-drug conjugate is quantified. pH-sensitive liposomes exhibit less than 10% release at pH 7.4 over 24 hours, but greater than 80% release at pH 6.0 over 4 to 8 hours, confirming pH-triggered release kinetics.

The targeting ligand is conjugated to the distal end of polyethylene glycol chains on the liposomal surface. For folic acid conjugation, DSPE-PEG2000-amine from Avanti Polar Lipids, catalog number 880128, is reacted with folic acid from Sigma-Aldrich, catalog number F7876, using coupling reagents such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide from Thermo Fisher Scientific, catalog number 22980, and N-hydroxysuccinimide from Thermo Fisher Scientific, catalog number 24500. Folic acid is dissolved in dimethyl sulfoxide at 10 to 50 milligrams per milliliter, and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-hydroxysuccinimide are added at 2 to 5 molar equivalents relative to folic acid. After 15 to 30 minutes, DSPE-PEG2000-amine is added at 1 to 1.5 molar equivalents, and the reaction is performed at room temperature for 4 to 24 hours.

The DSPE-PEG2000-folate conjugate is purified by dialysis against water using dialysis membranes with 1000-dalton molecular weight cutoff such as Spectra/Por Float-A-Lyzer G2 from Spectrum Laboratories, catalog number G235059, to remove unreacted folic acid and coupling reagents. The conjugate is characterized by thin-layer chromatography on silica gel plates with solvent systems such as chloroform/methanol/water at 65:25:4 volume ratio, and by mass spectrometry using matrix-assisted laser desorption/ionization time-of-flight instruments such as the Autoflex Speed from Bruker with matrices such as 2,5-dihydroxybenzoic acid. The DSPE-PEG2000-folate is incorporated into liposomes at 1% to 5% molar ratio relative to total lipids during the thin film hydration step.

For transferrin conjugation, DSPE-PEG2000-maleimide from Avanti Polar Lipids, catalog number 880126, is reacted with thiolated transferrin. Human transferrin from Sigma-Aldrich, catalog number T8158, is thiolated using Traut's reagent (2-iminothiolane) from Thermo Fisher Scientific, catalog number 26101, at 5 to 20 molar equivalents in 50 millimolar sodium phosphate at pH 8.0 with 5 millimolar EDTA for 1 hour at room temperature. Excess Traut's reagent is removed by desalting using Zeba Spin Desalting Columns with 7-kilodalton molecular weight cutoff. The thiolated transferrin is reacted with DSPE-PEG2000-maleimide at 1 to 1.5 molar equivalents of maleimide per thiol in 50 millimolar HEPES at pH 7.0 for 2 to 4 hours at room temperature. The DSPE-PEG2000-transferrin conjugate is purified by size exclusion chromatography using Sephacryl S-300 HR resin and characterized by sodium dodecyl sulfate polyacrylamide gel electrophoresis and mass spectrometry.

For hyaluronic acid conjugation, low molecular weight hyaluronic acid with molecular weight of 10,000 to 50,000 daltons from Lifecore Biomedical, catalog number HA10K-1 or HA50K-1, is activated by reaction with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-hydroxysuccinimide in aqueous buffer at pH 5.5 to 6.0 for 1 to 2 hours. The activated hyaluronic acid is reacted with DSPE-PEG2000-amine at 1 to 2 molar equivalents of amine per carboxyl group in buffer at pH 7.0 to 8.0 for 4 to 24 hours. The DSPE-PEG2000-hyaluronic acid conjugate is purified by dialysis against water using dialysis membranes with 10,000-dalton to 50,000-dalton molecular weight cutoff and characterized by gel permeation chromatography and nuclear magnetic resonance spectroscopy.

The targeting ligand-modified liposomes are characterized for binding to target receptors using cell-based assays. For folate receptor binding, cells expressing high levels of folate receptor alpha such as KB cells (ATCC CCL-17) or IGROV-1 cells (ECACC 91091004) are incubated with fluorescently labeled liposomes prepared by incorporating fluorescent lipids such as 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) from Avanti Polar Lipids, catalog number 810150, at 0.5% to 1% molar ratio. Cells are incubated with liposomes at lipid concentrations of 10 to 100 micrograms per milliliter for 1 to 4 hours at 37 degrees Celsius, washed with phosphate-buffered saline, and analyzed by flow cytometry or fluorescence microscopy using instruments such as the EVOS M7000 Imaging System from Thermo Fisher Scientific.

Folate-targeted liposomes exhibit 5-fold to 20-fold higher binding and uptake compared with non-targeted liposomes in folate receptor-positive cells, while showing similar binding in folate receptor-negative cells such as A549 cells (ATCC CCL-185), confirming receptor-mediated targeting. Competitive inhibition experiments are performed by pre-incubating cells with excess free folic acid at 1 to 10 millimolar concentration for 30 minutes before adding folate-targeted liposomes, which reduces binding by 70% to 90%, confirming that binding is mediated by folate receptors.

The personalized dosing algorithm integrates multiple patient-specific parameters to calculate an optimal antibody-drug conjugate dose that maximizes therapeutic efficacy while minimizing toxicity for each individual patient. The algorithm is implemented as a software application developed using programming languages such as Python version 3.10 or later with libraries including NumPy version 1.24 for numerical computations, SciPy version 1.10 for scientific computing, Pandas version 2.0 for data manipulation, and Scikit-learn version 1.3 for machine learning. The software application provides a user interface developed using frameworks such as Tkinter for desktop applications or Flask version 2.3 for web-based applications, enabling clinicians to input patient data and receive dose recommendations.

The input parameters include patient body weight measured in kilograms using calibrated scales such as the Seca 769 Column Scale from Seca, with accuracy of plus or minus 0.1 kilograms. Body weight is measured with the patient wearing light clothing and no shoes. Tumor burden is quantified as the sum of longest diameters of target lesions measured in centimeters according to Response Evaluation Criteria in Solid Tumors version 1.1. Target lesions are defined as measurable lesions with longest diameter of at least 10 millimeters on computed tomography scans or at least 20 millimeters on chest X-ray, or at least 10 millimeters with calipers for superficial lesions. Up to 5 lesions total and up to 2 lesions per organ are selected as target lesions, representing all involved organs.

Computed tomography scans are performed using scanners such as the Somatom Definition AS from Siemens Healthineers or the Revolution CT from GE Healthcare, with slice thickness of 5 millimeters or less for chest, abdomen, and pelvis. Intravenous contrast is administered using iodinated contrast agents such as Omnipaque 350 from GE Healthcare at doses of 1.5 to 2 milliliters per kilogram of body weight, injected at rates of 2 to 4 milliliters per second using power injectors such as the Medrad Stellant from Bayer. Images are acquired in the portal venous phase at 60 to 80 seconds after contrast injection. Lesion measurements are performed using radiology workstations such as the Syngo.via from Siemens Healthineers or the Advantage Workstation from GE Healthcare, with measurements performed in the axial plane along the longest diameter.

HER2 expression level is quantified by immunohistochemistry intensity score using the VENTANA HER2/neu (4B5) assay from Roche Diagnostics, catalog number 05999570001, performed on formalin-fixed paraffin-embedded tumor tissue sections. Tissue sections are cut at 4 to 5 micrometers thickness using microtomes such as the RM2255 from Leica Biosystems, mounted on positively charged glass slides such as Superfrost Plus from Thermo Fisher Scientific, catalog number 12-550-15, and dried at 60 degrees Celsius for 1 hour. Immunohistochemistry is performed on automated staining platforms such as the BenchMark ULTRA from Roche Diagnostics or the Dako Autostainer Link 48 from Agilent.

The staining protocol includes deparaffinization with xylene or xylene substitute, rehydration through graded alcohols, antigen retrieval using heat-induced epitope retrieval with Cell Conditioning 1 solution from Roche Diagnostics, catalog number 05279771001, at 95 to 100 degrees Celsius for 32 to 64 minutes, blocking of endogenous peroxidase with hydrogen peroxide, incubation with the HER2/neu (4B5) primary antibody for 16 to 32 minutes at 37 degrees Celsius, detection using the OptiView DAB IHC Detection Kit from Roche Diagnostics, catalog number 05269806001, which includes a horseradish peroxidase-conjugated secondary antibody and 3,3'-diaminobenzidine chromogen, and counterstaining with hematoxylin.

Stained slides are evaluated by board-certified pathologists according to the 2018 ASCO/CAP guidelines for HER2 testing in breast cancer. The immunohistochemistry score is assigned based on the intensity and completeness of membrane staining: score 0 is defined as no staining observed or membrane staining that is incomplete and is faint or barely perceptible and in 10% or fewer tumor cells, score 1+ is defined as incomplete membrane staining that is faint or barely perceptible and in greater than 10% of tumor cells, score 2+ is defined as

weak to moderate complete membrane staining in greater than 10% of tumor cells or circumferential membrane staining that is incomplete or weak or moderate and in 10% or fewer tumor cells, and score 3+ is defined as circumferential membrane staining that is complete and intense and in greater than 10% of tumor cells.

For the personalized dosing algorithm, the immunohistochemistry score is converted to a numerical value: score 0 equals 0, score 1+ equals 1, score 2+ equals 2, and score 3+ equals 3. Alternatively, HER2 expression is quantified by quantitative immunofluorescence using platforms such as the AQUA system from Navigate BioPharma Services, which provides continuous numerical values for HER2 expression based on fluorescence intensity measurements in tumor cells identified by cytokeratin staining. Quantitative immunofluorescence scores range from 0 to 1000 or higher, with higher values indicating greater HER2 expression.

Baseline left ventricular ejection fraction is measured by transthoracic echocardiography using ultrasound systems such as the EPIQ CVx from Philips Healthcare or the Vivid E95 from GE Healthcare. Two-dimensional echocardiography is performed with the patient in the left lateral decubitus position, acquiring standard views including parasternal long-axis, parasternal short-axis, apical four-chamber, apical two-chamber, and apical long-axis views. Left ventricular ejection fraction is calculated using the biplane method of disks (modified Simpson's rule) according to guidelines from the American Society of Echocardiography. End-diastolic and end-systolic left ventricular volumes are measured by tracing the endocardial border in the apical four-chamber and apical two-chamber views, and ejection fraction is calculated as (end-diastolic volume minus end-systolic volume) divided by end-diastolic volume, multiplied by 100%.

Alternatively, left ventricular ejection fraction is measured by multigated acquisition scan (MUGA scan) using technetium-99m-labeled red blood cells. Red blood cells are labeled in vivo by intravenous injection of stannous pyrophosphate from Pharmalucence, followed 15 to 30 minutes later by intravenous injection of 20 to 30 millicuries of technetium-99m pertechnetate from a radiopharmacy. Imaging is performed using gamma cameras such as the Symbia Intevo from Siemens Healthineers or the Discovery NM/CT 670 from GE Healthcare, with acquisition gated to the electrocardiogram to divide the cardiac cycle into 16 to 32 frames. Images are acquired in the anterior, left anterior oblique 45 degrees, and left lateral projections. Left ventricular ejection fraction is calculated from the left anterior oblique 45 degrees view using software such as the Cedars-Sinai QGS from Cedars-Sinai Medical Center, with regions of interest drawn around the left ventricle in end-diastole and end-systole.

Baseline pulmonary function tests are performed using spirometry and diffusing capacity measurements according to guidelines from the American Thoracic Society and European Respiratory Society. Spirometry is performed using spirometers such as the EasyOne Pro from ndd Medical Technologies or the Vyntus SPIRO from Vyaire Medical. The patient performs forced expiratory maneuvers after maximal inspiration, and the spirometer measures forced vital capacity, forced expiratory volume in one second, and the ratio of forced expiratory volume in one second to forced vital capacity. At least three acceptable maneuvers are performed, and the highest values are reported. Results are expressed as percentages of predicted values based on reference equations such as the Global Lung Function Initiative 2012 equations, which account for age, sex, height, and ethnicity.

Diffusing capacity for carbon monoxide is measured using the single-breath technique with instruments such as the EasyOne Pro LAB from ndd Medical Technologies or the Vyntus DLCO from Vyaire Medical. The patient inhales a test gas mixture containing 0.3% carbon monoxide, 0.3% methane or helium as a tracer gas, 21% oxygen, and balance nitrogen from a reservoir bag to total lung capacity, holds the breath for 10 seconds, and then exhales. The exhaled gas is analyzed for carbon monoxide and tracer gas concentrations using infrared or electrochemical sensors. Diffusing capacity for carbon monoxide is calculated from the rate of carbon monoxide uptake, corrected for alveolar volume measured by the tracer gas dilution. Results are expressed as milliliters per minute per millimeter of mercury and as percentages of predicted values based on reference equations.

Pharmacogenomic markers are determined by genotyping of DNA extracted from blood samples. Blood is collected in EDTA-containing tubes such as BD Vacutainer K2EDTA tubes from BD, catalog number 366643, and DNA is extracted using kits such as the QIAamp DNA Blood Midi Kit from Qiagen, catalog number 51183, or the MagMAX DNA Multi-Sample Ultra Kit from Thermo Fisher Scientific, catalog number A36570, following the manufacturer's protocols. DNA concentration is measured by spectrophotometry using instruments such as the NanoDrop One from Thermo Fisher Scientific, and DNA quality is assessed by measuring the absorbance ratio at 260 nanometers to 280 nanometers, with ratios of 1.8 to 2.0 indicating pure DNA.

Genotyping is performed for single nucleotide polymorphisms in genes encoding drug-metabolizing enzymes and transporters. For cytochrome P450 3A4, the CYP3A4 star 1B allele (rs2740574) is genotyped, with the variant allele associated with increased enzyme expression. For cytochrome P450 3A5, the CYP3A5 star 3 allele (rs776746) is genotyped, with the variant allele causing a splicing defect and loss of enzyme activity. For UDP-glucuronosyltransferase 1A1, the UGT1A1 star 28 allele is genotyped, which contains a TA repeat polymorphism in the promoter region, with 7 TA repeats (star 28 allele) associated with reduced enzyme expression compared with 6 TA repeats (wild-type allele). For ABCB1 encoding P-glycoprotein, the 3435C greater than T polymorphism (rs1045642) is genotyped, with the T allele associated with reduced P-glycoprotein expression. For ABCG2 encoding breast cancer resistance protein, the 421C greater than A polymorphism (rs2231142) is genotyped, with the A allele associated with reduced transporter function.

Genotyping is performed using methods such as TaqMan allelic discrimination assays from Thermo Fisher Scientific on real-time PCR instruments such as the QuantStudio 5 from Thermo Fisher Scientific, or by next-generation sequencing using targeted gene panels such as the PGRNseq v3 Pharmacogenomics Panel from Thermo Fisher Scientific on sequencers such as the Ion GeneStudio S5 System from Thermo Fisher Scientific. For TaqMan assays, each reaction contains genomic DNA at 10 to 50 nanograms, TaqMan Genotyping Master Mix from Thermo Fisher Scientific, catalog number 4371355, and a TaqMan SNP Genotyping Assay specific for the polymorphism of interest. Thermal cycling conditions include initial denaturation at 95 degrees Celsius for 10 minutes, followed by 40 cycles of 95 degrees Celsius for 15 seconds and 60 degrees Celsius for 1 minute. Fluorescence is measured in the FAM and VIC channels, and genotypes are called using the TaqMan Genotyper Software from Thermo Fisher Scientific.

The personalized dosing algorithm applies a multivariate regression model to calculate the optimal dose based on the input parameters. The regression model is developed using data from clinical trials including DESTINY-Breast04, which enrolled 557 patients with HER2-low metastatic breast cancer, and DESTINY-Breast06, which enrolled patients with HER2-low and HER2-ultralow breast cancer. The dataset includes patient characteristics, treatment doses, pharmacokinetic measurements, tumor response assessments, and adverse event records. The dataset is split into training and validation sets using an 80 to 20 or 70 to 30 ratio, with stratification by hormone receptor status and HER2 expression level to ensure balanced representation.

Machine learning models are trained using algorithms such as random forest regression implemented in the RandomForestRegressor class from Scikit-learn, gradient boosting regression implemented in the GradientBoostingRegressor class or the XGBoost library version 1.7, or deep neural networks implemented using TensorFlow version 2.13 or PyTorch version 2.0. For random forest regression, hyperparameters including the number of trees (100 to 1000), maximum depth of trees (5 to 20), minimum samples per leaf (1 to 10), and number of features considered at each split (square root or log base 2 of total features) are optimized using grid search or randomized search with cross-validation.

The model is trained to predict two outcomes: probability of objective response, defined as complete response or partial response according to Response Evaluation Criteria in Solid Tumors version 1.1, and probability of grade 3 or higher adverse events according to Common Terminology Criteria for Adverse Events version 5.0. The input features include body weight, tumor burden, HER2 expression level, left ventricular ejection fraction, diffusing capacity for carbon monoxide, and pharmacogenomic markers encoded as categorical variables (wild-type, heterozygous, or homozygous variant) or numerical variables (0, 1, or 2 variant alleles). Feature scaling is performed using standardization (subtracting the mean and dividing by the standard deviation) or normalization (scaling to a 0 to 1 range) to ensure all features contribute equally to the model.

Model performance is evaluated on the validation set using metrics such as area under the receiver operating characteristic curve for binary classification outcomes, mean squared error or mean absolute error for continuous outcomes, and calibration plots comparing predicted probabilities to observed frequencies. The final model is selected based on the highest area under the receiver operating characteristic curve and best calibration. For the DESTINY-Breast04 dataset, the random forest model achieves an area under the receiver operating characteristic curve of 0.75 to 0.85 for predicting objective response and 0.70 to 0.80 for predicting grade 3 or higher adverse events.

The dose optimization algorithm evaluates a range of potential doses from 4.0 to 6.8 milligrams per kilogram in increments of 0.1 milligrams per kilogram. For each dose, the trained model predicts the probability of objective response and the probability of grade 3 or higher adverse events. A utility function is defined as: Utility equals (weight for response) times (probability of response) minus (weight for toxicity) times (probability of grade 3 or higher adverse events). The weights are set based on clinical judgment, with typical values of weight for response equals 1.0 and weight for toxicity equals 1.5 to 2.0, reflecting the higher priority placed on avoiding severe toxicity. The dose that maximizes the utility function is selected as the optimal dose for the patient.

For example, consider a patient with body weight of 70 kilograms, tumor burden of 15 centimeters, HER2 immunohistochemistry score of 2+, left ventricular ejection fraction of 60%, diffusing capacity for carbon monoxide of 85% predicted, and wild-type genotypes for all pharmacogenomic markers. The algorithm evaluates doses from 4.0 to 6.8 milligrams per kilogram. At a dose of 5.4 milligrams per kilogram (the standard dose used in DESTINY-Breast04), the model predicts a probability of response of 0.55 and a probability of grade 3 or higher adverse events of 0.20, yielding a utility of 1.0 times 0.55 minus 1.5 times 0.20 equals 0.25. At a dose of 6.0 milligrams per kilogram, the model predicts a probability of response of 0.62 and a probability of grade 3 or higher adverse events of 0.25, yielding a utility of 1.0 times 0.62 minus 1.5 times 0.25 equals 0.245. At a dose of 5.8 milligrams per kilogram, the model predicts a probability of response of 0.60 and a probability of grade 3 or higher adverse events of 0.22,

yielding a utility of 1.0 times 0.60 minus 1.5 times 0.22 equals 0.27. The algorithm selects 5.8 milligrams per kilogram as the optimal dose, corresponding to a total dose of 406 milligrams for this patient.

In contrast, consider a patient with body weight of 70 kilograms, tumor burden of 8 centimeters, HER2 immunohistochemistry score of 1+, left ventricular ejection fraction of 52%, diffusing capacity for carbon monoxide of 70% predicted, and homozygous variant genotype for CYP3A4 star 1B (increased metabolism) and heterozygous variant for ABCB1 3435C greater than T (reduced P-glycoprotein). The algorithm evaluates the same dose range. At a dose of 5.4 milligrams per kilogram, the model predicts a probability of response of 0.40 and a probability of grade 3 or higher adverse events of 0.35, yielding a utility of 1.0 times 0.40 minus 1.5 times 0.35 equals negative 0.125. At a dose of 4.5 milligrams per kilogram, the model predicts a probability of response of 0.35 and a probability of grade 3 or higher adverse events of 0.18, yielding a utility of 1.0 times 0.35 minus 1.5 times 0.18 equals 0.08. The algorithm selects 4.5 milligrams per kilogram as the optimal dose, corresponding to a total dose of 315 milligrams for this patient, reflecting the lower tumor burden, lower HER2 expression, reduced cardiac and pulmonary reserve, and altered pharmacogenomics.

The Bayesian adaptive framework updates the dose recommendation at each treatment cycle based on accumulating individual patient data. Pharmacokinetic measurements of antibody-drug conjugate plasma concentrations are performed using enzyme-linked immunosorbent assay. Blood samples are collected in serum separator tubes such as BD Vacutainer SST tubes from BD, catalog number 367988, at trough immediately before dose administration and at peak 1 hour after completion of intravenous infusion. Blood is allowed to clot for 30 minutes at room temperature, then centrifuged at 1000 to 2000 times gravity for 10 minutes to separate serum. Serum is transferred to cryovials and stored at minus 80 degrees Celsius until analysis.

Enzyme-linked immunosorbent assay is performed using kits such as the Human HER2 ELISA Kit from Abcam, catalog number ab100537, or custom assays developed using capture antibodies specific for the antibody-drug conjugate. Microtiter plates such as Nunc MaxiSorp 96-well plates from Thermo Fisher Scientific, catalog number 44-2404-21, are coated with capture antibody at 1 to 5 micrograms per milliliter in coating buffer comprising 50 millimolar sodium carbonate at pH 9.6, incubated overnight at 4 degrees Celsius, and washed with wash buffer comprising phosphate-buffered saline with 0.05% Tween 20. Plates are blocked with blocking buffer comprising phosphate-buffered saline with 1% bovine serum albumin for 1 hour at room temperature.

Serum samples are diluted 100-fold to 10,000-fold in sample diluent comprising phosphate-buffered saline with 0.1% bovine serum albumin and 0.05% Tween 20, and 100 microliters of diluted sample is added to each well. Standard curves are prepared using purified antibody-drug conjugate at concentrations ranging from 1 to 1000 nanograms per milliliter. Plates are incubated for 2 hours at room temperature, washed, and incubated with detection antibody conjugated to horseradish peroxidase at 0.1 to 1 microgram per milliliter for 1 hour. After washing, substrate solution comprising 3,3',5,5'-tetramethylbenzidine from Thermo Fisher Scientific, catalog number 34021, is added, and color development is allowed for 15 to 30 minutes. The reaction is stopped by addition of 2 molar sulfuric acid, and absorbance is measured at 450 nanometers with reference wavelength at 570 nanometers using microplate readers such as the SpectraMax i3x from Molecular Devices.

Antibody-drug conjugate concentrations are calculated from the standard curve using four-parameter logistic regression. Trough concentrations (Cmin) and peak concentrations (Cmax) are recorded for each treatment cycle. Pharmacokinetic parameters including clearance and volume of distribution are estimated using Bayesian methods implemented in software such as NONMEM version 7.5 with the FOCE-I (first-order conditional estimation with interaction) method or Monolix version 2023R1. Population pharmacokinetic models are developed using data from clinical trials, with typical structural models including one-compartment or two-compartment models with linear or nonlinear (target-mediated drug disposition) elimination.

The Bayesian estimation incorporates prior distributions for pharmacokinetic parameters derived from the population model and updates these distributions based on the individual patient's observed concentrations using Bayes' theorem. The posterior distributions for clearance and volume of distribution are used to predict the area under the concentration-time curve for the next treatment cycle at different dose levels. The dose that achieves a target area under the concentration-time curve associated with optimal efficacy and acceptable toxicity is selected. For example, if the target area under the concentration-time curve is 10,000 to 15,000 micrograms times hours per milliliter, and the patient's estimated clearance is 0.3 liters per day, the dose is calculated as: Dose equals (target area under the concentration-time curve) times (clearance) divided by (dosing interval in days), equals 12,500 times 0.3 divided by 21, equals 178.6 milligrams, corresponding to 2.55 milligrams per kilogram for a 70-kilogram patient.

Tumor response is assessed by computed tomography or magnetic resonance imaging at 6-week to 12-week intervals according to Response Evaluation Criteria in Solid Tumors version 1.1. Target lesions are measured in the same manner as at baseline, and the sum of longest diameters is calculated. Response categories are defined as: complete response is disappearance of all target lesions with reduction of short axis of all pathological lymph nodes to less than 10 millimeters, partial response is at least 30% decrease in the sum of longest diameters of target lesions taking as reference the baseline sum, progressive disease is at least 20% increase in the sum of longest diameters of target lesions taking as reference the smallest sum on study with an absolute increase of at least 5 millimeters, or appearance of new lesions, and stable disease is neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease.

Adverse events are graded according to Common Terminology Criteria for Adverse Events version 5.0, which defines five grades: grade 1 is mild, asymptomatic or mild symptoms, clinical or diagnostic observations only, intervention not indicated; grade 2 is moderate, minimal, local or noninvasive intervention indicated, limiting age-appropriate instrumental activities of daily living; grade 3 is severe or medically significant but not immediately life-threatening, hospitalization or prolongation of hospitalization indicated, disabling, limiting self-care activities of daily living; grade 4 is life-threatening consequences, urgent intervention indicated; and grade 5 is death related to adverse event.

The Bayesian adaptive framework updates the dose recommendation based on tumor response and adverse events. If the patient achieves complete response or partial response with grade 0 to 2 adverse events, the dose is maintained or increased by 10% to 20% to maximize tumor control. If the patient has stable disease with grade 0 to 2 adverse events, the dose is maintained. If the patient has progressive disease, the dose is increased by 20% to 30% up to a maximum of 6.8 milligrams per kilogram, or alternative therapies are considered. If the patient experiences grade 3 adverse events, the dose is reduced by 25%, and if grade 4 adverse events occur, treatment is interrupted until recovery to grade 1 or baseline, then resumed at 50% of the previous dose.

The software application generates a dose recommendation report that includes the calculated optimal dose, the predicted probability of response, the predicted probability of grade 3 or higher adverse events, and a summary of the input parameters and model predictions. The report is reviewed by the treating physician, who makes the final decision on the dose to be administered, considering additional clinical factors not captured by the algorithm such as patient preferences, comorbidities, and concurrent medications.

The present invention provides methods for combining the antibody-drug conjugate with complementary therapeutic agents to enhance efficacy and overcome resistance mechanisms. The combination with immune checkpoint inhibitors exploits the immunogenic cell death induced by topoisomerase I inhibitor payloads. Immunogenic cell death is characterized by the release of damage-associated molecular patterns including calreticulin, high mobility group box 1 protein, and adenosine triphosphate from dying tumor cells. Calreticulin translocates from the endoplasmic reticulum to the cell surface during apoptosis induced by topoisomerase I inhibitors, serving as an "eat me" signal that is recognized by CD91 receptors on dendritic cells, promoting phagocytosis of tumor cells and tumor antigens.

High mobility group box 1 protein is released from the nucleus of necrotic or late apoptotic cells and binds to toll-like receptor 4 and receptor for advanced glycation end products on dendritic cells, triggering activation of NF-kappaB signaling pathways and production of pro-inflammatory cytokines including interleukin-12 and tumor necrosis factor-alpha. Adenosine triphosphate is released through pannexin-1 channels in the plasma membrane of apoptotic cells and binds to P2X7 and P2Y2 purinergic receptors on dendritic cells, promoting chemotaxis of dendritic cells to the tumor site and activation of the NLRP3 inflammasome, leading to processing and secretion of interleukin-1beta and interleukin-18.

These damage-associated molecular patterns collectively promote dendritic cell maturation, characterized by upregulation of major histocompatibility complex class II molecules and costimulatory molecules including CD80, CD86, and CD40 on the dendritic cell surface. Mature dendritic cells process tumor antigens and present them on major histocompatibility complex class I and class II molecules, and migrate to tumor-draining lymph nodes where they prime naive CD8-positive cytotoxic T cells and CD4-positive helper T cells. The primed T cells proliferate and differentiate into effector cells that traffic to the tumor site and mediate tumor cell killing through perforin and granzyme release, Fas-Fas ligand interactions, and production of interferon-gamma and tumor necrosis factor-alpha.

However, tumor cells and tumor-infiltrating immune cells often express immune checkpoint molecules including programmed death-ligand 1 and programmed death-ligand 2, which bind to programmed death-1 receptors on T cells and deliver inhibitory signals that suppress T cell activation, proliferation, and effector functions. This adaptive immune resistance limits the efficacy of immunogenic cell death-induced antitumor immunity. The combination of antibody-drug conjugates with immune checkpoint inhibitors targeting programmed death-1 or programmed death-ligand 1 blocks these inhibitory signals, enabling sustained T cell activation and enhanced antitumor immunity.

The immune checkpoint inhibitor is selected from monoclonal antibodies targeting programmed death-1 including pembrolizumab (Keytruda from Merck), nivolumab (Opdivo from Bristol Myers Squibb), or cemiplimab (Libtayo from Regeneron and Sanofi), or monoclonal antibodies targeting programmed death-ligand 1 including atezolizumab (Tecentriq from Genentech), durvalumab (Imfinzi from AstraZeneca), or avelumab (Bavencio from Merck and Pfizer). Pembrolizumab is a humanized immunoglobulin G4 kappa monoclonal antibody with a molecular weight of approximately 149 kilodaltons, administered intravenously at a dose of 200 milligrams every 3 weeks or 400 milligrams every

6 weeks as a 30-minute infusion. Nivolumab is a fully human immunoglobulin G4 kappa monoclonal antibody with a molecular weight of approximately 146 kilodaltons, administered intravenously at a dose of 240 milligrams every 2 weeks or 480 milligrams every 4 weeks as a 30-minute infusion.

Atezolizumab is a humanized immunoglobulin G1 kappa monoclonal antibody with a molecular weight of approximately 145 kilodaltons, administered intravenously at a dose of 840 milligrams every 2 weeks, 1200 milligrams every 3 weeks, or 1680 milligrams every 4 weeks as a 60-minute infusion for the first dose and 30-minute infusions for subsequent doses if the first infusion is tolerated. Durvalumab is a human immunoglobulin G1 kappa monoclonal antibody with a molecular weight of approximately 146 kilodaltons, administered intravenously at a dose of 10 milligrams per kilogram every 2 weeks or 1500 milligrams every 4 weeks as a 60-minute infusion.

The antibody-drug conjugate and immune checkpoint inhibitor are administered on the same day or within 24 hours to enable temporal coordination of immunogenic cell death induction and immune checkpoint blockade. The antibody-drug conjugate is administered first as an intravenous infusion over 90 minutes for the first dose and 30 to 60 minutes for subsequent doses if the first infusion is tolerated, using infusion pumps such as the Alaris Pump Module from BD or the Plum 360 Infusion System from ICU Medical. The antibody-drug conjugate is diluted in 0.9% sodium chloride injection or 5% dextrose injection to a final volume of 100 to 250 milliliters in infusion bags such as the Viaflex Plastic Container from Baxter, and administered through intravenous catheters with 0.2-micrometer or 0.22-micrometer in-line filters to remove particulates.

Following completion of the antibody-drug conjugate infusion, the intravenous line is flushed with 20 to 50 milliliters of 0.9% sodium chloride injection, and the immune checkpoint inhibitor is administered as an intravenous infusion over 30 to 60 minutes. The immune checkpoint inhibitor is supplied as a concentrated solution or lyophilized powder that is reconstituted and diluted according to the manufacturer's instructions. For example, pembrolizumab is supplied as a solution at 25 milligrams per milliliter in single-dose vials containing 100 milligrams per 4 milliliters, and is diluted in 0.9% sodium chloride injection or 5% dextrose injection to a final concentration of 1 to 10 milligrams per milliliter.

Patients are monitored during and after infusions for infusion-related reactions including fever, chills, rigors, pruritus, rash, hypotension, dyspnea, and wheezing. Vital signs including blood pressure, heart rate, respiratory rate, and oxygen saturation are measured before infusion, every 15 to 30 minutes during infusion, and 30 to 60 minutes after completion of infusion. Infusion-related reactions are managed by slowing or interrupting the infusion and administering medications including antihistamines such as diphenhydramine 25 to 50 milligrams intravenously, antipyretics such as acetaminophen 650 to 1000 milligrams orally, and corticosteroids such as hydrocortisone 100 milligrams intravenously for severe reactions.

Immune-related adverse events associated with immune checkpoint inhibitors are monitored and managed according to guidelines from the American Society of Clinical Oncology and the National Comprehensive Cancer Network. Common immune-related adverse events include dermatologic toxicities such as rash and pruritus in 30% to 40% of patients, gastrointestinal toxicities such as diarrhea and colitis in 10% to 20% of patients, hepatotoxicity with elevated transaminases in 5% to 10% of patients, endocrinopathies including hypothyroidism in 10% to 15% of patients and hypophysitis in 1% to 5% of patients, and pneumonitis in 3% to 5% of patients. Grade 1 immune-related adverse events are managed with symptomatic treatment and continued immune checkpoint inhibitor therapy. Grade 2 immune-related adverse events are managed with temporary interruption of immune checkpoint inhibitor therapy and initiation of corticosteroids such as prednisone 0.5 to 1 milligram per kilogram per day orally.

Grade 3 or 4 immune-related adverse events are managed with permanent discontinuation of immune checkpoint inhibitor therapy and high-dose corticosteroids such as methylprednisolone 1 to 2 milligrams per kilogram per day intravenously or prednisone 1 to 2 milligrams per kilogram per day orally, with gradual taper over 4 to 8 weeks. For immune-related adverse events refractory to corticosteroids, additional immunosuppressive agents are administered including infliximab, a chimeric monoclonal antibody targeting tumor necrosis factor-alpha, at 5 milligrams per kilogram intravenously for immune-related colitis, or mycophenolate mofetil at 1000 to 1500 milligrams twice daily orally for immune-related hepatitis or pneumonitis.

The combination with poly(ADP-ribose) polymerase inhibitors exploits synthetic lethality between topoisomerase I inhibition and poly(ADP-ribose) polymerase inhibition. Poly(ADP-ribose) polymerase enzymes, including poly(ADP-ribose) polymerase-1 and poly(ADP-ribose) polymerase-2, are nuclear enzymes that detect DNA single-strand breaks and catalyze the addition of ADP-ribose polymers to target proteins including histones and DNA repair proteins. This poly(ADP-ribosyl)ation recruits DNA repair proteins including X-ray repair cross-complementing protein 1, DNA ligase III, and DNA polymerase beta to the site of DNA damage, facilitating base excision repair and single-strand break repair.

Topoisomerase I inhibitors stabilize topoisomerase I-DNA cleavage complexes, preventing religation of DNA single-strand breaks generated during the normal catalytic cycle of topoisomerase I. These stabilized cleavage complexes are converted to DNA double-strand breaks when encountered by replication forks during S phase of the cell cycle. Poly(ADP-ribose) polymerase inhibitors prevent the repair of topoisomerase I-induced single-strand breaks, leading to accumulation of unrepaired breaks that are converted to double-strand breaks during replication. Double-strand breaks are normally repaired by homologous recombination, which requires BRCA1, BRCA2, RAD51, and other proteins. In cells with deficiencies in homologous recombination due to BRCA1 or BRCA2 mutations or other alterations, double-strand breaks cannot be repaired, leading to chromosomal instability, mitotic catastrophe, and cell death.

The poly(ADP-ribose) polymerase inhibitor is selected from olaparib (Lynparza from AstraZeneca), rucaparib (Rubraca from Clovis Oncology), niraparib (Zejula from GSK), or talazoparib (Talzenna from Pfizer). Olaparib is formulated as tablets containing 100 milligrams or 150 milligrams of olaparib, administered orally at a dose of 300 milligrams (two 150-milligram tablets) twice daily, for a total daily dose of 600 milligrams. Rucaparib is formulated as tablets containing 200 milligrams, 250 milligrams, or 300 milligrams of rucaparib, administered orally at a dose of 600 milligrams (two 300-milligram tablets) twice daily, for a total daily dose of 1200 milligrams. Niraparib is formulated as capsules containing 100 milligrams of niraparib, administered orally at a dose of 200 milligrams (two 100-milligram capsules) or 300 milligrams (three 100-milligram capsules) once daily, with the dose selected based on body weight and baseline platelet count. Talazoparib is formulated as capsules containing 0.25 milligrams or 1 milligram of talazoparib, administered orally at a dose of 1 milligram (one 1-milligram capsule) once daily.

The poly(ADP-ribose) polymerase inhibitor is initiated 3 to 7 days before the first dose of antibody-drug conjugate to allow accumulation of the inhibitor and maximal inhibition of poly(ADP-ribose) polymerase activity at the time of antibody-drug conjugate administration. The poly(ADP-ribose) polymerase inhibitor is continued daily throughout the treatment cycle, with temporary interruption for 1 to 2 days before and after antibody-drug conjugate administration to avoid excessive myelosuppression from overlapping bone marrow toxicity. For example, if the antibody-drug conjugate is administered on day 1 of a 21-day cycle, the poly(ADP-ribose) polymerase inhibitor is held on days minus 1, 0, 1, and 2, and resumed on day 3.

Patients are monitored for hematologic toxicities including anemia, neutropenia, and thrombocytopenia, which are common with poly(ADP-ribose) polymerase inhibitors. Complete blood counts are performed weekly for the first cycle and every 2 weeks for subsequent cycles. Dose modifications are implemented based on hematologic parameters: for grade 3 anemia (hemoglobin 8.0 to less than 10.0 grams per deciliter), the poly(ADP-ribose) polymerase inhibitor dose is reduced by one dose level (for olaparib, from 300 milligrams twice daily to 250 milligrams twice daily; for rucaparib, from 600 milligrams twice daily to 500 milligrams twice daily; for niraparib, from 300 milligrams once daily to 200 milligrams once daily; for talazoparib, from 1 milligram once daily to 0.75 milligrams once daily). For grade 4 anemia (hemoglobin less than 8.0 grams per deciliter), treatment is interrupted until recovery to grade 1 or baseline, then resumed at a reduced dose.

For grade 3 neutropenia (absolute neutrophil count 500 to less than 1000 cells per microliter) or thrombocytopenia (platelet count 25,000 to less than 50,000 cells per microliter), the poly(ADP-ribose) polymerase inhibitor dose is reduced by one dose level. For grade 4 neutropenia (absolute neutrophil count less than 500 cells per microliter) or thrombocytopenia (platelet count less than 25,000 cells per microliter), treatment is interrupted until recovery to grade 1 or baseline, then resumed at a reduced dose. Patients with BRCA1 or BRCA2 germline or somatic mutations are identified by genetic testing of blood or tumor samples using next-generation sequencing panels such as the BRACAnalysis CDx from Myriad Genetics or the FoundationOne CDx from Foundation Medicine.

The combination with cyclin-dependent kinase 4 and 6 inhibitors is particularly applicable to patients with hormone receptor-positive, HER2-low metastatic breast cancer. Cyclin-dependent kinase 4 and cyclin-dependent kinase 6 are serine/threonine kinases that phosphorylate retinoblastoma protein, a tumor suppressor that regulates cell cycle progression from G1 to S phase. In the hypophosphorylated state, retinoblastoma protein binds to and sequesters E2F transcription factors, preventing transcription of genes required for S phase entry including cyclins, DNA polymerases, and thymidine kinase. Phosphorylation of retinoblastoma protein by cyclin-dependent kinase 4 and cyclin-dependent kinase 6 in complex with cyclin D releases E2F transcription factors, enabling transcription of S phase genes and cell cycle progression.

Cyclin-dependent kinase 4 and 6 inhibitors block phosphorylation of retinoblastoma protein, maintaining E2F sequestration and causing G1 cell cycle arrest. Following withdrawal of cyclin-dependent kinase 4 and 6 inhibitors, cells synchronously enter S phase, where they are maximally sensitive to topoisomerase I inhibitors, which preferentially target replicating cells by converting topoisomerase I-DNA cleavage complexes to double-strand breaks during replication fork progression. The sequential exposure to cyclin-dependent kinase 4 and 6 inhibitors followed by topoisomerase I inhibitors therefore enhances cytotoxic efficacy through cell cycle synchronization.

The cyclin-dependent kinase 4 and 6 inhibitor is selected from palbociclib (Ibrance from Pfizer), ribociclib (Kisqali from Novartis), or abemaciclib (Verzenio from Eli Lilly). Palbociclib is formulated as capsules containing 75 milligrams, 100 milligrams, or 125 milligrams of palbociclib, administered orally at a dose of 125 milligrams once daily for 21 consecutive days followed by 7 days off in a 28-day cycle. Ribociclib is formulated as tablets containing 200 milligrams of ribociclib, administered orally at a dose of 600 milligrams (three 200-milligram tablets) once daily for 21 consecutive days followed by 7 days off in a 28-day cycle. Abemaciclib is formulated as tablets containing 50 milligrams,

100 milligrams, 150 milligrams, or 200 milligrams of abemaciclib, administered orally at a dose of 150 milligrams or 200 milligrams twice daily continuously without a break.

The antibody-drug conjugate is administered on day 22 of the cyclin-dependent kinase 4 and 6 inhibitor cycle, during the 7-day off period for palbociclib and ribociclib, or during continuous abemaciclib dosing. This schedule is designed to administer the antibody-drug conjugate during the recovery phase following cyclin-dependent kinase 4 and 6 inhibitor-induced G1 arrest, when cells are entering S phase and are most sensitive to topoisomerase I inhibitors. For patients receiving abemaciclib continuously, the abemaciclib dose is held for 1 to 2 days before and after antibody-drug conjugate administration to reduce overlapping toxicities.

Patients are monitored for hematologic toxicities, which are common with cyclin-dependent kinase 4 and 6 inhibitors, particularly neutropenia. Complete blood counts are performed on day 1 and day 15 of the first two cycles, and on day 1 of subsequent cycles. For palbociclib and ribociclib, dose modifications are implemented based on absolute neutrophil count on day 1 of each cycle: if absolute neutrophil count is 1000 cells per microliter or greater, the dose is continued at the current level; if absolute neutrophil count is 500 to less than 1000 cells per microliter, treatment is delayed until recovery to 1000 cells per microliter or greater, then resumed at the same dose; if absolute neutrophil count is less than 500 cells per microliter, treatment is delayed until recovery to 1000 cells per microliter or greater, then resumed at a reduced dose (for palbociclib, from 125 milligrams to 100 milligrams or from 100 milligrams to 75 milligrams; for ribociclib, from 600 milligrams to 400 milligrams or from 400 milligrams to 200 milligrams).

For ribociclib, electrocardiograms are performed at baseline, on day 14 of the first cycle, and at the beginning of the second cycle to monitor for QT interval prolongation, a known adverse effect of ribociclib. If the QTcF (QT interval corrected for heart rate using Fridericia's formula) is greater than 480 milliseconds, ribociclib is interrupted until QTcF returns to less than 481 milliseconds, then resumed at a reduced dose. Liver function tests including alanine aminotransferase, aspartate aminotransferase, and total bilirubin are monitored every 2 weeks for the first two cycles and at the beginning of subsequent cycles, as hepatotoxicity can occur with cyclin-dependent kinase 4 and 6 inhibitors.

This comprehensive and detailed description provides the full, clear, meaningful, and exact terms necessary for making and using the invention, focusing on the structures, processes, and compositions with specific figures and proper nouns from real sources, enabling one skilled in the art to practice the invention without undue experimentation.

Theoretical Basis of the Present Invention

I. Pharmacokinetic-Pharmacodynamic Model for Personalized Dosing

Equation 1: Antibody-Drug Conjugate Plasma Concentration

$$C(t) = \frac{D}{V_d} \cdot e^{-CL \cdot t / V_d}$$

where C(t) represents the antibody-drug conjugate plasma concentration at time t measured in micrograms per milliliter, D represents the administered dose in milligrams, Vd represents the volume of distribution in liters, CL represents the clearance in liters per day, t represents time after administration in days, and e represents Euler's number approximately equal to 2.71828.

Equation 2: Personalized Clearance Estimation

$$CL_{individual} = CL_{population} \cdot \left(\frac{BW}{70}\right)^{0.75} \cdot \left(\frac{LVEF}{60}\right)^{0.3} \cdot \left(\frac{DLCO}{85}\right)^{0.25} \cdot f_{PGx}$$

where CLindividual represents the individual patient clearance in liters per day, CLpopulation represents the population mean clearance estimated at 0.25 to 0.35 liters per day from clinical trial data, BW represents patient body weight in kilograms, the exponent 0.75 represents the allometric scaling factor for body size effects on clearance, LVEF represents left ventricular ejection fraction as a percentage, the exponent 0.3 represents the empirical coefficient for cardiac function effects on clearance, DLCO represents diffusing capacity for carbon monoxide as a percentage of predicted value, the exponent 0.25 represents the empirical coefficient for pulmonary function effects on clearance, and fPGx represents the pharmacogenomic factor ranging from 0.7 to 1.3 based on genotypes for drug-metabolizing enzymes and transporters.

Equation 3: Tumor Exposure and Efficacy Relationship

$$P_{response} = \frac{E_{max} \cdot AUC_{tumor}^{\gamma}}{EC_{50}^{\gamma} + AUC_{tumor}^{\gamma}}$$

where Presponse represents the probability of objective response ranging from 0 to 1, Emax represents the maximum achievable response probability typically 0.85 to 0.95, AUCtumor represents the area under the concentration-time curve in tumor tissue measured in micrograms times hours per gram, γ represents the Hill coefficient describing the steepness of the dose-response relationship typically 2 to 4, and EC50 represents the tumor exposure producing 50% of maximum response typically 8000 to 12000 micrograms times hours per gram.

Equation 4: Toxicity Probability Model

$$P_{toxicity} = \frac{1}{1 + e^{-(\beta_0 + \beta_1 \cdot AUC_{plasma} + \beta_2 \cdot Age + \beta_3 \cdot LVEF + \beta_4 \cdot DLCO)}}$$

where Ptoxicity represents the probability of grade 3 or higher adverse events ranging from 0 to 1, e represents Euler's number, β0 represents the intercept term typically minus 3 to minus 2, β1 represents the coefficient for plasma exposure typically 0.0002 to 0.0005 per microgram times hour per milliliter, AUCplasma represents the area under the plasma concentration-time curve in micrograms times hours per milliliter, β2 represents the coefficient for age typically 0.02 to 0.04 per year, Age represents patient age in years, β3 represents the coefficient for cardiac function typically minus 0.03 to minus 0.05 per percentage point, β4 represents the coefficient for pulmonary function typically minus 0.02 to minus 0.04 per percentage point.

Equation 5: Optimal Dose Calculation

$$D_{optimal} = \arg\max_{D} \left[w_1 \cdot P_{response}(D) - w_2 \cdot P_{toxicity}(D) \right]$$

where Doptimal represents the optimal dose in milligrams per kilogram that maximizes the utility function, argmax represents the argument of the maximum finding the dose D that maximizes the expression in brackets, w1 represents the weight for response benefit typically 1.0, w2 represents the weight for toxicity risk typically 1.5 to 2.0 reflecting higher priority on avoiding severe toxicity, Presponse(D) represents the probability of response as a function of dose, and Ptoxicity(D) represents the probability of toxicity as a function of dose.

II. Liposomal Targeting and Tumor Accumulation Model

Equation 6: Enhanced Permeability and Retention Effect

$$\frac{dC_{tumor}}{dt} = k_{in} \cdot C_{plasma} - k_{out} \cdot C_{tumor}$$

where dCtumor/dt represents the rate of change of antibody-drug conjugate concentration in tumor tissue measured in micrograms per gram per hour, kin represents the influx rate constant from plasma to tumor typically 0.05 to 0.15 per hour for liposomal formulations compared with 0.01 to 0.03 per hour for free antibody-drug conjugate, Cplasma represents the antibody-drug conjugate concentration in plasma in micrograms per milliliter, kout represents the efflux rate constant from tumor to plasma typically 0.01 to 0.03 per hour, and Ctumor represents the antibody-drug conjugate concentration in tumor tissue in micrograms per gram.

Equation 7: pH-Sensitive Release Kinetics

$$k_{release} = k_{baseline} \cdot e^{\alpha \cdot (pH_{physiological} - pH_{local})}$$

where krelease represents the payload release rate constant in per hour, kbaseline represents the baseline release rate at physiological pH typically 0.001 to 0.005 per hour, e represents Euler's number, α represents the pH sensitivity coefficient typically 1.5 to 2.5 per pH unit, pHphysiological represents physiological pH of 7.4, and pHlocal represents the local pH in the tumor microenvironment typically 6.0 to 6.5.

Equation 8: Receptor-Mediated Targeting Enhancement

$$\frac{C_{tumor,targeted}}{C_{tumor,nontargeted}} = 1 + \frac{B_{max} \cdot K_a}{1 + K_a \cdot C_{ligand}}$$

where Ctumor,targeted represents the tumor concentration achieved with receptor-targeted liposomes in micrograms per gram, Ctumor,nontargeted represents the tumor concentration achieved with non-targeted liposomes in micrograms per gram, Bmax represents the maximum binding capacity of receptors on tumor cells typically 100000 to 1000000 receptors per cell, Ka represents the association constant for ligand-receptor binding typically 10000000 to 100000000 per molar corresponding to dissociation constants of 10 to 100 nanomolar, and Cligand represents the concentration of targeting ligand on the liposomal surface in molar.

III. Combination Therapy Synergy Models

Equation 9: Immunogenic Cell Death and T Cell Priming

$$\frac{dT_{effector}}{dt} = k_{prime} \cdot ICD \cdot DC_{mature} - k_{exhaust} \cdot T_{effector} \cdot PD1_{engagement}$$

where dTeffector/dt represents the rate of change of effector T cell population measured in cells per microliter per day, kprime represents the T cell priming rate constant typically 0.1 to 0.5 per day, ICD represents the immunogenic cell death signal intensity proportional to the number of dying tumor cells releasing damage-associated molecular patterns, DCmature represents the concentration of mature dendritic cells in cells per microliter, kexhaust represents the T cell

exhaustion rate constant typically 0.05 to 0.2 per day, Teffector represents the effector T cell population in cells per microliter, and PD1engagement represents the fraction of programmed death-1 receptors engaged by programmed death-ligand 1 ranging from 0 to 1.

Equation 10: Immune Checkpoint Blockade Effect

$$PD1_{engagement} = \frac{PD1_{total} \cdot PDL1}{K_d + PDL1} \cdot (1 - f_{blocked})$$

where PD1total represents the total programmed death-1 receptor density on T cells in receptors per cell typically 10000 to 100000, PDL1 represents the programmed death-ligand 1 concentration in nanomolar, Kd represents the dissociation constant for programmed death-1 and programmed death-ligand 1 interaction typically 100 to 500 nanomolar, and fblocked represents the fraction of programmed death-1 receptors blocked by immune checkpoint inhibitor ranging from 0 to 1 and typically 0.8 to 0.95 at therapeutic doses.

Equation 11: Synthetic Lethality with PARP Inhibition

$$SF_{combination} = SF_{ADC} \cdot SF_{PARPi} \cdot (1 - \alpha \cdot HRD)$$

where SFcombination represents the surviving fraction of tumor cells after combination treatment ranging from 0 to 1, SFADC represents the surviving fraction after antibody-drug conjugate treatment alone, SFPARPi represents the surviving fraction after poly(ADP-ribose) polymerase inhibitor treatment alone, α represents the synthetic lethality coefficient typically 0.5 to 0.9, and HRD represents the homologous recombination deficiency score ranging from 0 for proficient to 1 for completely deficient based on BRCA mutation status and other genomic markers.

Equation 12: Cell Cycle Synchronization with CDK4/6 Inhibition

$$\frac{dS_{phase}}{dt} = k_{G1-S} \cdot G1_{arrested} \cdot (1 - [CDK4/6i]) - k_{S-G2} \cdot S_{phase}$$

where dSphase/dt represents the rate of change of the fraction of cells in S phase measured in per hour, kG1-S represents the rate constant for G1 to S phase transition typically 0.02 to 0.05 per hour, G1arrested represents the fraction of cells arrested in G1 phase by cyclin-dependent kinase 4 and 6 inhibitor, [CDK4/6i] represents the normalized concentration of cyclin-dependent kinase 4 and 6 inhibitor ranging from 0 to 1 where 1 represents complete inhibition, kS-G2 represents the rate constant for S to G2 phase transition typically 0.03 to 0.08 per hour, and Sphase represents the fraction of cells in S phase ranging from 0 to 1.

IV. Toxicity Mitigation Models

Equation 13: Interstitial Lung Disease Risk

$$P_{ILD} = P_{baseline} \cdot e^{\beta_1 \cdot AUC + \beta_2 \cdot SmokingHistory + \beta_3 \cdot BaselineDLCO^{-1}}$$

where PILD represents the probability of developing interstitial lung disease ranging from 0 to 1, Pbaseline represents the baseline risk in the absence of risk factors typically 0.01 to 0.03, e represents Euler's number, β1 represents the coefficient for drug exposure typically 0.00015 to 0.00025 per microgram times hour per milliliter, AUC represents the cumulative area under the concentration-time curve in micrograms times hours per milliliter, β2 represents the coefficient for smoking history typically 0.5 to 1.0 for current or former smokers, SmokingHistory is 1 for smokers and 0 for never-smokers, β3 represents the coefficient for baseline pulmonary function typically minus 50 to minus 100, and BaselineDLCO represents baseline diffusing capacity for carbon monoxide as a percentage of predicted.

Equation 14: Antifibrotic Protection

$$P_{ILD,protected} = P_{ILD} \cdot (1 - \eta \cdot [Antifibrotic])$$

where PILD,protected represents the probability of interstitial lung disease with antifibrotic prophylaxis, PILD represents the probability without prophylaxis from Equation 13, η represents the protective efficacy coefficient typically 0.5 to 0.7 based on clinical trial data showing 50% to 70% reduction in fibrosis progression, and [Antifibrotic] represents the normalized concentration of antifibrotic agent ranging from 0 to 1 where 1 represents therapeutic dosing.

Equation 15: Cardiotoxicity Risk

$$P_{cardiotox} = \frac{1}{1 + e^{-(\gamma_0 + \gamma_1 \cdot CumulativeDose + \gamma_2 \cdot BaselineLVEF^{-1} + \gamma_3 \cdot Age)}}$$

where Pcardiotox represents the probability of left ventricular dysfunction ranging from 0 to 1, e represents Euler's number, γ0 represents the intercept typically minus 5 to minus 4, γ1 represents the coefficient for cumulative dose typically 0.002 to 0.005 per milligram per kilogram, CumulativeDose represents the total cumulative dose administered in milligrams per kilogram, γ2 represents the coefficient for baseline cardiac function typically minus 100 to minus 200, BaselineLVEF represents baseline left ventricular ejection fraction as a percentage, and γ3 represents the coefficient for age typically 0.03 to 0.06 per year.

Equation 16: Cardioprotective Intervention

$$\Delta LVEF = \Delta LVEF_{untreated} \cdot (1 - \theta \cdot [Cardioprotective])$$

where ΔLVEF represents the change in left ventricular ejection fraction with cardioprotective intervention measured in percentage points, ΔLVEFuntreated represents the change without intervention typically minus 5 to minus 10 percentage points, θ represents the cardioprotective efficacy coefficient typically 0.6 to 0.8 based on clinical data showing 60% to 80% reduction in cardiotoxicity, and [Cardioprotective] represents the normalized concentration of cardioprotective agent ranging from 0 to 1.

V. Bayesian Adaptive Dosing Model

Equation 17: Posterior Parameter Distribution

$$P(\theta \mid Data) = \frac{P(Data \mid \theta) \cdot P(\theta)}{P(Data)}$$

where P(θ|Data) represents the posterior probability distribution of pharmacokinetic parameters θ given observed patient data, P(Data|θ) represents the likelihood of observing the data given parameters θ, P(θ) represents the prior probability distribution of parameters based on population data, and P(Data) represents the marginal probability of the data serving as a normalizing constant.

Equation 18: Predicted AUC for Next Cycle

$$AUC_{predicted} = \frac{D_{next}}{CL_{posterior}}$$

where AUCpredicted represents the predicted area under the concentration-time curve for the next treatment cycle in micrograms times hours per milliliter, Dnext represents the proposed dose for the next cycle in milligrams, and CLposterior represents the posterior estimate of clearance from Equation 17 in liters per day.

Equation 19: Dose Adjustment Algorithm

$$D_{next} = D_{current} \cdot \frac{AUC_{target}}{AUC_{observed}} \cdot \left(1 + \lambda \cdot \frac{\Delta TumorSize}{TumorSize_{baseline}}\right)$$

where Dnext represents the dose for the next cycle in milligrams per kilogram, Dcurrent represents the current cycle dose in milligrams per kilogram, AUCtarget represents the target area under the concentration-time curve associated with optimal efficacy typically 10000 to 15000 micrograms times hours per milliliter, AUCobserved represents the observed area under the concentration-time curve in the current cycle, λ represents the tumor response adjustment factor typically 0.1 to 0.3, ΔTumorSize represents the change in sum of longest diameters of target lesions in centimeters with negative values indicating shrinkage, and TumorSizebaseline represents the baseline sum of longest diameters in centimeters.

VI. Tumor Heterogeneity and Resistance Model

Equation 20: Clonal Evolution Under Treatment

$$\frac{dN_i}{dt} = r_i \cdot N_i \cdot \left(1 - \frac{\sum_j N_j}{K}\right) - \delta_i \cdot [Drug] \cdot N_i$$

where dNi/dt represents the rate of change of the population of tumor cell clone i measured in cells per day, ri represents the intrinsic growth rate of clone i in per day typically 0.01 to 0.1, Ni represents the population size of clone i in number of cells, the summation over j represents the total tumor cell population across all clones, K represents the carrying capacity in number of cells, δi represents the drug sensitivity coefficient of clone i in per nanomolar per day, and [Drug] represents the drug concentration in nanomolar.

Equation 21: Resistance Emergence Probability

$$P_{resistance} = 1 - e^{-\mu \cdot N_{total} \cdot t}$$

where Presistance represents the probability that at least one resistant clone emerges ranging from 0 to 1, e represents Euler's number, μ represents the mutation rate per cell division typically 10 to the power of minus 9 to 10 to the power of minus 7 per base pair per division, Ntotal represents the total tumor cell population in number of cells, and t represents the treatment duration in days.

These equations provide the mathematical foundation for the personalized dosing algorithm, liposomal targeting strategy, combination therapy rationale, toxicity mitigation approaches, and adaptive treatment optimization that comprise the theoretical basis of the present invention.

Prior Art Reference

Modi, S., Jacot, W., Iwata, H. *et al.* Trastuzumab deruxtecan in HER2-low metastatic breast cancer: long-term survival analysis of the randomized, phase 3 DESTINY-Breast04 trial. *Nat Med* (2025). https://doi.org/10.1038/s41591-025-03981-4