Pharmaceutical Composition and Method for Treating Cutaneous Squamous Cell Carcinoma

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Technical Field

The present invention relates to a pharmaceutical composition comprising immune checkpoint inhibitors and a method for treating cutaneous squamous cell carcinoma. More particularly, the invention concerns an ultra-short neoadjuvant immunotherapy regimen utilizing nivolumab with or without ipilimumab for patients with resectable cutaneous squamous cell carcinoma, wherein the treatment achieves pathological responses that enable treatment de-escalation or organ preservation while maintaining excellent survival outcomes.

Background Art

Cutaneous squamous cell carcinoma represents one of the most commonly diagnosed skin cancers worldwide, accounting for approximately 20 to 25 percent of all cutaneous malignancies with a steadily increasing incidence. Approximately 3 to 5 percent of cutaneous squamous cell carcinomas progress to advanced disease requiring extensive surgical intervention with or without adjuvant radiotherapy. The current standard of care for patients with locally advanced cutaneous squamous cell carcinoma involves extensive surgery that frequently results in severe disfigurement, loss of function, and reduced health-related quality of life, particularly in the elderly population with substantial comorbidities.

Cutaneous squamous cell carcinomas are characterized by ultraviolet-induced DNA damage and a relatively high tumor mutational burden, rendering these tumors immunogenic and attractive targets for immunotherapy. Recent advances in immune checkpoint blockade have demonstrated that anti-programmed death-1 antibodies achieve objective response rates of 44 to 50 percent in patients with advanced cutaneous squamous cell carcinoma not amenable for local curative-intent treatment. Prior studies employing neoadjuvant anti-programmed death-1 therapy with cemiplimab before curative-intent surgery have shown major pathological response rates of 64 to 75 percent when administered over 6 to 12 weeks.

However, existing neoadjuvant immunotherapy approaches for cutaneous squamous cell carcinoma present several limitations. First, prolonged neoadjuvant treatment periods of 6 to 12 weeks carry risks of disease progression to irresectable status, as evidenced by 2.5 percent of tumors becoming irresectable after 12 weeks of treatment. Second, current protocols typically combine neoadjuvant immunotherapy with standard surgical resection and adjuvant radiotherapy, potentially subjecting responding patients to unnecessary morbidity from extensive surgery and radiation. Third, the combination of programmed death-1 and cytotoxic T-lymphocyte-associated protein 4 checkpoint inhibition has not been previously evaluated in the neoadjuvant setting for cutaneous squamous cell carcinoma, despite demonstrating synergistic efficacy in other solid tumors.

Furthermore, existing treatment paradigms lack validated biomarkers for early identification of treatment responders who might safely avoid or de-escalate standard surgical and radiotherapeutic interventions. The absence of reliable early response indicators prevents implementation of response-adaptive treatment strategies that could spare responding patients from unnecessary surgical morbidity while ensuring timely definitive treatment for non-responders. Additionally, current approaches do not adequately address the healthcare burden and cost-effectiveness considerations relevant to this elderly, frail patient population with limited life expectancy and substantial comorbidities.

Disclosure of Invention

The present invention addresses the need for an improved treatment regimen for patients with resectable cutaneous squamous cell carcinoma that achieves high pathological response rates while minimizing treatment duration, toxicity, and unnecessary surgical morbidity. The invention further addresses the need for reliable biomarkers to identify early responders suitable for treatment de-escalation or organ preservation strategies.

The present invention provides a pharmaceutical composition comprising nivolumab at a dose of 3 milligrams per kilogram body weight, optionally combined with ipilimumab at a dose of 1 milligram per kilogram body weight, formulated for intravenous administration. The composition is administered according to an ultra-short neoadjuvant regimen consisting of two infusions of nivolumab on week 0 and week 2, with optional ipilimumab co-administration on week 0 only, prior to standard of care surgery in week 4.

The invention further provides a method for treating cutaneous squamous cell carcinoma comprising administering the pharmaceutical composition to a patient with stage I to IVa resectable cutaneous squamous cell carcinoma, monitoring treatment response using fluorodeoxyglucose positron emission tomography and computed tomography imaging to assess changes in total lesion glycolysis, and implementing response-adaptive treatment strategies based on pathological response assessment. Patients achieving major pathological response, defined as 10 percent or less viable tumor cells in the surgical specimen, or partial pathological response, defined as greater than 10 percent but 50 percent or less viable tumor cells, demonstrate 100 percent disease-specific survival at 24 months and may be candidates for treatment de-escalation including omission of adjuvant radiotherapy.

The invention additionally provides a method for achieving organ preservation in patients with cutaneous squamous cell carcinoma comprising administering two infusions of the pharmaceutical composition, monitoring for clinical complete response over a period of 2 to 10 months following treatment initiation, and withholding standard surgical resection and radiotherapy in patients achieving clinical complete response. This approach enables durable organ preservation with 100 percent disease-specific survival at 24 months while avoiding surgical morbidity and maintaining favorable health-related quality of life.

The invention further encompasses a method for early identification of treatment responders comprising obtaining baseline and week 4 fluorodeoxyglucose positron emission tomography and computed tomography scans, calculating the percentage change in total lesion glycolysis between baseline and week 4, and classifying patients with decreased total lesion glycolysis as responders with 94 percent sensitivity, 86 percent specificity, and 92 percent accuracy for primary tumors. For patients with lymph node metastases, assessment of total lesion glycolysis change in the index lymph node identifies responders with 88 percent sensitivity, 100 percent specificity, and 91 percent accuracy.

The invention also provides a method for pathological response assessment comprising obtaining tumor biopsies at week 4, evaluating the proportion of viable tumor cells, and correlating biopsy findings with overall treatment response, wherein pathological response in week 4 biopsies defined as 50 percent or less remaining viable tumor identifies treatment responders with 92 percent sensitivity, 64 percent specificity, and 82 percent accuracy. For patients with lymph node metastases, pathological response assessment of the index lymph node reflects the overall regional lymph node response with 100 percent accuracy.

The present invention provides several significant advantages over existing treatment approaches for cutaneous squamous cell carcinoma. First, the ultra-short neoadjuvant regimen consisting of only two infusions over 4 weeks achieves pathological response rates of 55 percent with nivolumab monotherapy and 80 percent with nivolumab plus ipilimumab, comparable to or exceeding response rates observed with longer 6 to 12 week treatment courses while minimizing the risk of disease progression to irresectable status. Second, the invention demonstrates that patients achieving major pathological response or partial pathological response experience 100 percent disease-specific survival at 24 months without adjuvant immunotherapy, enabling substantial treatment de-escalation compared to current protocols employing up to 48 weeks of adjuvant treatment.

Third, the invention establishes that clinical complete response can be achieved in a subset of patients using only two infusions of checkpoint inhibition without any surgical resection or radiotherapy, with 100 percent of such patients maintaining durable organ preservation and cancer-free status at median follow-up of 34 months. This represents a paradigm shift from the current standard requiring extensive surgery and radiotherapy for all patients regardless of treatment response. Fourth, the safety profile of the ultra-short regimen is favorable, with grade 3 immune-related adverse events occurring in only 12 percent of patients receiving nivolumab and 8 percent receiving nivolumab plus ipilimumab, without any grade 4 or 5 toxicities or surgical delays.

Fifth, the invention provides validated biomarkers for early response assessment, with fluorodeoxyglucose positron emission tomography-based total lesion glycolysis changes demonstrating approximately 90 percent accuracy in identifying responders by week 4, enabling implementation of response-adaptive treatment strategies that spare responders from unnecessary surgical morbidity while ensuring timely definitive treatment for non-responders. Sixth, patients achieving clinical complete response and avoiding surgery demonstrate statistically significant and clinically relevant improvements in role functioning, social functioning, and global quality of life compared to patients undergoing surgery and radiotherapy.

Seventh, the invention demonstrates cost-effectiveness advantages, with patients treated with immunotherapy alone incurring average costs of 28,777 euros and gaining 1.48 quality-adjusted life-years, compared to 52,651 euros and 1.28 quality-adjusted life-years for patients receiving neoadjuvant immunotherapy plus standard of care, resulting in both quality-adjusted life-year gains and cost savings for the immunotherapy-alone approach. Eighth, the invention addresses the specific needs of the elderly, frail patient population affected by cutaneous squamous cell carcinoma, for whom minimizing treatment-related morbidity and healthcare utilization is particularly important given substantial comorbidities and limited life expectancy.

Mode for Carrying Out the Invention

The present invention provides a pharmaceutical composition and comprehensive method for treating cutaneous squamous cell carcinoma that achieves high pathological response rates while enabling treatment de-escalation or organ preservation in responding patients. The invention encompasses detailed protocols for patient selection, treatment administration, response monitoring, biomarker assessment, and implementation of response-adaptive treatment

strategies based on extensive clinical trial data from the MATISSE trial conducted at the Netherlands Cancer Institute Antoni van Leeuwenhoek Hospital and University Medical Center Utrecht.

The pharmaceutical composition of the present invention comprises nivolumab as the primary active ingredient. Nivolumab is a fully human immunoglobulin G4 kappa monoclonal antibody with a molecular weight of approximately 146 kilodaltons that specifically binds to the programmed death-1 receptor expressed on activated T lymphocytes, B lymphocytes, natural killer cells, and myeloid cells. The antibody blocks the interaction between programmed death-1 and its ligands programmed death-ligand 1 and programmed death-ligand 2, thereby preventing the inhibitory signals that suppress T cell activation and proliferation in the tumor microenvironment. Nivolumab is produced by recombinant DNA technology in Chinese hamster ovary cell suspension culture and is formulated as a sterile, preservative-free, clear to opalescent, colorless to pale yellow solution for intravenous infusion.

The nivolumab formulation contains 10 milligrams per milliliter of nivolumab in a solution comprising sodium citrate dihydrate at a concentration of 5.88 milligrams per milliliter, sodium chloride at 5.84 milligrams per milliliter, mannitol at 50 milligrams per milliliter, pentetic acid at 0.05 milligrams per milliliter, polysorbate 80 at 0.5 milligrams per milliliter, sodium hydroxide for pH adjustment, and hydrochloric acid for pH adjustment, with water for injection as the vehicle. The pH of the formulation is maintained at 6.0. The solution is supplied in single-dose vials containing 40 milligrams in 4 milliliters, 100 milligrams in 10 milliliters, or 240 milligrams in 24 milliliters of nivolumab. The vials are stored under refrigeration at 2 to 8 degrees Celsius and protected from light until use.

In the combination therapy embodiment, the pharmaceutical composition further comprises ipilimumab as a second active ingredient. Ipilimumab is a fully human immunoglobulin G1 kappa monoclonal antibody with a molecular weight of approximately 148 kilodaltons that specifically binds to cytotoxic T-lymphocyte-associated protein 4 expressed on the surface of activated T lymphocytes. The antibody blocks the interaction between cytotoxic T-lymphocyte-associated protein 4 and its ligands CD80 and CD86 on antigen-presenting cells, thereby augmenting T cell activation and proliferation. Ipilimumab is produced by recombinant DNA technology in Chinese hamster ovary cell culture and is formulated as a sterile, preservative-free, clear to slightly opalescent, colorless to pale yellow solution for intravenous infusion.

The ipilimumab formulation contains 5 milligrams per milliliter of ipilimumab in a solution comprising tris hydrochloride at 1.21 milligrams per milliliter, sodium chloride at 5.85 milligrams per milliliter, mannitol at 50 milligrams per milliliter, pentetic acid at 0.04 milligrams per milliliter, polysorbate 80 at 0.1 milligrams per milliliter, sodium hydroxide for pH adjustment, and hydrochloric acid for pH adjustment, with water for injection as the vehicle. The pH of the formulation is maintained at 7.0. The solution is supplied in single-dose vials containing 50 milligrams in 10 milliliters or 200 milligrams in 40 milliliters of ipilimumab. The vials are stored under refrigeration at 2 to 8 degrees Celsius and protected from light until use.

For administration, the required dose of nivolumab is calculated based on the patient's body weight at 3 milligrams per kilogram. The calculated volume of nivolumab is withdrawn from the vial using aseptic technique and diluted in either 0.9 percent sodium chloride injection or 5 percent dextrose injection to prepare an infusion solution with a final concentration ranging from 1 to 10 milligrams per milliliter. The diluted solution is mixed by gentle inversion and is stable for up to 24 hours under refrigeration at 2 to 8 degrees Celsius or up to 8 hours at room temperature up to 25 degrees Celsius from the time of preparation to the end of infusion. The infusion solution is administered intravenously over 60 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein-binding in-line filter with a pore size of 0.2 to 1.2 micrometers.

When ipilimumab is co-administered with nivolumab, the required dose of ipilimumab is calculated based on the patient's body weight at 1 milligram per kilogram. The calculated volume of ipilimumab is withdrawn from the vial using aseptic technique and diluted in either 0.9 percent sodium chloride injection or 5 percent dextrose injection to prepare an infusion solution with a final concentration ranging from 1 to 4 milligrams per milliliter. The diluted solution is mixed by gentle inversion and is stable for up to 24 hours under refrigeration at 2 to 8 degrees Celsius or up to 4 hours at room temperature up to 25 degrees Celsius from the time of preparation to the end of infusion. When both antibodies are administered on the same day, ipilimumab is infused first over 90 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein-binding in-line filter with a pore size of 0.2 to 1.2 micrometers, followed by flushing of the line, and then nivolumab is infused over 60 minutes through the same line.

Patient selection for treatment with the pharmaceutical composition involves comprehensive clinical, pathological, and radiological assessment to identify individuals with cutaneous squamous cell carcinoma who are appropriate candidates for neoadjuvant immunotherapy followed by curative-intent surgery with or without adjuvant radiotherapy. Eligible patients include those with histologically confirmed cutaneous squamous cell carcinoma of any anatomical site, staged as American Joint Committee on Cancer Guidelines 8th edition stage I, II, III, or IVa disease, who have an indication for extensive surgical resection that would result in significant functional impairment or disfigurement, or who have multifocal disease requiring multiple surgical procedures.

Specifically, patients with stage III to IVa disease are eligible if they have primary tumors classified as T1, T2, T3, or T4 with regional lymph node involvement classified as N1, N2, or N3, or if they have regional lymph node metastases only without an identifiable primary tumor classified as T0 with N1, N2, or N3 disease. Stage III disease is defined as T1 to T4 tumors with N1 lymph node involvement, or T3 to T4 tumors with N0 lymph nodes but with high-risk features. Stage IVa disease is defined as T1 to T4 tumors with N2 or N3 lymph node involvement. Patients with stage I to II disease are eligible if they have multifocal tumors requiring disfiguring surgery or surgery that would result in significant functional impairment, such as tumors involving the eyelid, nose, ear, or lip where surgical resection would compromise organ function or cosmesis.

The primary tumor must be measurable by clinical examination or radiological imaging, with a minimum diameter of 10 millimeters for primary tumors or lymph node metastases. For patients with lymph node metastases, at least one lymph node must be accessible for ultrasound-guided fine needle aspiration cytology to confirm the presence of metastatic cutaneous squamous cell carcinoma prior to treatment initiation. The tumor must be deemed resectable with curative intent by a multidisciplinary tumor board comprising surgical oncologists, radiation oncologists, medical oncologists, radiologists, and pathologists, with the expectation that complete surgical resection with negative margins can be achieved.

Patients must be at least 18 years of age with no upper age limit, reflecting the predominantly elderly population affected by advanced cutaneous squamous cell carcinoma. The median age of patients in the MATISSE trial was 76 years with a range of 32 to 92 years, demonstrating the feasibility and safety of neoadjuvant immunotherapy in elderly patients. Patients must have a World Health Organization performance status of 0 or 1, indicating that they are fully active or restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature. This performance status requirement ensures that patients are sufficiently fit to undergo the planned surgical resection and potential adjuvant radiotherapy.

Patients must have adequate organ function as demonstrated by laboratory testing within 14 days prior to treatment initiation. Adequate bone marrow function is defined as absolute neutrophil count of 1.5 times 10 to the power of 9 per liter or greater, platelet count of 100 times 10 to the power of 9 per liter or greater, and hemoglobin of 90 grams per liter or greater. Adequate hepatic function is defined as total bilirubin of 1.5 times the upper limit of normal or less, aspartate aminotransferase and alanine aminotransferase of 3 times the upper limit of normal or less, and alkaline phosphatase of 2.5 times the upper limit of normal or less. Adequate renal function is defined as serum creatinine of 1.5 times the upper limit of normal or less or calculated creatinine clearance of 40 milliliters per minute or greater using the Cockcroft-Gault formula.

Patients must have adequate coagulation function as demonstrated by international normalized ratio of 1.5 or less and activated partial thromboplastin time of 1.5 times the upper limit of normal or less, unless the patient is receiving anticoagulation therapy in which case these parameters must be within the therapeutic range of intended use of anticoagulants. Patients must have adequate thyroid function as demonstrated by thyroid-stimulating hormone within normal limits, or if thyroid-stimulating hormone is abnormal, total thyroxine or free thyroxine must be within normal limits. Patients with controlled hypothyroidism on stable thyroid hormone replacement therapy are eligible.

Key exclusion criteria are designed to identify patients at increased risk of severe immune-related adverse events or those for whom neoadjuvant immunotherapy would be inappropriate. Patients with distant metastases classified as M1 disease are excluded, as the treatment intent is curative rather than palliative. Patients with tumors deemed irresectable by the multidisciplinary tumor board are excluded, as the neoadjuvant approach requires the ability to perform definitive surgical resection. Patients who have received prior radiotherapy to the current tumor bed are excluded due to potential alterations in the tumor microenvironment and increased risk of surgical complications. Patients who have received prior systemic therapy for cutaneous squamous cell carcinoma, including chemotherapy, targeted therapy, or immunotherapy, are excluded to ensure a treatment-naive population.

Patients with active autoimmune disease requiring systemic immunosuppressive therapy within the past 2 years are excluded due to the risk of exacerbation of autoimmune conditions with checkpoint inhibitor therapy. Exceptions include patients with vitiligo, resolved childhood asthma or atopy, type 1 diabetes mellitus on stable insulin regimen, hypothyroidism on stable thyroid hormone replacement, psoriasis not requiring systemic therapy, or conditions not expected to recur in the absence of an external trigger. Patients with a history of life-threatening autoimmune disease or requiring systemic corticosteroids at doses exceeding 10 milligrams per day of prednisone equivalent are excluded.

Patients with known human immunodeficiency virus infection are excluded due to potential interactions between antiretroviral therapy and immunotherapy and concerns about immune reconstitution. Patients with active hepatitis B virus infection, defined as positive hepatitis B surface antigen, or active hepatitis C virus infection, defined as detectable hepatitis C virus RNA, are excluded due to the risk of viral reactivation with immunotherapy. Patients with active tuberculosis or other active infections requiring systemic antimicrobial therapy are excluded until the infection is adequately treated and controlled.

Patients with a history of severe hypersensitivity reactions to monoclonal antibodies or any component of the nivolumab or ipilimumab formulations are excluded. Patients with a history of interstitial lung disease or pneumonitis

requiring systemic corticosteroids are excluded due to the risk of immune-related pneumonitis with checkpoint inhibitor therapy. Patients with uncontrolled intercurrent illness including symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness that would limit compliance with study requirements are excluded.

Patients who are pregnant or breastfeeding are excluded due to potential risks to the fetus or nursing infant. Women of childbearing potential must have a negative serum or urine pregnancy test within 24 hours prior to treatment initiation and must agree to use highly effective contraception during treatment and for at least 5 months after the last dose of nivolumab or ipilimumab. Men with female partners of childbearing potential must agree to use effective contraception during treatment and for at least 7 months after the last dose of nivolumab or ipilimumab.

Comprehensive baseline assessment is performed within 28 days prior to treatment initiation to establish disease extent, obtain tissue and blood samples for biomarker analyses, and document baseline health-related quality of life. Clinical examination includes detailed inspection and palpation of the primary tumor site and regional lymph node basins, with measurement of tumor dimensions in three perpendicular planes using calipers. The anatomical location, size, depth of invasion, presence of ulceration, and relationship to critical structures are documented. Regional lymph node basins are systematically examined by palpation to identify clinically enlarged or suspicious lymph nodes.

Clinical photography is performed using standardized protocols with the patient positioned to optimally visualize the tumor and surrounding anatomy. Multiple views are obtained including frontal, lateral, and oblique angles as appropriate for the anatomical location. A ruler or other size reference is included in the photographs to enable accurate measurement. The photographs are obtained using a digital camera with consistent lighting, distance, and exposure settings to enable comparison with on-treatment and post-treatment images.

Fluorodeoxyglucose positron emission tomography and computed tomography imaging is performed using a dedicated positron emission tomography and computed tomography scanner following a standardized protocol. Patients fast for at least 6 hours prior to imaging and blood glucose is confirmed to be less than 200 milligrams per deciliter before fluorodeoxyglucose administration. Fluorodeoxyglucose is administered intravenously at a dose of 3.7 to 5.2 megabecquerels per kilogram body weight, and imaging is performed 60 minutes after injection. The positron emission tomography acquisition covers from the skull base to the mid-thigh with an acquisition time of 2 to 3 minutes per bed position. Low-dose computed tomography is performed for attenuation correction and anatomical localization using 120 kilovolt peak, 50 to 100 milliampere-seconds, 5 millimeter slice thickness, and a pitch of 1.5.

Positron emission tomography images are reconstructed using ordered subset expectation maximization iterative reconstruction with 2 iterations and 8 subsets, with a gaussian filter of 5 millimeters full width at half maximum. Computed tomography images are reconstructed with a slice thickness of 3 millimeters. The images are reviewed on a dedicated workstation by a nuclear medicine physician with expertise in oncological imaging. For each fluorodeoxyglucose-avid lesion corresponding to the primary tumor or lymph node metastases, the maximum standardized uptake value, mean standardized uptake value, metabolic tumor volume, and total lesion glycolysis are measured.

The metabolic tumor volume is defined as the volume of tissue with standardized uptake value equal to or greater than 50 percent of the maximum standardized uptake value within the tumor, calculated using automated segmentation software with manual adjustment as needed to exclude physiological uptake in adjacent normal tissues. The mean standardized uptake value is calculated as the average standardized uptake value of all voxels within the metabolic tumor volume. The total lesion glycolysis is calculated as the product of the metabolic tumor volume and the mean standardized uptake value, providing a measure that incorporates both the volume and metabolic activity of the tumor.

Magnetic resonance imaging of the primary tumor site and regional lymph node basins is performed using a 3 Tesla magnetic resonance imaging scanner with dedicated surface coils appropriate for the anatomical region. The imaging protocol includes T2-weighted sequences with 4 millimeter slice thickness, short tau inversion recovery sequences with 5 millimeter slice thickness for fat suppression and edema detection, T1-weighted sequences with 4 millimeter slice thickness, and post-contrast T1-weighted sequences with 4 millimeter slice thickness with and without fat suppression following intravenous administration of gadoteric acid at a dose of 0.2 milliliters per kilogram body weight.

Diffusion-weighted imaging is performed using both echo-planar imaging sequences with b-values of 0, 200, and 1000 seconds per square millimeter, and split acquisition of fast spin echo signals for diffusion imaging sequences with b-values ranging from 0 to 1000 seconds per square millimeter in 10 steps, each with 4 millimeter slice thickness. Apparent diffusion coefficient maps are generated from the diffusion-weighted images. The images are reviewed by a radiologist with expertise in head and neck imaging, and tumor dimensions are measured in three perpendicular planes on the sequence providing optimal tumor visualization.

Ultrasound examination of regional lymph node basins is performed using a high-frequency linear array transducer with a frequency of 10 to 15 megahertz. Lymph nodes are assessed for size, shape, echogenicity, presence of fatty hilum, and vascularity pattern on color Doppler imaging. Suspicious features include short axis diameter greater than 10 millimeters, round shape with short to long axis ratio greater than 0.5, hypoechoic echotexture, absence of fatty hilum, and peripheral or mixed vascularity pattern. For lymph nodes with suspicious features, ultrasound-guided fine needle aspiration cytology is performed using a 23 to 25 gauge needle with multiple passes to obtain adequate cellular material for cytological examination.

The aspirated material is smeared on glass slides, air-dried, and stained with May-Grünwald-Giemsa stain or fixed in 95 percent ethanol and stained with Papanicolaou stain. The slides are examined by a cytopathologist, and the presence of malignant squamous cells confirms lymph node metastasis. The lymph node with the largest short axis diameter on magnetic resonance imaging or computed tomography imaging is designated as the index lymph node and its location is documented to enable correlation with pathological examination of the surgical specimen.

Tumor biopsies are obtained from the primary tumor and, when present and with additional patient consent, from lymph node metastases. For primary tumors, 4 to 6 core biopsies are obtained using a 14 to 16 gauge core biopsy needle under local anesthesia, targeting the viable tumor periphery while avoiding necrotic or keratinized central areas. For lymph node metastases, ultrasound-guided core biopsies are obtained using a 16 to 18 gauge core biopsy needle. The biopsies are divided for multiple purposes: one to two cores are placed in formalin for routine histopathological examination and confirmation of cutaneous squamous cell carcinoma diagnosis, two to three cores are snap-frozen in liquid nitrogen for whole-exome sequencing and bulk RNA sequencing, and one to two cores are placed in culture medium for ex vivo functional assays.

The formalin-fixed paraffin-embedded tissue sections are stained with hematoxylin and eosin and examined by a pathologist to confirm the diagnosis of cutaneous squamous cell carcinoma and assess histological features including degree of differentiation, presence of perineural invasion, lymphovascular invasion, and depth of invasion. Immunohistochemical staining is performed for programmed death-ligand 1 using the 28-8 pharmDx assay on the Dako Autostainer Link 48 platform, with results reported as the percentage of tumor cells with membranous staining at any intensity. Additional immunohistochemical markers may be performed as needed to confirm squamous differentiation or exclude other diagnoses.

Blood samples are collected by venipuncture into multiple tubes for different analyses. Whole blood is collected in ethylenediaminetetraacetic acid tubes for complete blood count, differential white blood cell count, and isolation of peripheral blood mononuclear cells. Serum is collected in serum separator tubes for chemistry panel including electrolytes, renal function tests, liver function tests, and lactate dehydrogenase. Plasma is collected in heparin tubes for cytokine and chemokine analyses. Peripheral blood mononuclear cells are isolated by density gradient centrifugation using Ficoll-Paque Plus, with cells counted and viability assessed by trypan blue exclusion. Cells are cryopreserved in freezing medium containing 90 percent fetal bovine serum and 10 percent dimethyl sulfoxide and stored in liquid nitrogen for subsequent analyses.

Health-related quality of life is assessed using validated questionnaires administered in the patient's native language. The European Organisation for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30 version 3.0 is a 30-item questionnaire assessing global health status, functional scales including physical functioning, role functioning, emotional functioning, cognitive functioning, and social functioning, and symptom scales including fatigue, nausea and vomiting, pain, dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial difficulties. Responses are recorded on a 4-point Likert scale from 1 indicating not at all to 4 indicating very much, except for the global health status questions which use a 7-point scale from 1 indicating very poor to 7 indicating excellent.

The EuroQol-5 Dimension 5-Level questionnaire assesses health status across five dimensions: mobility, self-care, usual activities, pain or discomfort, and anxiety or depression. Each dimension has five levels: no problems, slight problems, moderate problems, severe problems, and extreme problems or unable to perform. Patients also rate their overall health on a visual analog scale from 0 indicating the worst health imaginable to 100 indicating the best health imaginable. The questionnaires are self-administered by patients in a quiet area of the clinic, with assistance from research staff available if needed.

Tumor staging is performed according to the American Joint Committee on Cancer Guidelines 8th edition for cutaneous squamous cell carcinoma of the head and neck. The primary tumor is classified as T1 if the tumor is 2 centimeters or less in greatest dimension, T2 if greater than 2 centimeters but not more than 4 centimeters, T3 if greater than 4 centimeters or with minor bone erosion or perineural invasion or deep invasion, or T4 if with gross cortical bone or marrow invasion or skull base invasion or skull base foramen invasion. Regional lymph nodes are classified as N0 if no regional lymph node metastasis, N1 if a single ipsilateral lymph node 3 centimeters or less in greatest dimension and extranodal extension negative, N2a if a single ipsilateral lymph node 3 centimeters or less in greatest dimension and extranodal extension positive or a single ipsilateral lymph node greater than 3 centimeters but not more than 6 centimeters in greatest dimension and extranodal extension negative, N2b if multiple ipsilateral lymph nodes not more than 6 centimeters in greatest dimension and extranodal extension negative, N2c if bilateral or contralateral lymph nodes not more than 6 centimeters in greatest dimension and extranodal extension negative, N3a if any lymph node greater than 6 centimeters in greatest dimension and extranodal extension negative, or N3b if any lymph node and extranodal extension positive.

The overall stage is determined by combining the T and N classifications: stage I is T1 N0, stage II is T2 N0, stage III is T3 N0 or T1 to T3 N1, and stage IVa is T1 to T3 N2 or any T N3 or T4 any N. Patients with distant metastases classified as M1 are stage IVb and are excluded from the study. The clinical tumor-node-metastasis classification is documented based on clinical examination and imaging findings prior to treatment initiation.

Treatment is initiated within 7 days of completion of baseline assessments. On day 0, which is designated as week 0, patients receive the first infusion of nivolumab. For patients randomized to the nivolumab monotherapy arm, nivolumab is administered at a dose of 3 milligrams per kilogram body weight intravenously over 60 minutes. The patient's body weight is measured on the day of infusion using a calibrated scale, and the dose is calculated by multiplying the body weight in kilograms by 3 milligrams per kilogram and rounding to the nearest milligram. The calculated dose is prepared by the pharmacy according to the dilution protocol described previously, and the infusion bag is labeled with the patient's name, medical record number, drug name, dose, volume, infusion rate, and expiration date and time.

Prior to infusion, baseline vital signs including blood pressure, heart rate, respiratory rate, temperature, and oxygen saturation are measured and documented. An intravenous catheter is inserted, typically in a peripheral vein of the forearm or hand, using aseptic technique. The infusion line is primed with the nivolumab solution, and the in-line filter is positioned between the infusion bag and the patient. The infusion is initiated at a rate calculated to deliver the total volume over 60 minutes using an infusion pump programmed with the appropriate rate in milliliters per hour.

During the infusion, the patient is monitored for signs and symptoms of infusion reactions including fever, chills, rigors, pruritus, rash, hypotension, hypertension, dyspnea, wheezing, bronchospasm, or angioedema. Vital signs are measured at 15 minutes after infusion start, at 30 minutes, at 45 minutes, at the end of infusion, and at 30 minutes after infusion completion. If an infusion reaction occurs, the infusion is interrupted and the patient is assessed. For mild to moderate reactions, the infusion may be resumed at a slower rate after symptoms resolve. For severe reactions, the infusion is permanently discontinued and appropriate medical management is provided including antihistamines, corticosteroids, bronchodilators, or epinephrine as clinically indicated.

After completion of the nivolumab infusion, the intravenous line is flushed with 0.9 percent sodium chloride injection to ensure delivery of the complete dose. The intravenous catheter is removed and pressure is applied to the insertion site until hemostasis is achieved. The patient remains in the infusion area for at least 30 minutes of observation after infusion completion to monitor for delayed reactions. If no concerning symptoms develop, the patient is discharged with instructions to report any new or worsening symptoms including fever, rash, diarrhea, abdominal pain, dyspnea, cough, or other concerning symptoms.

For patients randomized to the combination therapy arm, ipilimumab is administered in addition to nivolumab on day 0. The ipilimumab dose is calculated at 1 milligram per kilogram body weight based on the same body weight measurement used for nivolumab dose calculation. The ipilimumab infusion is prepared by the pharmacy according to the dilution protocol described previously. The ipilimumab infusion is administered first, over 90 minutes, using the same intravenous access and monitoring protocol described for nivolumab. After completion of the ipilimumab infusion, the intravenous line is flushed with at least 50 milliliters of 0.9 percent sodium chloride injection or 5 percent dextrose injection to clear the line of ipilimumab.

The nivolumab infusion is then administered over 60 minutes through the same intravenous line, with the same monitoring protocol. The total time for both infusions including line flushing is approximately 3 hours. After completion of both infusions, the patient remains under observation for at least 30 minutes before discharge. Patients are provided with written information about potential immune-related adverse events and instructions to contact the treating physician immediately if concerning symptoms develop.

On day 14, which is designated as week 2, patients return for the second infusion of nivolumab. Prior to infusion, an interim history is obtained to assess for any adverse events since the first infusion, and a focused physical examination is performed. Blood samples are collected for safety laboratories including complete blood count, comprehensive metabolic panel, and thyroid function tests. A tumor biopsy is obtained from the primary tumor using the same technique as the baseline biopsy, targeting the same anatomical location to enable comparison of sequential samples. The biopsy is processed for whole-exome sequencing and bulk RNA sequencing as described for the baseline biopsy.

The patient's body weight is measured, and the nivolumab dose is calculated at 3 milligrams per kilogram. The infusion is prepared and administered using the same protocol as the first infusion. Patients randomized to the combination therapy arm receive only nivolumab at week 2, not ipilimumab, as the protocol specifies ipilimumab administration only at week 0. After completion of the infusion and observation period, the patient is discharged with instructions to return for week 4 assessments and surgery.

Between week 2 and week 4, patients are contacted by telephone at week 3 to assess for adverse events and ensure that any concerning symptoms are promptly evaluated and managed. Patients are instructed to report any new or worsening symptoms immediately rather than waiting for the scheduled week 4 visit. If grade 3 or 4 immune-related adverse events occur, treatment with high-dose corticosteroids is initiated according to established management algorithms, and surgery may be delayed if necessary to allow resolution of the adverse event.

On day 28, which is designated as week 4, patients return for comprehensive on-treatment assessment prior to surgery. An interval history is obtained focusing on any symptoms that may represent immune-related adverse events, including dermatological symptoms such as rash, pruritus, or blistering; gastrointestinal symptoms such as diarrhea, abdominal pain, or blood in stool; pulmonary symptoms such as dyspnea, cough, or chest pain; endocrine symptoms such as fatigue, headache, or visual changes; hepatic symptoms such as jaundice, dark urine, or right upper quadrant pain; musculoskeletal symptoms such as myalgia, arthralgia, or muscle weakness; neurological symptoms such as numbness, tingling, or weakness; and constitutional symptoms such as fever, chills, or unintentional weight loss.

A complete physical examination is performed including vital signs, performance status assessment, examination of the skin for rash or other dermatological manifestations, examination of the primary tumor site and regional lymph node basins, and examination of other organ systems as clinically indicated. The primary tumor dimensions are measured in three perpendicular planes using calipers and compared to baseline measurements to assess clinical response. Clinical photography is performed using the same standardized protocol as baseline to document on-treatment tumor appearance.

Blood samples are collected for comprehensive safety laboratories including complete blood count with differential, comprehensive metabolic panel including electrolytes, blood urea nitrogen, creatinine, glucose, calcium, total protein, albumin, total bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, thyroid function tests including thyroid-stimulating hormone and free thyroxine, lipase, amylase, and C-reactive protein. Additional tests are performed as clinically indicated based on symptoms, such as cortisol and adrenocorticotropic hormone for suspected adrenal insufficiency, or creatine kinase for suspected myositis. Peripheral blood mononuclear cells are isolated and cryopreserved for immunological analyses.

Fluorodeoxyglucose positron emission tomography and computed tomography imaging is repeated using the same protocol and scanner as the baseline scan to enable direct comparison. The scan is performed at a median of 3 days before surgery with a range of 0 to 9 days. The same nuclear medicine physician who interpreted the baseline scan reviews the on-treatment scan and measures the maximum standardized uptake value, mean standardized uptake value, metabolic tumor volume, and total lesion glycolysis for the primary tumor and each lymph node metastasis. The percentage change in total lesion glycolysis is calculated for each lesion by subtracting the baseline total lesion glycolysis from the week 4 total lesion glycolysis, dividing by the baseline total lesion glycolysis, and multiplying by 100 percent.

A decrease in total lesion glycolysis, defined as a negative percentage change, indicates metabolic response to treatment. An increase in total lesion glycolysis, defined as a positive percentage change, indicates metabolic progression or pseudo-progression. Pseudo-progression is characterized by initial increase in metabolic activity or tumor size due to immune cell infiltration, followed by subsequent decrease and eventual response. Pseudo-progression is distinguished from true progression by continued clinical improvement, subsequent imaging showing response, and pathological examination showing treatment effect with immune cell infiltration rather than viable tumor.

Magnetic resonance imaging is repeated using the same protocol and scanner as the baseline scan. The same radiologist who interpreted the baseline scan reviews the on-treatment scan and measures tumor dimensions in three perpendicular planes. Response is assessed according to Response Evaluation Criteria in Solid Tumors version 1.1 modified for magnetic resonance imaging. Complete response is defined as disappearance of all target lesions with any pathological lymph nodes reduced to less than 10 millimeters in short axis. Partial response is defined as at least 30 percent decrease in the sum of diameters of target lesions. Progressive disease is defined as at least 20 percent increase in the sum of diameters of target lesions with an absolute increase of at least 5 millimeters, or appearance of new lesions. Stable disease is defined as neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease.

Ultrasound examination of regional lymph node basins is repeated, and ultrasound-guided fine needle aspiration cytology is performed on the index lymph node or other suspicious lymph nodes to assess for persistent metastatic disease. The cytological findings are compared to baseline to determine whether viable tumor cells are still present or whether the lymph node shows only inflammatory changes or necrosis without viable tumor.

Tumor biopsies are obtained from the primary tumor and, when feasible, from the index lymph node. For patients proceeding to surgery under general anesthesia, the biopsies are obtained in the operating room immediately before surgical resection. For patients who opt to forgo surgery, biopsies are obtained under local anesthesia in the outpatient setting. The biopsies are processed for formalin fixation and paraffin embedding, with hematoxylin and eosin-stained sections examined by a pathologist to assess the proportion of viable tumor cells, extent of treatment-related changes including fibrosis, necrosis, inflammation, and presence of multinucleated giant cells.

The pathological response in the week 4 biopsy is classified using the same criteria as the surgical specimen: major pathological response is defined as 10 percent or less viable tumor cells, partial pathological response is defined as

greater than 10 percent but 50 percent or less viable tumor cells, and no pathological response is defined as greater than 50 percent viable tumor cells. The pathological response in the biopsy is compared to the pathological response in the subsequent surgical specimen to assess the accuracy of biopsy-based response assessment. Additional biopsy cores are snap-frozen for whole-exome sequencing and bulk RNA sequencing to assess on-treatment changes in the tumor genome and transcriptome.

Health-related quality of life questionnaires are administered using the same instruments as baseline. Patients complete the European Organisation for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30 and the EuroQol-5 Dimension 5-Level questionnaire, with scores calculated and compared to baseline to assess changes in quality of life during neoadjuvant treatment. Particular attention is paid to functional scales including physical functioning, role functioning, and social functioning, as well as symptom scales including fatigue, pain, and dyspnea.

Based on the comprehensive week 4 assessment, patients are counseled regarding treatment options. Patients with clinical complete response, defined as no evidence of disease on physical examination, imaging, and biopsy, are informed that they have the option to proceed with standard of care surgery and radiotherapy as originally planned, or to defer surgery and undergo close surveillance with the possibility of achieving durable organ preservation. Patients with clinical partial response or stable disease are counseled that surgery remains the recommended treatment, but that the neoadjuvant immunotherapy may have reduced the extent of surgery required or eliminated the need for adjuvant radiotherapy depending on the pathological response.

Patients with progressive disease are counseled that surgery should proceed as planned and that additional systemic therapy may be considered in the adjuvant setting depending on pathological findings. The decision regarding whether to proceed with surgery is made through shared decision-making between the patient and the multidisciplinary treatment team, taking into account the patient's values, preferences, and goals of care, as well as the clinical, radiological, and pathological response to neoadjuvant treatment.

For patients who elect to proceed with surgery, the procedure is performed at a median of 28 days after treatment initiation with a range of 15 to 34 days. The surgical approach and extent of resection are determined by the multidisciplinary tumor board based on the location and extent of disease, taking into account the response to neoadjuvant treatment. The goal of surgery is complete resection of all gross disease with negative microscopic margins, defined as no tumor cells at the inked margin of the specimen.

For primary tumors of the head and neck region, which account for 90 percent of cases in the MATISSE trial, surgical approaches include wide local excision with primary closure or reconstruction using local flaps, regional flaps, or free tissue transfer depending on the size and location of the defect. For tumors involving critical structures such as the orbit, skull base, or major vessels, the surgical plan may include resection of these structures if necessary to achieve negative margins, with appropriate reconstruction. For tumors of other body sites including the trunk and extremities, wide local excision is performed with margins of 5 to 10 millimeters depending on tumor characteristics and anatomical constraints.

For patients with clinically node-positive disease at baseline, regional lymph node dissection is performed even if the lymph nodes are no longer palpable or visible on imaging after neoadjuvant treatment, as microscopic disease may persist despite clinical response. For head and neck tumors, this typically involves selective neck dissection of levels I through V, or modified radical neck dissection if there is extensive nodal involvement. The index lymph node is identified in the surgical specimen based on anatomical location documented on pre-treatment imaging and marked with a suture or clip to enable correlation with pathological response assessment.

The surgical specimen is oriented by the surgeon using sutures or clips to indicate anatomical landmarks and margins, and is immediately transported to the pathology department. The specimen is measured in three dimensions and weighed. The external surface is inked with different colors to identify different margins. The specimen is serially sectioned at 3 to 5 millimeter intervals perpendicular to the long axis. Each section is examined grossly to identify residual tumor, which appears as firm, white to tan tissue, distinct from surrounding fibrosis, necrosis, or normal tissue.

Representative sections are submitted for microscopic examination, including sections from the tumor bed showing the interface between tumor and surrounding tissue, sections from all margins, and sections from any grossly identifiable residual tumor. For lymph node dissection specimens, all lymph nodes are identified by careful palpation and dissection of the fibroadipose tissue, measured, and submitted in their entirety for microscopic examination. The index lymph node is submitted separately and labeled to enable correlation with imaging findings.

The tissue sections are processed using standard histological techniques including fixation in 10 percent neutral buffered formalin for 12 to 24 hours, dehydration through graded alcohols, clearing in xylene, infiltration with paraffin, embedding in paraffin blocks, sectioning at 4 micrometers thickness using a microtome, mounting on glass slides, and staining with hematoxylin and eosin. The slides are examined by an experienced head and neck pathologist who is blinded to the treatment arm but aware that the patient received neoadjuvant immunotherapy.

The pathological response is assessed according to the International Neoadjuvant Melanoma Consortium scoring system proposed by Tetzlaff and colleagues in 2018. For the primary tumor, the tumor bed is defined as the area that contains viable tumor cells, necrosis, keratinous debris, fibrosis, granulation tissue, multinucleated giant cells, and other features indicating the pre-treatment extent of tumor. The tumor bed is outlined on the slide, and the area is measured using digital pathology software or a calibrated ocular micrometer. Within the tumor bed, the areas containing viable tumor cells are outlined and measured separately.

The percentage of viable tumor is calculated by dividing the total area of viable tumor by the total area of the tumor bed and multiplying by 100 percent. Viable tumor cells are defined as malignant squamous cells with intact nuclei and cytoplasm, excluding areas of necrosis, keratinization without viable cells, and inflammatory infiltrate. Treatment-related changes are documented including extent of fibrosis, necrosis, inflammation, granulation tissue, multinucleated giant cells, hemosiderin-laden macrophages, and cholesterol clefts.

Pathological complete response is defined as 0 percent viable tumor, meaning complete absence of viable tumor cells in the entire tumor bed. Near pathological complete response is defined as greater than 0 percent but 10 percent or less viable tumor. Major pathological response is defined as 10 percent or less viable tumor, encompassing both pathological complete response and near pathological complete response. Partial pathological response is defined as greater than 10 percent but 50 percent or less viable tumor. No pathological response is defined as greater than 50 percent viable tumor.

For lymph node metastases, each lymph node is examined separately. The presence or absence of metastatic carcinoma is documented. For lymph nodes containing metastatic carcinoma, the percentage of viable tumor is calculated by estimating the proportion of the lymph node replaced by viable tumor cells versus treatment-related changes including necrosis, fibrosis, and inflammation. The pathological response category is assigned to each lymph node using the same criteria as the primary tumor. For patients with multiple lymph node metastases, the pathological responses of all lymph nodes are averaged to determine the overall nodal response.

The pathological response of the index lymph node is compared to the averaged response of all lymph nodes to assess whether the index lymph node is representative of the overall nodal response. In the MATISSE trial, the pathological response of the index lymph node reflected the averaged lymph node response with 100 percent accuracy in 11 patients with lymph node metastases, supporting the concept that assessment of the index lymph node alone may be sufficient to determine overall regional response.

Margin status is assessed by examining the relationship between residual tumor and the inked margins. Margins are classified as negative if no tumor cells are present at the inked margin, close if tumor cells are present within 5 millimeters of the margin but not at the margin, or positive if tumor cells are present at the inked margin. The distance from tumor to the closest margin is measured in millimeters. For patients with positive margins, re-excision is considered if anatomically feasible.

Pathological staging is performed according to the American Joint Committee on Cancer Guidelines 8th edition based on the findings in the surgical specimen. The pathological T classification is based on the size and extent of residual tumor, if present, or on the size of the tumor bed if no residual tumor is present. The pathological N classification is based on the number, size, and laterality of lymph nodes containing viable metastatic carcinoma, and the presence or absence of extranodal extension. Pathological down-staging is defined as a lower pathological stage compared to the clinical stage assigned at baseline.

Immunohistochemical staining is performed on selected cases to further characterize the tumor microenvironment and treatment response. Programmed death-ligand 1 staining is performed on pre-treatment and post-treatment specimens to assess changes in expression. CD3, CD8, and CD4 staining is performed to quantify T lymphocyte infiltration. CD68 and CD163 staining is performed to assess macrophage infiltration. Cytokeratin staining is performed to highlight residual tumor cells and facilitate accurate quantification of viable tumor percentage.

The pathology report includes a detailed description of the gross and microscopic findings, the pathological response category, the percentage of viable tumor, the extent and character of treatment-related changes, the margin status, the pathological tumor-node-metastasis classification, and the pathological stage. The report is reviewed at a multidisciplinary tumor board meeting attended by surgeons, medical oncologists, radiation oncologists, radiologists, and pathologists to determine the need for adjuvant therapy.

The decision regarding adjuvant radiotherapy is made according to national and institutional guidelines based on pathological risk factors. In the MATISSE trial, 53 percent of patients had an upfront indication for adjuvant radiotherapy based on clinical stage and tumor characteristics. Indications for adjuvant radiotherapy include pathological T3 or T4 primary tumors, close or positive margins, perineural invasion, lymphovascular invasion, poorly differentiated histology, pathological N2 or N3 nodal disease, extranodal extension, or multiple positive lymph nodes.

However, the pathological response to neoadjuvant immunotherapy is taken into account when making adjuvant radiotherapy decisions. Patients achieving pathological complete response with negative margins may have adjuvant

radiotherapy omitted even if they had an upfront indication based on clinical staging, particularly if they had pathological down-staging from clinical stage III or IVa to pathological stage 0 or I. In the MATISSE trial, adjuvant radiotherapy was omitted in 5 patients with pathological complete response despite upfront indications.

For patients who receive adjuvant radiotherapy, treatment is initiated 4 to 8 weeks after surgery to allow adequate wound healing. Radiotherapy planning includes clinical examination, review of pre-treatment and post-treatment imaging, review of the pathology report, and consultation with the surgeon to define the tumor bed and areas at risk for microscopic disease. A planning computed tomography scan is performed with the patient immobilized in the treatment position using a thermoplastic mask for head and neck cases or other immobilization devices for other anatomical sites.

The gross tumor volume is defined as any gross residual disease visible on post-operative imaging, which is typically absent in patients who underwent complete resection. The clinical target volume is defined as the tumor bed plus a margin to account for microscopic disease, typically 5 to 10 millimeters, and includes the regional lymph node basins at risk. The planning target volume is defined as the clinical target volume plus a margin to account for setup uncertainty and organ motion, typically 3 to 5 millimeters.

Radiotherapy is delivered using intensity-modulated radiation therapy or volumetric modulated arc therapy techniques to achieve conformal dose distribution and spare adjacent normal tissues. The prescription dose is typically 60 to 66 Gray to the tumor bed delivered in 30 to 33 fractions of 2 Gray per fraction over 6 to 6.5 weeks for patients with negative margins, or 66 to 70 Gray in 33 to 35 fractions for patients with positive margins. Regional lymph node basins receive 50 to 54 Gray in 25 to 27 fractions if not dissected, or 54 to 60 Gray if dissected and containing metastatic disease.

Dose constraints for organs at risk are applied according to institutional protocols to limit toxicity. For head and neck radiotherapy, constraints include mean dose to the parotid glands less than 26 Gray to reduce risk of xerostomia, maximum dose to the spinal cord less than 45 Gray to prevent myelopathy, maximum dose to the brainstem less than 54 Gray, mean dose to the oral cavity less than 40 Gray to reduce mucositis, and maximum dose to the mandible less than 70 Gray to reduce risk of osteoradionecrosis.

Patients are seen weekly during radiotherapy for on-treatment assessment including evaluation of acute toxicities, supportive care, and encouragement of treatment compliance. Common acute toxicities include radiation dermatitis, mucositis, dysphagia, odynophagia, xerostomia, dysgeusia, fatigue, and weight loss. Toxicities are graded according to Common Terminology Criteria for Adverse Events version 5.0 and managed with supportive measures including topical corticosteroids for dermatitis, viscous lidocaine and opioid analgesics for mucositis and odynophagia, artificial saliva for xerostomia, nutritional supplementation, and antiemetics as needed.

After completion of all treatment including surgery and adjuvant radiotherapy if administered, patients enter a structured follow-up program to monitor for disease recurrence, late treatment-related toxicities, and health-related quality of life. Follow-up visits are scheduled at 3-month intervals for the first 2 years, then at 6-month intervals for years 3 through 5, then annually thereafter. At each follow-up visit, an interval history is obtained focusing on symptoms that may indicate disease recurrence such as new lumps, pain, bleeding, or functional impairment, as well as symptoms of late immune-related adverse events or radiotherapy toxicities.

A complete physical examination is performed including examination of the primary tumor site for local recurrence, palpation of regional lymph node basins for nodal recurrence, and examination of distant sites for metastatic disease. Clinical photography is performed at each visit to document the appearance of the surgical site and enable comparison over time. Blood tests are performed including complete blood count, comprehensive metabolic panel, and thyroid function tests to monitor for late immune-related adverse events such as hypothyroidism or adrenal insufficiency.

Imaging surveillance is performed according to institutional protocols based on risk of recurrence. For patients at high risk of recurrence, such as those with no pathological response or partial pathological response, imaging is performed every 3 to 6 months for the first 2 years. For patients at low risk of recurrence, such as those with major pathological response, imaging may be performed less frequently or only if clinically indicated. Imaging modalities include computed tomography of the primary site and regional lymph node basins, or fluorodeoxyglucose positron emission tomography and computed tomography if there is clinical suspicion of recurrence.

Health-related quality of life questionnaires are administered at each follow-up visit using the same instruments as during treatment. The European Organisation for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30 and EuroQol-5 Dimension 5-Level questionnaire are completed, with scores calculated and tracked over time to assess long-term quality of life outcomes. Particular attention is paid to functional outcomes such as ability to eat, speak, and perform activities of daily living, as well as cosmetic outcomes and patient satisfaction with treatment.

Disease recurrence is classified as local recurrence if it occurs at or near the primary tumor site, regional recurrence if it occurs in regional lymph node basins, or distant recurrence if it occurs at distant sites. Biopsy confirmation of recurrence is obtained whenever feasible. Treatment of recurrence is determined by the multidisciplinary tumor board and may include surgical resection, radiotherapy, systemic therapy with checkpoint inhibitors or other agents, or palliative care depending on the extent and location of recurrence and the patient's performance status and preferences.

Survival outcomes are calculated from defined timepoints. Overall survival is calculated from the date of randomization to the date of death from any cause, with patients who are alive censored at the date of last contact. Disease-specific survival is calculated from the date of randomization to the date of death from cutaneous squamous cell carcinoma, with patients who die from other causes or who are alive censored at the date of death or last contact. Recurrence-free survival is calculated from the last day of treatment, defined as the date of surgery or last dose of radiotherapy or immunotherapy, to the date of disease recurrence or death from any cause, with patients who are alive without recurrence censored at the date of last contact.

In the MATISSE trial, patients achieving major pathological response or partial pathological response demonstrated 100 percent disease-specific survival at 24 months, indicating that no patients died from cutaneous squamous cell carcinoma during this follow-up period. The 24-month overall survival was 100 percent for patients with major pathological response and 88 percent for patients with partial pathological response, with deaths in the partial pathological response group due to non-cancer causes including interstitial lung disease from amiodarone use and traumatic injury from a fall, both unrelated to the cancer or its treatment.

Patients who achieved clinical complete response without undergoing surgery demonstrated 100 percent disease-specific survival, recurrence-free survival, and overall survival at 24 months, with a median follow-up of 34 months. All 9 patients with clinical complete response remained cancer-free without any disease recurrence, supporting the feasibility of organ preservation in carefully selected patients who achieve complete response to ultra-short neoadjuvant immunotherapy.

This comprehensive method for carrying out the invention demonstrates that ultra-short neoadjuvant immunotherapy with nivolumab or nivolumab plus ipilimumab achieves high pathological response rates in patients with resectable cutaneous squamous cell carcinoma, enables treatment de-escalation in responding patients, and supports organ preservation strategies in patients achieving clinical complete response, all while maintaining excellent long-term survival outcomes and favorable health-related quality of life.

Industrial Applicability

The present invention is industrially applicable in the pharmaceutical and medical fields for the treatment of cutaneous squamous cell carcinoma. The pharmaceutical composition comprising nivolumab with or without ipilimumab can be manufactured using standard pharmaceutical production methods and formulated for intravenous administration according to established protocols. The treatment method can be implemented in oncology centers and hospitals equipped to administer intravenous checkpoint inhibitors and perform surgical resection of cutaneous squamous cell carcinoma.

The invention provides particular industrial applicability for pharmaceutical companies developing and marketing immune checkpoint inhibitors for cancer treatment. The ultra-short neoadjuvant regimen consisting of only two infusions over 4 weeks represents an efficient use of expensive checkpoint inhibitor drugs while achieving pathological response rates comparable to or exceeding those observed with longer treatment courses. The ability to identify responders early using fluorodeoxyglucose positron emission tomography-based total lesion glycolysis changes and pathological assessment of week 4 tumor biopsies enables implementation of response-adaptive treatment strategies that optimize resource utilization.

The invention further provides industrial applicability for healthcare systems seeking to improve cost-effectiveness of cancer care. The demonstrated quality-adjusted life-year gains and cost savings associated with immunotherapy-alone approaches in patients achieving clinical complete response, compared to standard combinations of neoadjuvant immunotherapy plus surgery and radiotherapy, support adoption of response-adaptive treatment de-escalation strategies. The reduction in healthcare utilization, surgical complications, and radiotherapy-related toxicities in responding patients provides additional economic and quality-of-life benefits.

The invention additionally provides industrial applicability for diagnostic companies developing and marketing fluorodeoxyglucose positron emission tomography and computed tomography imaging systems and analysis software. The validated use of total lesion glycolysis changes as an early biomarker for treatment response creates demand for standardized imaging protocols and quantitative analysis tools to support implementation of response-adaptive treatment strategies in clinical practice. The high sensitivity, specificity, and accuracy of total lesion glycolysis-based response assessment support its adoption as a standard biomarker for guiding treatment decisions.

The invention is readily scalable for widespread clinical implementation, as nivolumab and ipilimumab are already approved and commercially available for treatment of various cancers, and the required imaging and pathological assessment methods are standard procedures available in most comprehensive cancer centers. The treatment protocol is straightforward to implement, requiring only two infusions over 4 weeks followed by standard surgical resection in week 4, with response assessment using widely available fluorodeoxyglucose positron

emission tomography and computed tomography imaging and routine pathological examination of surgical specimens or tumor biopsies.

Theoretical Basis of the Present Invention

The present invention is based on several theoretical frameworks that can be expressed mathematically to describe the mechanisms of action, response prediction, and treatment optimization.

Immune Checkpoint Blockade Efficacy Model

The efficacy of immune checkpoint blockade can be modeled as:

$$E_{ICB} = \alpha \cdot TMB \cdot \frac{IFN\gamma}{1 + \beta \cdot T_{reg}} \cdot (1 - e^{-k \cdot [Ab]})$$

where E_{ICB} represents the immune checkpoint blockade efficacy measured as the probability of achieving major pathological response, α is a proportionality constant representing baseline immune competence of the patient, TMB is the tumor mutational burden measured in mutations per megabase, IFNγ is the interferon-gamma signature score representing pre-existing anti-tumor immunity, β is a coefficient representing the immunosuppressive effect, T_{reg} is the regulatory T cell infiltration score, k is the binding affinity constant for antibody-receptor interaction, and [Ab] is the concentration of checkpoint inhibitor antibody at the tumor site.

Synergistic Combination Therapy Model

The synergistic effect of combining nivolumab and ipilimumab can be expressed as:

$$E_{combo} = E_{NIVO} + E_{IPI} + \gamma \cdot E_{NIVO} \cdot E_{IPI}$$

where E_{combo} represents the combined efficacy of nivolumab plus ipilimumab, E_{NIVO} is the efficacy of nivolumab monotherapy targeting programmed death-1, E_{IPI} is the efficacy of ipilimumab monotherapy targeting cytotoxic T-lymphocyte-associated protein 4, and γ is the synergy coefficient representing the additional benefit from simultaneous blockade of both checkpoints beyond additive effects.

Pathological Response Quantification

The pathological response is quantified as:

$$PR = \left(1 - \frac{V_{viable}}{V_{tumor_{bed}}}\right) \times 100\,\%$$

where PR represents the pathological response percentage, V_{viable} is the volume or area of viable tumor cells remaining in the surgical specimen, and V $_{tumor_{bed}}$ is the total volume or area of the tumor bed including viable tumor, necrosis, fibrosis, and immune infiltrate.

Response Classification Function

The classification of pathological response categories can be expressed as:

$$R_{category} = \begin{cases} PCR & \text{if } PR = 100\,\% \\ MPR & \text{if } 90\,\% \leq PR < 100\,\% \\ PPR & \text{if } 50\,\% < PR < 90\,\% \\ NPR & \text{if } PR \leq 50\,\% \end{cases}$$

where $R_{category}$ represents the response category, PCR is pathological complete response, MPR is major pathological response, PPR is partial pathological response, and NPR is no pathological response.

Metabolic Response Assessment

The change in total lesion glycolysis as a biomarker for response is calculated as:

$$\Delta TLG_{50\%} = \frac{TLG_{week4} - TLG_{baseline}}{TLG_{baseline}} \times 100\,\%$$

where $\Delta TLG_{50\%}$ represents the percentage change in total lesion glycolysis, TLG_{week4} is the total lesion glycolysis measured at week 4 before surgery, and $TLG_{baseline}$ is the total lesion glycolysis measured at baseline before treatment initiation.

The total lesion glycolysis itself is defined as:

$$TLG_{50\%} = MTV_{50\%} \times SUV_{mean}$$

where $MTV_{50\%}$ is the metabolic tumor volume defined as the volume of voxels with standardized uptake value equal to or greater than 50 percent of the maximum standardized uptake value, and SUV_{mean} is the mean standardized uptake value of all voxels within the metabolic tumor volume.

Predictive Accuracy Metrics

The accuracy of fluorodeoxyglucose positron emission tomography-based response prediction is expressed as:

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN}$$

$$Sensitivity = \frac{TP}{TP + FN}$$

$$Specificity = \frac{TN}{TN + FP}$$

where TP represents true positives defined as patients with decreased total lesion glycolysis who achieved major pathological response or partial pathological response, TN represents true negatives defined as patients with increased total lesion glycolysis who had no pathological response, FP represents false positives defined as patients with decreased total lesion glycolysis who had no pathological response, and FN represents false negatives defined as patients with increased total lesion glycolysis who achieved major pathological response or partial pathological response.

Survival Probability Function

The disease-specific survival probability can be modeled using the Kaplan-Meier estimator:

$$S(t) = \prod_{t_i \leq t} \left(1 - \frac{d_i}{n_i}\right)$$

where S(t) represents the survival probability at time t, t_i represents the time of each event, d_i is the number of disease-specific deaths at time t_i, and n_i is the number of patients at risk just before time t_i.

Quality-Adjusted Life Years Calculation

The quality-adjusted life years gained are calculated as:

$$QALY = \sum_{i=1}^{n} U_i \times T_i$$

where QALY represents the total quality-adjusted life years, U_i is the utility score derived from EuroQol-5 Dimension 5-Level questionnaire at time interval i ranging from 0 for death to 1 for perfect health, T_i is the duration of time interval i in years, and n is the number of time intervals over the evaluation period.

Cost-Effectiveness Ratio

The incremental cost-effectiveness ratio comparing treatment strategies is expressed as:

$$ICER = \frac{C_{IT+SOC} - C_{IT}}{QALY_{IT+SOC} - QALY_{IT}}$$

where ICER represents the incremental cost-effectiveness ratio in euros per quality-adjusted life year, C_{IT+SOC} is the total healthcare cost for patients receiving immunotherapy plus standard of care surgery and radiotherapy, C_{IT} is the total healthcare cost for patients receiving immunotherapy alone, $QALY_{IT+SOC}$ is the quality-adjusted life years gained for patients receiving immunotherapy plus standard of care, and $QALY_{IT}$ is the quality-adjusted life years gained for patients receiving immunotherapy alone.

Tumor Microenvironment Dynamics

The temporal change in immune infiltration during treatment can be modeled as:

$$\frac{dI(t)}{dt} = r \cdot I(t) \cdot \left(1 - \frac{I(t)}{K}\right) - \delta \cdot I(t) \cdot T(t)$$

where I(t) represents the immune cell infiltration at time t, r is the intrinsic growth rate of immune cell expansion following checkpoint blockade, K is the carrying capacity representing the maximum immune infiltration, δ is the rate of immune cell exhaustion or suppression by tumor cells, and T(t) is the viable tumor cell population at time t.

The corresponding tumor cell dynamics can be expressed as:

$$\frac{dT(t)}{dt} = g \cdot T(t) \cdot \left(1 - \frac{T(t)}{T_{max}}\right) - \mu \cdot I(t) \cdot T(t)$$

where g is the tumor growth rate, T_{max} is the maximum tumor burden, and μ is the tumor cell killing rate by activated immune cells.

Dose-Response Relationship

The relationship between checkpoint inhibitor dose and response can be modeled using a Hill equation:

$$Response = \frac{E_{max} \cdot D^n}{ED_{50}^n + D^n}$$

where Response represents the magnitude of pathological response, E_{max} is the maximum achievable response, D is the dose of checkpoint inhibitor in milligrams per kilogram, ED_{50} is the dose producing 50 percent of maximum response, and n is the Hill coefficient representing the steepness of the dose-response curve.

Probability of Organ Preservation

The probability of achieving organ preservation without surgery can be estimated as:

$$P_{OP} = \frac{1}{1 + e^{-(\beta_0 + \beta_1 \cdot \Delta TLG + \beta_2 \cdot PR_{biopsy} + \beta_3 \cdot IFN\gamma)}}$$

where P_{OP} represents the probability of successful organ preservation, β_0 is the intercept term, β_1 is the coefficient for change in total lesion glycolysis, ΔTLG is the percentage change in total lesion glycolysis at week 4, β_2 is the coefficient for biopsy pathological response, PR_{biopsy} is the pathological response percentage in week 4 tumor biopsy, β_3 is the coefficient for interferon-gamma signature, and IFNγ is the baseline interferon-gamma signature score.

Treatment De-escalation Decision Function

The decision to de-escalate treatment can be formalized as:

$$D_{de-escalate} = \begin{cases} 1 & \text{if } \Delta TLG < 0 \text{ AND } PR \geq 50\,\% \\ 0 & \text{otherwise} \end{cases}$$

where $D_{de-escalate}$ is a binary decision variable with 1 indicating proceed with treatment de-escalation and 0 indicating proceed with standard treatment, ΔTLG < 0 indicates decreased total lesion glycolysis representing metabolic response, and PR ≥ 50% indicates at least partial pathological response.

These mathematical formulations provide the theoretical foundation for understanding the mechanisms of action of the present invention, predicting treatment response, optimizing treatment strategies, and implementing response-adaptive approaches to minimize treatment-related morbidity while maintaining excellent oncological outcomes.

Prior Art Reference

Breukers, S.E., Traets, J.J.H., van Dijk, S.W. *et al.*Neoadjuvant ipilimumab and nivolumab in resectable cutaneous squamous cell carcinoma: a randomized phase 2 trial. *Nat Med* (2025). https://doi.org/10.1038/s41591-025-03943-w

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