

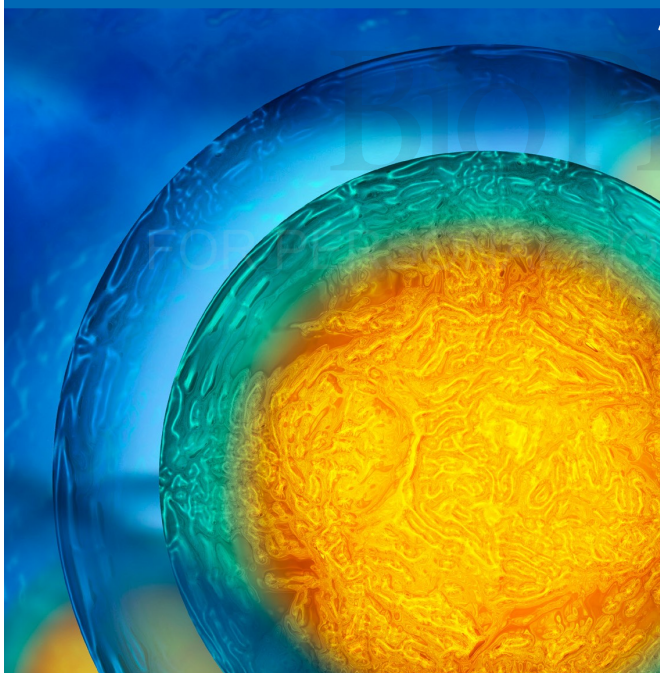
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## PROCESS ANALYTICAL TECHNOLOGIES FOR MANUFACTURING CELL AND GENE THERAPIES



DEVELOPMENT  
FORMULATING AUTOLOGOUS  
THERAPIES FOR CANCER

UPSTREAM PROCESSING  
SOURCING RAW MATERIALS

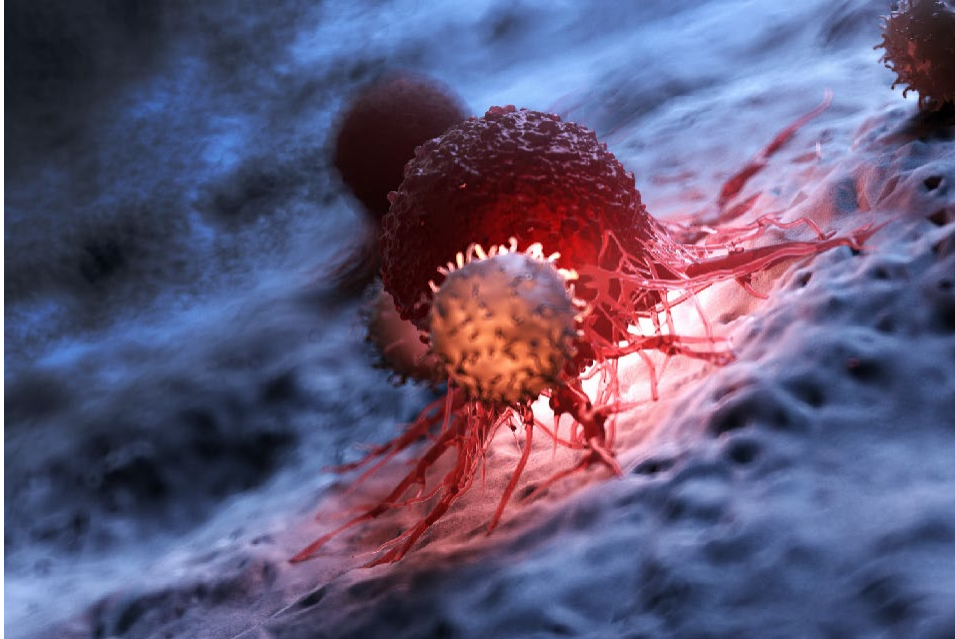
DOWNSTREAM  
PROCESSING  
MANAGING RESIDUAL  
IMPURITIES

MANUFACTURING  
TRENDS IN ISOLATOR  
EQUIPMENT

ANALYTICS  
STREAMLINING GLYCOSYLATION  
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QUALITY/REGULATIONS  
GMP<sub>s</sub> IN ASEPTIC PROCESSING

OUTSOURCING  
ASSAY DEVELOPMENT  
AND TRANSFERS



# Formulating Autologous Therapies for Cancer

Autologous tumor cells engineered for immune system stimulation can target unique metabolic, genomic, and phenotypic characteristics of cancer cells.

RAYMOND P. GOODRICH, AMANDA GUTH, JON WESTON, TERRY OPGENORTH, AND GARY GORDON

**T**raditional approaches to treating patients with cancer are based primarily on maximizing the destruction of tumor cells while minimizing the damage to healthy cells. The ability of chemical agents, radiation, and surgical procedures to destroy or remove tumor cells while preserving the functionality of healthy tissue is the basis of every form of cancer therapy. The result of this quest has yielded therapeutic formulations targeting unique metabolic, genomic, and phenotypic characteristics of cancer cells for the treatment of patients with cancer.

Advances in our understanding of immunology and the body's ability to defend itself against aberrant cells and tissue have afforded new tools in our search for better treatments and cures. Most of these approaches rely on the body's inherent ability to distinguish between cancerous cells and healthy tissue and to respond through both the production of antibodies (humoral immunity) and the generation of cell-based immune responses. New formulations of autologous therapies for cancer are produced by culturing and expanding specific immune cell populations, specifically T cells (T lymphocyte), *ex vivo*, and training them for seek-and-destroy missions

when re-infused. These therapies have produced life-saving outcomes in patients, such as those suffering from B-cell lymphomas. Nonetheless, these methods have limitations such as non-specific targeting (e.g., destroying all B cells and not just the cancerous ones), cost of production, and complexities related to *ex-vivo* expansion of the engineered cells.

The complexity of steps involved in the production of targeted T cells require *ex-vivo* expansion and manipulation of the immune cells, which in turn requires costly reagents to produce enough cells for administration. These manufacturing steps drive up cost, introduce logistical issues, and reduce the ability to treat the diverse nature of most tumors. For autologous therapies for cancer, new therapeutic formulations not

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only use the patient's own immune cells but also their own tumor cells to generate therapeutic preparations for treating their specific cancer. This approach may address many of the current limitations of cell-based immunotherapy.

### PATIENT-SPECIFIC IMMUNE THERAPY LIMITATIONS

The development of methods to manipulate immune cells from a patient *ex vivo* has greatly increased the utility and benefit of cellular therapy and autologous, patient-specific immune therapies. The most recent example of Chimeric Antigen Receptor (CAR) T-cell therapy targeting B-cell lymphoma is perhaps the most specific illustration of how personalized cell therapy approaches can be effectively implemented with great benefit to patients (1). By developing methods to target CD19 antigen, *ex vivo*, T cells are generated with the ability to seek and destroy all cells expressing CD19 antigen. Both tumor cells and healthy B cells with this antigen are eliminated. The resulting reset of the immune system through triggering mechanisms that silence these altered T-cell clones can lead to the cure of the lymphoma that the patient has on board.

Unfortunately, a reset such as this is not possible with other cell types. While it is feasible for an individual to survive for a period without B cells, it is not possible for them to survive if all lung, colon, liver, and/or other solid organ tissue were similarly targeted for elimination. The limitation of T-cell therapies of this type are thus dependent on identifying antigens that are specific to the tumor (neoantigens) but not present on the healthy cells. The presence of such specific antigens allows the immune system to distinguish between healthy and cancerous and thus eliminate the tumor more specifically. Unfortunately, targeting against a specific neoantigen may prove insufficient since tumors may contain a plethora of these tumor-specific antigens, which are not expressed across every cell in an

individual's tumor. This issue can lead to the escape of some tumor cells, resulting in tumor regrowth if the targeting is too specific. In recent years, multiple new cell therapies have entered clinical trials and are reporting improved efficacy over the standard of care (2). These therapies are using genomic analysis and artificial intelligence to identify or predict neoantigens on individual patient tumors. Researchers then construct peptide vaccines or potentiate harvested patient immune cells to the neoantigens for reinfusion.

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T-cell culturing and dendritic cell manipulation *ex vivo* also require a complex logistical framework for manufacture. First, immune cells must be harvested from the patient in sufficient quantities to allow subsequent culturing and expansion. These processes often take weeks of *ex-vivo* manipulation in cell bioreactors using reagents and culture conditions that promote cell expansion of specific cell types to the exclusion of others. An insufficient yield of cells at the end of culture could mean that the patient is unable to subsequently receive an effective

therapeutic dose of the altered cells. The *ex-vivo* manipulation of the product required also introduces challenges in maintaining sterility and covering high costs associated with the reagents and procedures needed to drive cell differentiation in culture along the desired pathways. The average cost of CAR-T therapy exceeded \$400,000 per treatment in the initial introduction, and reimbursement today remains at \$240,000 in most settings (3). This limits the number of patients for whom such treatment is accessible.

### AN ALTERNATIVE APPROACH: CAR-T THERAPY IN REVERSE

An alternative method to *ex-vivo* manipulation of the immune cells is to modulate immune responses *in vivo* by presenting the tumor antigens to the immune cells in an environment separate from the tumor microenvironment where immune suppressors may be expressed by the tumor cells, thus reducing immune response at the local site of the tumor. Such an approach would allow the type of cell modification that CAR-T therapy achieves *ex vivo* to occur *in vivo* through the presentation of tumor antigen to the immune system that the patient has on board.

Several methods using specific tumor antigens or whole tumor cells, which have been inactivated before administration, have been utilized previously with moderate success (4). Single antigen approaches, however, face the same challenges that CAR-T cell therapy faces. Elimination of specific clones of a tumor may not lead to complete clearance of all tumor cells in a patient. The use of whole, inactivated cells requires that the cells be rendered incapable of replication post-infusion to prevent the regrowth of tumors in the patient. To assure complete inactivation, common methods employ chemical agents such as formalin, ultraviolet (UV) light, or gamma irradiation of sufficient energy and dose to destroy cell replication processes. The harsh and non-spe-

cific nature of the chemistry that these methods invoke results in cells that are not only inactivated but also rendered altered from the native antigen state that these tumor cells express. As a result, these methods often require large amounts of tumor material to induce a strong immune response, effectively increasing the dose to levels at which sufficient amounts of the native tumor antigen are still capable of generating immune reaction to those antigens.

Currently, we are exploring the ability of a method initially developed to treat blood products for the prevention of transfusion-transmitted infections to create inactivated tumor cells that can subsequently be used, in combination with Th1-promoting adjuvants, to stimulate an immune response *in vivo* in pre-clinical models (5). This photochemical approach uses Riboflavin (vitamin B2) and UV light of specific wavelengths to generate specific modifications to tumor cell DNA that prevent replication of these cells (6). The specific nature of the chemistry for nucleic acid modification without alteration of proteins and antigens in the tumor cells yields cells that lack the ability to replicate but maintain their native antigen profile. In addition, these treated cells maintain cell metabolism and protein expression following treatment like blood cells treated similarly without the ability to replicate (7).

Tumor cells treated by this process were evaluated in immune-competent mouse models and shown to slow tumor growth, reduce metastatic disease, and stimulate immune cell responses to tumor tissue (8). In canine studies, preparations of tumor cells at doses as low as  $1E+06$  tumor cells per dose stimulate immune cytokine production and reduce tumor cell immunosuppressive cytokine expression. Doses of product sufficient to treat autochthonous tumors in dogs without inducing adverse acute reactions were reported previously (9).

The production of these materials does not require *ex-vivo* expansion of the cells, thus reducing turnaround

time and complexity of providing the therapy to patients. In a canine cancer trial conducted at the Flint Animal Cancer Center at Colorado State University, we tested the logistical practicality of using a biopsy or excised tumor tissue obtained at the Cancer Center, processing it at the PhotonPharma labs at the Research Innovation Center on campus, and returning the products to the canine subjects within one to seven days of their initial clinic visit. Production of the inactivated cells requires less than two hours using equipment and disposables that already received a Conformité Européenne Mark and are available in routine clinical use in blood centers globally (10).

Injection of the inactivated cells in an autologous fashion allows for the generation of immune response targeted to the patient's specific tumor type and all tumor antigens that may be present in that patient's tumor. The extensive experience of using treated blood products, which include nucleated leukocytes and lymphocytes from allogeneic donors, demonstrates the safety of the infusion of nucleated cells treated by this process. Riboflavin is a substance that has an extensive toxicology profile showing its safety after exposure to UV light in the process that is utilized here for creating inactivated tumor cells (11).

The low-cost profile of the equipment and disposables required for producing the inactivated cells via this method also has distinct advantages that may increase the accessibility of this therapy in patients with solid-organ tumors. As extensive *ex-vivo* manipulation of the product is not required, production costs for such a therapy can also be limited and made more accessible for patients, healthcare providers, and national health services. Such an approach could make cellular therapy methods available to populations in many regions of the world today for which these procedures are currently inaccessible due to cost and complexity of implementation.

Our initial focus of work is the development of this therapy for treating patients with breast cancer. These tumors are generally accessible via biopsy or surgical excision. Breast cancer patients also experience high levels of metastatic relapse potential due to the nature of these tumors and the fact that chemotherapy or radiation therapy may allow for the survival of small populations of resistant cells. An autologous therapy approach as outlined in this article has the potential to reduce the likelihood of disease relapse in this patient population. Such an approach could also add another dimension to treating other solid organ tumors. This could include follow-up in such patients in a way that boosts the efficacy of more conventional treatments. The specificity of the therapy to the patient's own tumor makes a response in each patient more relevant to their specific tumor composition, providing a lower likelihood of immunological escape. In the end, tumor material that is otherwise discarded can be applied to defeat the disease that led to its existence to begin with.

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