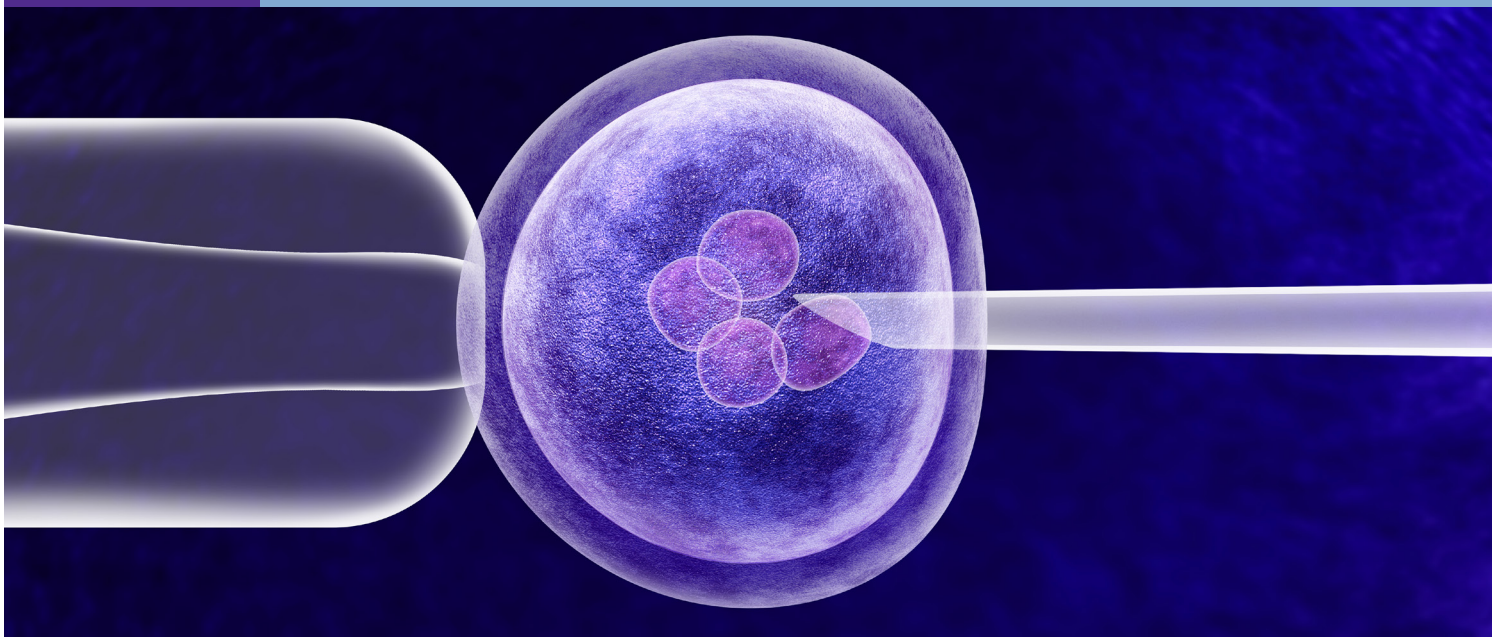
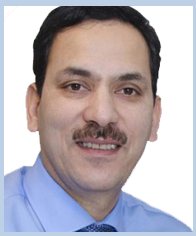
WHITE PAPER

Gene-Edited and Engineered Cell Therapy for Cancer: From Bench to Bedside



For cell-based therapies to progress successfully from preclinical testing into clinical care, immuno-oncology researchers need to design tumor biology-driven cell therapies that are tested on physiologically relevant, realistic, and reliable preclinical models that depict the human tumor microenvironment.

Gene-editing technology has evolved significantly over the last decade. Today, researchers can isolate T cells from a patient, reprogram them, and put them back into the same patient. Stem cells can migrate to multiple targets simultaneously equipped with anti-tumor agents and kill switches. One patient's cancer cells can even be used to kill another person's cancer cells.



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One pioneer in cell-based therapies for glioblastoma, Khalid Shah, MS, PhD, Director, Center for Stem Cell Therapeutics and Imaging, Brigham and Women's Hospital, has created a research program on the vision of "creating an 'off-the-shelf' therapeutic offering for cancer that would positively impact the quality of life of individuals affected across the globe."

Types of Cell-Based Therapies Tools for Cancer

Manufacturing methods separate available cell-based therapies into two categories: allogeneic and autologous.

Allogeneic Therapies

Allogeneic therapies, created from unrelated donor tissue samples (e.g., bone marrow, adipose fat) can be produced in large batches using Good Manufacturing Practices (GMP), which offer researchers multiple advantages (Bartel, n.d.):

- Readily available ("off the shelf")
- Used to treat a large number of patients
- Quality controlled product

Examples of allogeneic therapies include the following:

- Engineered stem cells
  - Mesenchymal stem cells (MSCs), which are derived from adult stem cells, umbilical cord, or adipose fat.
- Induced pluripotent stem cells (iPSCs) derived natural killer (NK) cells, which are produced from peripheral blood cells (Zeng, 2017).

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Given that glioblastoma patients and providers have only 2-3 weeks between diagnosis and surgery, Shah said he has to be realistic in his choice of therapies, which is why he leans toward allogeneic cells. Having worked with many engineered stem cell types, Shah's lab has settled on mesenchymal.

Autologous Therapies

Autologous therapies are custom produced for an individual patient. Tissue is removed and returned to the same individual (Bartel, n.d.). Autologous therapies take more time and are not produced under GMP. This biologic product is often produced on-site where the patient is receiving care and requires enhanced measures to ensure the sample is returned to the correct patient (Bartel, n.d.).

Examples of autologous therapies include the following:

- Engineered cancer cells
- Chimeric antigen receptor (CAR) T cell therapy

CAR-T cells are still challenging in solid tumors, according to Shah, requiring researchers to consider treatment resistance, PD-L1/PD-1 expression, tumor microenvironment, and the role of macrophages.

"The more we understand the biology of the tumor, all the different cells within the tumor microenvironment, the more we can overcome these challenges," said Shah.

Challenges in Immunotherapeutic Development

Cell-based immunotherapies offer enormous potential to transform the standard of care in cancer. Many cell therapies, however, do not progress into clinical care. Researchers may observe variable response rates (Sambi, 2019). Promising results in simple preclinical models may not be generated in more complex models (Hegde, 2020).

To overcome the most common challenges in developing cell-based therapies, Shah outlines three basic principles:

- Understand the mechanism behind the function of therapeutic cells.
- Develop tumor models that mimic the clinical setting of tumor growth.
- Understand the current treatment regimen for each cancer type.

Understanding Tumor Biology

"The molecular mechanisms of tumors should drive cell-based therapies," said Shah.

Profiling tumors reveals new targets for engineered stem cells. Shah has designed stem cell therapies for a range of anti-tumor agents with many targets, including the following:

- **Proapoptotic proteins:** Stem cells engineered to express therapeutic proteins (tumor necrosis factor-related apoptosis-inducing ligand [TRAIL]) that bind to antibodies on tumor cells (death receptors [DR4/5]) (Kavari and Shah, 2019).
- **Oncolytic viruses:** MSCs armed with oncolytic herpes simplex virus (oHSV) and PD-L1 blockade to increase IFNγ-producing CD8+ tumor-infiltrating T lymphocytes (Du, 2017).
- **Immune therapy:** Engineered MSCs, encapsulated in synthetic extracellular matrix, which secrete IFNβ, to boost the immune response from CD4/CD8 T cells, when placed in a tumor resection cavity (Choi S. H., 2017).

By targeting multiple receptors, researchers can work to overcome common obstacles such as limited efficacy from poor pharmacokinetics, therapy resistance, and the heterogeneity of tumor cells, while also helping to improve precision and efficacy (Kavari and Shah, 2019).

The Multiple Target Approach

In glioblastoma, CAR-T cells have been shown to be effective in crossing the blood-brain barrier and penetrating dense tumor tissue, but with isolated success in regressing advanced disease in clinical studies (Hughes-Parry, 2019).

Tumor cell heterogeneity is one factor contributing to the lack of consistent outcomes. Researchers at Massachusetts General Hospital targeted EGFRvIII for the first-in-human clinical trial of CAR-T cells in glioblastoma because EGFRvIII is a mutation found in tumor cells, not healthy cells. However, not all glioblastoma cells express EGFRvIII, which resulted in a partial response.

To target EGFRvIII-negative tumor cells, researchers added a target for a second antigen, wild-type EGFR. To avoid targeting healthy cells expressing EGFR, they added a bi-specific T cell engager or "BiTE" to create a dual-binding CAR (Choi B. D., 2019).

Better models needed to move discoveries out of the laboratory

To align the preclinical testing of gene-edited and engineered cell therapies with the complex tumor microenvironment, researchers must adopt physiologically relevant models that mimic what happens in the clinic.

"We don't have real tumor models," said Shah. "We think we can just take a cancer cell out of a patient and put it in the mouse brain to test a treatment. We can't."

Traditional models do not replicate how cancer cells adapt and evolve. The tumor microenvironment is fluid, evolving — responding to therapeutic interventions and developing resistance.

For many years Shah's lab focused on treating solid intact brain tumors. Upon resection, tumor tissue was used in patient-derived xenograft (PDX) models to study treatments for glioblastoma. The resected tumors, however, did not reflect a brain tumor in the clinical setting. After excision, tumors were often treated with temozolomide and radiation.

In Shah's PDX models, resected tumors grew faster than those in patients. "When you resect the tumor, you refresh the tumor, you basically bring growth factors back in," said Shah. "You reduce the amount of EGFR and you reduce hypoxia. The tumors are refreshed, and they grow much faster."

Shah then worked to develop a better mouse model by implanting encapsulated mesenchymal cells in hydrogel (hyaluronic acid-based gel), which were implanted into the cavities left by excision (Shah, 2013).

Anticipating Clinical Realities

It is essential to understand the current treatment regimen for each cancer type to translate novel therapies into clinical settings. In the case of glioblastoma, Shah has had to anticipate the impact of temozolomide on stem cells or CAR-T cells. His decision to use allogeneic stem cells was driven by the compressed time frame between diagnosis and surgery.

The impact of therapies used before surgery can affect the tumor microenvironment. In glioblastoma, adjuvant therapy enhances the number of functional T-cells in the tumor. Resection removes blood-derived macrophages at the core of the tumor but may leave macrophages at the edges of the tumor that contain microglia. To prevent macrophages from enhancing the survival of tumor cells left behind after surgery, Shah is looking at re-educating macrophages by targeting them with CSF1R.

It is crucial to stratify patients to anticipate resistance and the full range of responses to immunotherapy, including toxic cytokine storm syndromes. "Every individual's immune system is going to react differently to cell-based therapies," said Shah. "Some individuals will respond well, and others will not."

Shah anticipates the field will evolve over the next five years to include immune profiling, potentially capable of examining the impact of the microbiome on treatment response. "We will probably find out that most of our immune system resides in the gut," he said.

It is this broader perspective, said Shah, that will help investigators move cell-based therapies from the bench to the bedside: "Investigators must step out of their comfort zone to anticipate the downstream challenges faced by patients, clinicians, and manufacturing."

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