## Cellular Therapies for Neuroblastoma

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### Introduction:

The mainstay of treatment for children with newly diagnosed neuroblastoma includes combination of chemotherapy, surgery, high-dose chemotherapy with stem cell rescue, radiation therapy, immunotherapy and isotretinoin. Despite improvements in these therapies over the past decades, many children are not cured and experience significant short and long term toxicities (1). One promising class of treatment is immunotherapy, which harnesses a patient's immune system to recognize and fight the tumor (2). New techniques are emerging to manipulate the immune system to better recognize, effectively eliminate and prevent the reappearance of cancer cells. The success of this work has translated to 5 main types of immunotherapies to fight cancer:

**1) Monoclonal antibodies** are protein molecules that binds to a specific target on tumor cells to initiate an immune response. They are usually given by IV infusion and once they enter the blood stream and bind to their tumor target, these proteins activate other components of the immune system to kill cancer cells (3).

**2) Bi-specific T cell engagers (BITEs):** These are monoclonal antibodies that have two binding sites instead of one. On one end, they bind to tumor cells as with conventional monoclonal antibodies; however, they can also simultaneously bind to other immune cells, like T cells. BiTE and similar molecules recruit the patient's own immune system to help kill the cancer better. Blinatumomab is an example of a BITE that has been FDA approved for use in children and has had led to significant improvements in certain types of leukemia (4) .

**3) Immune checkpoint inhibitors:** These are a class of medications that block proteins which inhibit the body's own immune response to cancer and in return unleash immune cells to attack cancer cells. Typically, immune checkpoints are integral parts of the immune system and prevent the body from responding too harshly to an infection or other injury; however, cancers have an ability to use this to their advantage and as such avoid elimination. Blocking immune checkpoints enable immune cells, like T cells, to recognize and kill cancer cells more efficiently. Several drugs have been developed to this for many cancers. One example is Pembrolizumab, which targets the checkpoint inhibitor PD-1 and has been very helpful in the treatment of a variety of solid tumors as well as some types of lymphoma (5).

**4) Cancer vaccines:** Vaccines are already successful in fighting viral infections. They do this by introducing the body's immune system to specific molecules that stimulate

elimination of an infection and induce an immune memory response, so that in the future should the patient be infected with the actual infection, the immune system can eliminate it before causing medical issues. Several vaccines are currently implemented in the fight against COVID19. Cancer treatment vaccines work in a similar way. Particles unique to a specific tumor are inserted into a vaccine which are then injected into the patient. This tells the patient's immune system to develop antibodies and cancer specific cells that find and destroy any cancer cells that have that fragment on their surface. Vaccines can be personalized (i.e. tumor fragments are taken from a patient's own tumor biopsy and then built into the vaccine) or can be made from a common protein or particle that is known to be present on the tumor in general (6).

**5): Engineered Cell Therapies:** In this treatment, immune cells are genetically engineered in the laboratory so that they express a molecule called a chimeric antigen receptor (CAR) that binds directly to a target expressed on tumor cells. Once these CAR expressing cells are made, they can be given when needed. Based on the success in the laboratory, engineered cell therapies have rapidly evolved over the last two decades, with many patients with certain blood diseases (i.e. B-cell leukemia) successfully cured (7, 8). This therapy has evolved to use many different immune cell types.

***CAR T cells*:** One of the initial cell therapies used T cells to target leukemias and are called CAR T cell therapies. Based on the success of the therapy, the FDA has now approved four CAR T cell therapies for the treatment of patients with leukemia or lymphoma that has been resistant to aggressive therapies and one treatment for multiple myeloma. Patients on these treatments often develop fevers and other symptoms from inflammation that can last days to weeks. CAR T cells have also been developed for multiple solid tumors, including neuroblastoma with several clinical trials that have been completed or are currently ongoing (9, 10). Complete and partial remissions in patients in some patients with smaller tumor burdens have been reported with this treatment.

***CAR NK Cells:*** Other researchers have sought to find ways to improve and expedite the manufacturing process by genetically modifying another type of immune cell called a natural killer (NK) cells with CARs. These have been successful in treating a variety of tumors. They can target tumors similar to CAR T cells but be used "off the shelf" such that once a patient is identified, they would have cells more quickly available without the need for going through the manufacturing process. In contrast to CAR T cells, they can kill tumor cells more broadly since they do not require identification of a specific target of interest, and can help kill tumor cells that might lack the expression of that target on

 

their surface (11, 12), however their anti-tumor effect is generally more short lived than T cell based therapies.

***CAR NKT Cells:*** Lastly, CARs have been expressed with another immune cell called a natural killer T cell, called a CAR NKT cell. These are particularly helpful for the treatment of solid cancers, because NKT cells are very good at migrating to tumor tissue in the body and target tumor associated macrophages (TAMs), a type of immune cell that promotes tumor growth and spread. In addition, they can indirectly recruit NK and T cells to target the tumor as well (12). Therefore, CARNKTs can directly target neuroblastoma cells and indirectly by eliminating TAMs and recruiting other members of the immune system(13). A recent preliminary report showed their promise in treating children with relapsed or refractory high-risk neuroblastoma (14).

**Specific Treatments for Neuroblastoma:**

Significant progress has been in the development of immunotherapies as described, and many drugs have been developed from these modalities to treat neuroblastoma. Dinutuximab, for example, is a monoclonal antibody which targets GD2, a target highly expressed on neuroblastoma. While it has been an important addition to therapy, Its use has led to only modest improvements in survival rates alongside chemotherapy in high risk patients (15, 16).

Several phase 1 clinical trials using CAR T cells for neuroblastoma have been completed or are currently ongoing. The first CAR T cells to be used for neuroblastoma targeted the marker L1

cell adhesion molecule (L1-CAM), which is highly expressed on neuroblastoma tumor tissue. Though therapy was relatively safe, all patients ultimately had worsening of their disease with time(17). More recent CAR T cell trials have been targeting GD2 given its prevalence on neuroblastoma cells. One group treated 11 children with neuroblastoma using GD2-CAR T cells with and without PD-1 checkpoint inhibition. While treatment was safe, there was limited anti-tumor responses following therapy (18). Additionally, another trial treated 12 neuroblastoma patients with GD2+ CAR T cells at multiple dose levels. As in the prior study, they found that therapy was relatively safe but due to a lack of CAR T cell persistence, tumors ultimately progressed in all patients (19). Therefore, while these trials showed promise, more efforts are needed to improve the ability of CAR T cells to grow and persist in patient's following therapy (20).

**Next Generation Cellular Therapies for Neuroblastoma:**

 

There are several proposed mechanisms by which neuroblastomas can evade cancer therapies, and a new generation of cellular therapies are in development to counter these mechanisms. One mechanism is to genetically enhance CAR T cells to express one or multiple genes on their surface that stimulate growth and survival (21). The use of these CAR T cells, known as second and third generation CAR T cells, have led to improved anti-tumor effect in mice, which has led to follow up clinical trials, one of which out targeting L1-CAM which is currently in progress (NCT02311621). Additionally, studies are being developed to generate armored CAR T cells, whereby CAR T cells are genetically modified to express cytokines, or chemicals in the blood that stimulate CAR T cells to grow and remain in the patient's body. These armored CAR T cells have led to improved CAR T cell function in mice, and their success has been translated to clinical trials, including a GD2 targeted CAR T cell expressing the continuously signaling IL-7-receptor that is currently ongoing (NCT03635632) and a GD2-CAR T cell co-expressing the cytokine IL-15 for patients with solid tumors (NCT03721068) (22, 23).

In addition to enhancing the function of cell therapies, researchers are also improving how immune cells and cell therapy products migrate into neuroblastoma tumors. For example, recent work has focused on genetically modifying CAR NKT cells to target GD2 and co-express the cytokine IL15. This has shown significant promise in the laboratory and has led to a current phase 1 clinical trial which has shown safety and potentially effectiveness in children (NCT03294954) (13, 14).

**Table 1: List of currently recruiting Phase 1 immunotherapy clinical trials targeting Neuroblastoma in the United States, Canada, and United Kingdom.**

| NCT Number | Title of Study |
|---|---|
| **Engineered Cellular Therapies** | |
| **NCT02573896** | Immunotherapy of Relapsed Refractory Neuroblastoma with Expanded NK Cells |
| **NCT03294954** | GD2 Specific CAR and Interleukin-15 Expressing Autologous NKT Cells to Treat Children with Neuroblastoma |
| **NCT03721068** | Study of CAR T-Cells Targeting the GD2 with IL-15+iCaspase9 for Relapsed/Refractory Neuroblastoma or Relapsed/Refractory Osteosarcoma |
| **NCT02311621** | Engineered Neuroblastoma Cellular Immunotherapy (ENCIT)-01 |
| **NCT01757626** | Combination Therapy of Antibody Hu3F8 With Granulocyte- Macrophage Colony Stimulating Factor (GM-CSF) in Patients with Relapsed/Refractory High-Risk Neuroblastoma |
| **NCT03635632** | C7R-GD2.CART Cells for Patients with Relapsed or Refractory Neuroblastoma and Other GD2 Positive Cancers (GAIL-N) |

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| **NCT03860207** | Study of the Safety and Efficacy of Humanized 3F8 Bispecific Antibody (Hu3F8-BsAb) in Patients with Relapsed/Refractory Neuroblastoma, Osteosarcoma and Other Solid Tumor Cancers |
| **NCT04483778** | B7H3 CAR T Cell Immunotherapy for Recurrent/Refractory Solid Tumors in Children and Young Adults |
| **NCT03618381** | EGFR806 CAR T Cell Immunotherapy for Recurrent/Refractory Solid Tumors in Children and Young Adults |
| **Checkpoint Inhibition Therapies:** | |
| **NCT04500548** | Testing the Combination of Two Immunotherapy Drugs (Nivolumab and Ipilimumab) in Children, Adolescent, and Young Adult Patients with Relapsed/Refractory Cancers That Have an Increased Number of Genetic Changes, The 3CI Study |
| **NCT02914405** | Phase I Study of 131-I mIBG Followed by Nivolumab & Dinutuximab Beta Antibodies in Children with Relapsed/Refractory Neuroblastoma |
| **NCT03332667** | MIBG With Dinutuximab +/- Vorinostat |
| **NCT04239040** | GVAX Plus Checkpoint Blockade in Neuroblastoma |
| **Vaccine Therapies**: | |
| **NCT00911560** | Bivalent Vaccine with Escalating Doses of the Immunological Adjuvant OPT-821, in Combination with Oral Î²-glucan for High-Risk Neuroblastoma |

**Conclusions:**

Harnessing the immune system to target cancer effectively has come a long way in the last several years, with recent clinical trials showing that CAR T cells and CAR NKT cells are safe, and there is ongoing research to identify new targets as well as develop methods to further improve the next generation of immunotherapies. While there is still progress to be made for universally effective therapies, there are encouraging results from the laboratory and from clinical trials that should results in novel neuroblastoma treatments in the near future.

Please contact info@cncfhope.org with any comments

 

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