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The Role of Immunotherapy in Treating High-Risk Neuroblastoma

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Abstract

Neuroblastoma originates from the cells in the neural crest. High-risk neuroblastoma, patients have poor outcomes even with the multi-step treatment plans, including immunotherapy maintenance treatment. Researchers in developmental biology search for unique antigens in neuroblastoma cells to utilize monoclonal antibodies (mAbs). Currently, GD2 is the most effective antigen that scientists have isolated in the tumor; these anti-GD2 mAbs are administered in the forms of Dinutuximab or Dinutuximab beta to attack the tumor. Monoclonal antibodies are currently administered in neuroblastoma instead of CAR T (that has seen success in curing different types of leukemias) due to the heterogeneity of this tumor. Although GD2 treatment has made significant strides in outcomes, there is still a high rate of relapse and treatment failure. Scientists continue to pursue further developments to help cure high risk neuroblastoma through better technology and more research.

Introduction: Neuroblastoma accounts for 15% of all pediatric oncologic deaths and is the most common extracranial solid tumor in children (Kholodenko et. al., 2018). Through understanding the tumor's origin and developmental biology, researchers have zeroed in on unique markers to utilize natural killers (NK) in targeting and eliminating the cancer. In the high-risk phase, neuroblastoma is deadly and has a high rate of relapse. Despite improvements in outcome, rates of recurrent neuroblastoma are high; in about 50% of cases treatments fail (Jabbari,2019). Currently, the final part of the treatment plan is a monoclonal antibody immunotherapy that targets the GD2 antigens on the cancer cells.

Origin

It is important to understand the developmental biology and neuroblastoma origins because its proliferation, guide tissue morphogenesis, and differentiation resemble cancer cells' progression. From studying the unique physiology seen in the neural differentiation of the sympathoadrenal lineage, researchers can apply that knowledge to treat neuroblastoma. For example, one of the backbones of maintenance therapy is isotretinoin because scientists found that the neural differentiation is driven by retinoids in vitro (Cheung and Dyer,2013).

The neural crest cells that form neurons can be divided into five functional types: vagal, sacral, cranial, cardiac and trunk cells. The trunk cells then separate into two lineages in the early stages of embryonic development – sympathetic and adrenal. When the cells migrate from the neural crest, they undergo epithelial-mesenchymal transition (EMT). Both neural crest cells and tumor cells undergo similar EMT processes and express matrix metalloproteinases (MMPs), disintegrins and metalloproteinases (ADAMs) that facilitate cell invasion and migration (Kholendenko, 2018).

The migrating neural crest progenitor cells committed to the sympathoadrenal lineage initiate their differentiation due to the signaling of bone morphogenetic protein (BMP). The cells then commit to becoming either adrenal chromaffin cells or sympathetic ganglia. Members of the MYCN family are expressed throughout this process in the migrating trunk neural crest cells and are committed to the sympathoadrenal lineage (Cheung,2013).

Neuroblastoma arises from the progenitor cells of sympathoadrenal lineage of the neural crest during development. More specifically, scientists believe that neuroblastoma may develop from this chromaffin lineage because most tumors are localized in the adrenal gland region. The expression of different transcription factors is critical for neural crest development and is upregulated in cancers including Snail, Twist, SoxE, and FoxD families. The neural crest cells are a transitional type of cell that quickly pass from multipotent progenitors to a variety of differentiated cell types. Scientists previously thought that the neural crest cells gradually lose their multipotent properties and plasticity when they reach the postmigration stage, but it has been seen that is not the case. Adult neural crest-derived cells retain the properties of stem cells and even mimic the transcriptional expression profiles of both embryonic stem cells and neural crest progenitors. These progenitor cells of the neural crest are found in many types of tissues, including skin, dorsal root ganglia, adrenal medulla, bone marrow, among other tissues (Kholendenko et. al., 2018).

Presentation of Neuroblastoma

A 2016 study showed that 50% of neuroblastoma patients presented with metastases at diagnosis (Tolbert and Matthay 2018). Most cases of neuroblastoma are diagnosed in the abdomen or arise in the adrenal medulla or lumbar sympathetic ganglia. (Cheung, 2013). The tumor can appear anywhere from the neck to the pelvis because the origin is along the paraspinal and sympathetic ganglia. The clinical presentation varies based on the tumor's primary and secondary metastatic sites (Tolbert and Matthay, 2018). The most common metastatic site is the bone marrow, at 89%; therefore, bone marrow aspirates are used for diagnosing and responding to the cancer (Shumacher-Kuckelkorn, 2020). Due to the tumor's involvement with the central nervous system (CNS) other common metastatic sites are bones and regional lymph nodes. Tumors can present at the top of the paraspinal ganglia, causing Horner's syndrome in some patients because

neuroblastoma involves surrounding nerve roots of the sympathetic ganglia. Thoracic tumors present in only 5% of patients, arising from the posterior mediastinum and paraspinal ganglia, and, often, the tumor will invade the neural foramina. The neural foramina can be invaded anywhere adjacent to the spinal cord compressing it. In the abdominal cavity, masses can cause distention with or without pain. Furthermore, extensive liver involvement can be seen in infants and can cause liver disease such as coagulopathy as well as renal and lung dysfunction due to abdominal distention. The neurologic symptoms that can accompany pelvic tumors include bladder dysfunction, constipation, lower extremity pain or weakness due to nerve root involvement. Another symptom seen predominantly in infants' metastases in the skin is painless subcutaneous nodules that have a blue hue. If the tumor metastasizes in the bone of lower extremities, the patient may limp or refuse to bear weight. Only 80% of patients who need bone marrow infiltration will present with anemia and thrombocytopenia. The common symptom of racoon eyes is caused by bone lesions in the skull, within the periorbital region, that can cause periorbital bruising. The catecholamines released can cause flushing, hypertension, or tachycardia, and in rare cases, a paraneoplastic phenomenon will secrete vasoactive intestinal peptides causing profuse diarrhea. If children present with opsoclonus myoclonus syndrome (OMS) or varying neurologic symptoms such as opsoclonus, myoclonus, ataxia, or other cerebellar symptoms, they should be tested for neuroblastoma. Fifty to eighty percent of these patients will then be diagnosed with neuroblastoma, but only 2-3% of neuroblastoma patients are affected by OMS. (OMS can be attributed to the anti-neuronal antibodies cross-reacting with the cerebellum.) (Tolbert,2018)

Stages of Neuroblastoma

The international neuroblastoma community organized neuroblastoma into four distinct stages. The first L1, is a localized tumor confined to one body compartment, which does not involve vital structures as defined by the list of image-defined risk factors. The second stage L2, is a locoregional tumor with presence of one or more image-defined risk factors. The third stage is M: a distant metastatic disease. MS is confined to children younger than 18 months - metastases are confined to skin, liver and/or bone marrow. Further classification is based on age, histologic category, grade of tumor differentiation, MYCN amplification, 11q aberration and ploidy (Tolbert,2018). Patients with low-risk classifications have a favorable prognosis with >90% survival; in contrast, patients with high-risk neuroblastoma have a 5-year survival rate that is still below 50% (Weinke,2021).

Treatment Steps Overview

Most children who present with low and intermediate risk disease respond well to treatment plans, but children with high-risk neuroblastoma need an intense "multimodal" approach as the cure rates are estimated at <50% (Khan et. al., 2021). The current treatment for high-risk neuroblastoma has three phases and lasts approximately 18 months. Induction, the first phase, is where patients receive 5–8 cycles of intensive chemotherapy and start stem cell collection to prepare for their autologous stem cell transplant (ASCT). Surgery is usually preformed towards the end of this phase (Smith and Foster, 2018). Next is consolidation, which includes high dose chemotherapy followed by ASCT, shown to be especially beneficial in neuroblastoma, unlike in most other high-risk diseases with solid tumors (Khan, 2021). Radiation therapy usually starts after ASCT recovery. The last step is maintenance, which deals with the residual disease, trying to prevent the 50% relapse rate (Smith,2018). This phase uses immunotherapy which is a combination of monoclonal antibody targeting called disialoganglioside (GD2) / Chimeric antibody 14.18 (ch14.18), GM-CSF, and interleukin-2 (IL-2), which were added to previous maintenance therapy of just administering isotretinoin. In the cases when the patient relapses or the cancer becomes refractory, the cancer is rarely cured. Salvage therapy is administered to improve symptoms and quality of life (Tolbert and Mathey, 2018).

Monoclonal Antibodies' History

B-lymphocytes are activated when a foreign substance enters the body, and antibodies are produced in response to this antigen. Monoclonal antibodies (mAbs) are the main antibodies used in immunotherapy treatment for cancer. The history of mAbs began in 1975 with future Nobel Prize winners Georges Kohler and Cesar Milstein using hybridoma technology to produce mAbs, an antibody that only recognizes a single epitope. Antibodies that are made for immunotherapy are based on the Fv's region affinity for antibody targeting and the Fc's region ability to participate in the host's immune system. The two classes of mAbs are non-conjugated: naked mAbs and mAbs that work with chemo drugs or radioactive particles that target to enable the mAbs to reach the objective. MAbs use three different mechanisms of action via targeting and binding the target cell's antigen on the cell membrane and blocking the pathways that lead to the cells multiplying. Firstly, they inhibit the factors and receptors that activate the pathways allowing cancer cell proliferation. Second, they cause antibody dependent cell-mediated cytotoxicity (ADCC), where the mAbs bind to the tumor associated antigens in the surface of target cells. Then the

Fc receptors of immune cells recognize cell-bound mAbs, followed by cross-linking of these receptors, releasing cytotoxic agents that lead to apoptosis of tumor cells. The third way is through complement dependent cytotoxicity (CDC). The mAbs bind to the antigen on the target cell, causing a "complement cascade". These complements bind an attack complex and activate the cell's complement system lyses (Kimis-Gebologlu et. al., 2018).

GD2

Scientists isolated GD2 for the mAbs to target. GD2 in its humanized forms is called hu3F8 and ch14.18. GD2 is an oncofetal differentiation antigen that was identified by comparing neuronal differentiation and tumorigenesis (Cheung,2013). GD2 is a perfect candidate for antibody therapy because it is abundantly expressed in most neuroblastoma cells, and GD2 is limited in normal cells, including peripheral nerves (Nguyen et. al., 2018). It belongs to a unique class of T cell-independent carbohydrate antigens with high density, membrane proximity, homogeneity within and across neuroblastomas, and rare occurrence of antigen loss. Anti-GD2 mAbs attach tumor cells to NK cells and rescue NK cells from being suppressed or inhibited by neuroblastoma. GD2 is also ideal for tumor-selective delivery of radioisotopes, liposomes, or nanoparticles (Cheung,2013).

Further future applications of GD2 were seen in association with tumor-associated macrophages (TAMs). These TAMS are myeloid effectors that can become polarized into type 1 antitumor or type 2 pro-tumor phenotypes. But researchers discovered that in the presence of anti-GD2 mAbs in vitro, ADCC can turn protumor M-CSF-activated macrophages into antitumor killers. Even though mAb therapy is considered a passive immunotherapy, introducing a host anti-tumor following mAb therapy might benefit long-term tumor control (Cheung,2013).

MYCN, KIR-HLA, ALK, ATRX

In addition to GD2 researchers continue to search for unique features of the tumor that can lead to a cure. Scientists specifically look at the distinctive genes and mutations seen in a lot of cases and the effect they have on the outcomes.

Only less than two percent of patients have mutations in the signaling pathways involved with the sympathoadrenal line that causes familial neuroblastoma. PHOX2B was another mutation identified that promotes cell cycle exit and neural differentiation. Though in sporadic neuroblastomas, 6-10% carry somatic anaplastic lymphoma receptor tyrosine kinase (ALK) activating mutations, while 3-4% have a high frequency of ALK gene amplification. ALK plays a role in ensuring the balance between proliferation and differentiation. (PHOX2B and ALK gene have been linked because PHOX2B may directly regulate ALK expression) (Cheung,2013). ALK is expressed only in neural tissues, and mAbs designed to recognize ALK on neuroblastoma cell surfaces have shown increased ADCC of ALK-amplified NB cells. This ALK expression is linked to the MYCN expression (Jabbari,2019).

The most common focal genetic lesion in sporadic neuroblastoma is MYCN amplification. It is a major oncogenic driver since it controls proliferation, growth, differentiation, and survival of cells in the developing CNS (Cheung,2013). MYCN is expressed prenatally in different tissues, but its expression is lost during the first week postnatally. MYCN amplification is associated with metastases, reduced T-cell infiltration to TME, and invasiveness of the tumor and its progression during induction treatment. But MYCN-amplified patients were found to have better early response to treatment, however their survival rates were unaffected. Recent studies have employed miRNAs to suppress MYC family (Jabbari,2019).

ATRX mutation is another mutation seen in some neuroblastomas. ATRX is associated with increased telomere activity that is vital for the cancer cells to survive. This high telomere activity is found in 30% of neuroblastomas at diagnosis and is predictive of reduced EFS and overall survival. There are currently no molecular therapies targeting these pathways associated with telomeres (Cheung,2013).

NK cells are capable of inhibiting colony formation of human neuroblastoma cells and infusion of NK cells into mice bearing human metastatic neuroblastoma showing improvement in OS (Venstrom,2009). NK activity is regulated by inhibitory and activating signals following engagement of cell membrane receptors with their cognate ligands on target cells (Tarek,2012).

NK cells expressing inhibitory killer cell immunoglobulin-like receptors (KIR ligand) for self- human leukocyte antigen (HLA) class I molecules are equipped with effector function, ensuring that potentially autoreactive NK cells expressing KIR for non-self HLA ("missing ligand") are not able to work when they encounter cells lacking their cognate ligand. (Venstrom,2009). Untreated NB tumors and cell lines are widely reported to have reduced to no HLA class I expression, rendering them potentially susceptible to NK killings due to lack of engagement of HLA class I–specific inhibitory KIRs (Tarek,2012).

Clinically, the "missing ligand" KIR-HLA compound genotype is a strong predictor of response and survival. When treated with anti-GD2, patients with neuroblastoma who lack one or more HLA ligand for their inhibitory KIRs respond better to treatment, have lower rates of relapse, and survive longer compared with patients who possess

all HLA KIR ligand. This suggests that the unlicensed NK cells expressing KIRs for non–self-HLA ligands are more effective in tumor eradication than the licensed NK cells expressing KIRs for self HLA. HLA class I expression on neuroblastoma cell lines selectively inhibits licensed cell activity, allowing unlicensed NK cells to mediate ADCC, showing the importance of unlicensed NK cells for the antitumor role in patients undergoing mAb therapy. This explains the "missing KIR ligand" benefit in patients with neuroblastoma. Since licensed NK cells expressing S-KIRs have higher ADCC capacity in general, rescuing licensed NK activity from class I inhibition is advantageous because it could increase response in all patients, even those with all the KIR ligands present (Tarek,2012).

In addition, the use of exogenous NK cells in the treatment of patients with NB may be potentially useful if the patient lacks class I ligands for the donor inhibitory KIRs. Augmentation of innate immunity through adoptive transfer of allogeneic NK cells or the use of agents that increase endogenous NK cell number and activity, such as IL-2, lenalidomide, and anti-CD137 antibody, may all improve NB control, particularly in the presence of 3F8. While transplantation and mAb therapy are hardly normal physiological conditions, they both take advantage of the important pool of unlicensed NK cells, previously thought to be hyporesponsive and therefore potentially less clinically relevant. (Tarek,2012).

GM-CSF, IL 2, Retinoid Acid

Dinutuximab is augmented by GM-CSF and IL-2 because they stimulate the immune response and specifically the antitumor effect. IL-2 works to stimulate NK cells, and GM-CSF activates granulocyte and macrophage cytotoxicity (Armideo et. al.,2017). Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that enhances the activity of macrophages (Jabbari,2019). IL-2 is an important component of immunotherapy because it can improve the cytolytic function of NK cells against neuroblastoma cells. Though 23% of patients receiving IL-2 suffer from capillary leaks, IL-15 has been considered as an alternative to IL-2 for combination with anti-GD2 mAbs in neuroblastoma. Furthermore, administration of IL-15 is necessary for the NKT cells, an anti-neuroblastoma lymphocyte, to survive the hypoxic neuroblastoma environment (Cheung and Dyer, 2013). The International Society of Pediatric Oncology Europe Neuroblastoma (SIOPEN) has even removed IL-2 infusions with dinutuximab beta because of the toxicities related. They suggest dinutuximab beta and isotretinoin for maintenance therapy (Ladenstein et. al., 2020). The isotretinoin is used with anti-GD2 as a differentiating agent that induces the maturation of neuroblastoma cells since GD2 is a marker on only mature neurons (Armideo et. al., 2017).

Reasons CAR T does not Work in High-Risk Neuroblastoma:

Neuroblastoma is an extremely heterogeneous disease, meaning each tumor has unique molecular, cellular, and genetic features, all affecting the tumor's response to the treatment. It can continue to metastasize or even become refractory to a specific therapy. Furthermore, scientists are unsure how heterogeneity evolves with treatment and disease progression remains unknown. Neuroblastoma has a narrow epitope range hence the T cell-based therapy is not effective; therefore, antibody-based immunotherapy is used targeting GD2, and oncofetal antigens. Unlike its restriction in normal tissue, GD2 (disialogangliosides) present on tumors that arise from the neuroectoderm (neuroblastoma, melanoma, small cell lung cancer, and sarcomas) are expressed.. Also, there are few natural antibodies opposed to neuroblastoma. Further, fighting the rare variants of somatic mutations seen in neuroblastoma contrast with the lack of mutations in adult cancers. Then the intensive use of chemotherapy further damages those T cells that were attacking the neuroblastoma. Specifically, active adaptive immunity is harder for patients with high-risk neuroblastoma because of the primary and metastatic tumor's bulk together with its rapid proliferation that overwhelms the immature immune system in children (Cheung,2013). Another technique used to gain long term control over cancers is an immune checkpoint blockade that targets the immune system by activating previously exhausted or dysfunctional T cells. These mAbs (programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)) used in the blockade in adults' tumors are efficient, but in pediatric tumors, these checkpoint inhibitors have shown no significant benefit (Liu,2020).

Neuroblastoma recruits pro-tumor macrophages and silences natural killer (NK) cells. Furthermore, neuroblastoma evades T cells by downregulating or losing human leukocyte antigen (HLA) expression, but they simultaneously cause damage by re-expressing HLA to resist NK cell-mediated antibody dependent cell-mediated cytotoxicity (NK-ADCC). Once bound to GD2, anti-GD2 mAbs bind to Fc-receptors on the surface of granulocytes and neurokinin cells and eliminate NB cells through ADCC and CMC (Jabbari et. al., 2019).

Side Effects of Anti-GD2

As with all treatments when dinutuximab - using mouse

SP2/0 cells (and dinutuximab beta- In Europe) was recloned in Chinese hamster ovarian (CHO) cells), is administered, patients can experience a range of adverse effects (McKeage et. al., 2018; Ladenstein, 2020).

Dinutuximab beta was also found to be a cost-effective treatment option (Pennington,2019). Dinutuximab beta is an orphan medicine that is approved in the EU for the treatment of high-risk neuroblastoma in patients aged 12 months who have previously received induction chemotherapy, and achieved at least a partial response, followed by myeloablative therapy and SCT, relapsed or refractory neuroblastoma ± residual disease (Ladenstein, 2020). The continuous, 10-day infusion regimen appears to be associated with less toxicity than the once-daily infusion on 5 consecutive days (McKeage,2018). These side effects usually do not continue once treatment ends, but it may interrupt the treatment.

Doctors can prescribe medications during treatment to deal with some of the side effects. The most frequent of these is neuropathic pain. This appears to be caused by dinutuximab, which targets GD2, a protein that is present on the neuroblastoma cell and is also present on neurons and peripheral nerve fibers, which causes the pain (Bartholomew, 2017). The pain can be controlled to some extent by analgesic therapy, including intravenous morphine, prior to and during the infusion (McKeage et. al., 2018). Furthermore, infusing an antibody can cause severe reactions including anaphylaxis and cytokine-release syndrome (Bartholomew et. al., 2016). Since 39% of patients run a fever, acetaminophen is administered prophylactically prior to treatment (Armideo et. al., 2017). The fever may stem from a few different factors including, the IL-2 releasing pyrogenic factors, immune stimulation or the cytokines that are released when the antibody is administered (Bartholomew,2017). Patients may also require premedication of antihistamines before each infusion. Furthermore, Dinutuximab can cause hypersensitivity reactions (25%) and even more so when IL-2 and dinutuximab are administered together; this is due to immune stimulation and cytokine release (Aust Prescr,2020).

The 23% of patients that experience capillary leak syndrome (CLS) can be attributed to endothelial cell damage, a cytotoxic response of dinutuximab, GM-CSF, and IL-2. Common treatment for CLS uses furosemide, which has a higher association with hypokalemia (Bartholomew,2017). The rare side effect of a liver dysfunction can be evidenced through the elevated alanine transaminase (ALT), (23%); and aspartate transaminase (AST) (10%) along with electrolyte disturbances (Armideo,2017). A small population have ocular changes such as mydriasis and accommodation issues along with anisocoria and sluggish pupillary response; therefore, patients should be monitored for photophobia, papillary reactivity, and visual changes (Bartholomew,2017). Other common toxicities seen are infection (39%), hypokalemia (35%), hyponatremia (23%), gastrointestinal side effects (nausea, vomiting, diarrhea; 22%), hypotension (18%), hypoxia (13%), and urticaria. (Armideo,2017).

Immunosuppressive Environment:

Factors that are unique to pediatric solid tumors are the paucity of neoantigens, development of resistance, and an immunosuppressive environment. The neuroblastoma tumor microenvironment (TME) studies have identified tumor associated macrophages (TAMs) within the immunosuppressive microenvironment in neuroblastoma that specifically inhibit both innate and adaptive immune responses (Liu,2020).

Other immunosuppressive components affecting the T cells function in attacking the tumor are defects in antigen presenting machinery (APM) and low levels of MHC class I molecule displayed by neuroblastoma tumor cells that lead to decreased cytotoxic T-cell activation. Secretion of different solubles, including transforming growth factor-β (TGF-β), and galectin-1 by tumor cells, directly inhibits T cell function. Furthermore, some myeloid cells in the tumor may not fully differentiate into dendritic cells, macrophages, or granulocytes, but instead generate a heterogeneous population of immature immunosuppressive myeloid cells, MDSCs. Hypoxia-inducible factor 1α (HIF1α) is a soluble factor that promotes the differentiation of MDSCs into TAMs, creating a feedback loop to support immunosuppression. Targeting these MDSCs enhance anti-tumor immune responses in neuroblastoma, implying that MDSCs play roles in cancer-related inflammation, enhancing the tumor's progression (Liu and Joshi, 2020).

Cytokines can be used for intercellular communication; therefore, the cancer cells can use them to alter the TME according to their needs. When tumor cells release VEGF, which promotes angiogenesis within TME, it is usually associated with higher stages of neuroblastoma and its poor prognosis. IL-6 is a cytokine, that positively affects tumor growth and distant metastases while IL-10 is an immunosuppressive cytokine found in neuroblastoma. Blocking IL-10 receptors has been shown to enhance immune response to tumors and improve outcomes of treatment. Also, INF-γ, a cytokine, can also induce tumor regression by promoting ADCC (Jabbari et. al, 2019).

Traditionally the thymus eliminates T-cells through T-cell receptors (TCRs) with high affinity toward tumor (self)-antigens. T-cells with high-affinity TCRs can be eliminated or converted into regulatory T-cells (Tregs) (Jabbari,2019).

Treg cells exhibit their suppressive activity via several mechanisms including inhibition of antigen-presenting cell maturation through the CTLA-4 pathway, secretion of inhibitory cytokines such as IL-10, TGFβ, IL-35, and granzyme and perforin expression that kill effector T-cells. Studies are currently inconclusive of Treg's exact effect on patients with neuroblastoma, but they do correlate a higher proportion of Treg cells in the bone marrow and peripheral blood with MYCN amplification (Liu,2020). Since Tregs lack IL-7Rα, it can be exploited for increasing expansion and enhancing function of CAR T-cells without concomitant expansion of Tregs (Jabbari,2019).

Further challenges stem from the neuroblastoma cells. Gangliosides and sialic are highly expressed surface carbohydrates that are important for migration, adhesion, and metastasis, but are also poorly immunogenic and sometimes even immunosuppressive. Since natural anti-ganglioside antibodies are rare, it allows the neuroblastomas to survive in the circulation despite not having a complement decay accelerating factor (CD55). Myeloid suppressor cells, regulatory T cells, and it exploits protectin (CD59) to resist or suppress immunity (Cheung and Dyer, 2013).

Further Developments

Almost 80% of patients with the clinically aggressive disease do not show sustained responses to recent advances in anticancer therapy. More research is necessary to understand neuroblastoma's biology and an accurate representation of the tumor to identify agents that can be used in pediatric drug development. Additionally, since there is a low occurrence of neuroblastoma there are less clinical trials (Corallo et. al., 2020).

Current neuroblastoma studies primarily employ two-dimensional (2D) cell cultures. A three-dimensional (3D) culture would improve the research since it can reconstruct a physiologically relevant TME. Also, current models do not reflect the pediatric context of neuroblastoma, immature immune systems, differences in drug metabolism, and continuing developmental changes. A 3-D platform would also address the lack of clinical trials issue and can lead to more efficient treatment plans, hopefully reducing the exposure of pediatric patients to additional rounds of chemotherapy (Corallo et. al., 2020).

Scientists have looked at instances of spontaneously regressing neuroblastomas (NBL-4S) where an advanced metastasizing neuroblastoma that had spread to the skin, peripheral blood, bone marrow, and peripheral ganglia (but excluding the bone) suddenly regresses. This situation shows that neuroblastoma may be different and disconnected from the GD2 marker. Although GD2 is present in NBL-4S, the study showed its expression may be lower (this should infer a worst prognosis, but the exact opposite occurred). Scientists have not been able to define the immune response nature and signature of neuroblastoma rejection in NBL-4S, but they assume NK cells are involved (Rovigatti,2021).

Researchers are working on a vaccine to prevent neuroblastoma relapses. Once integrated to the cell genome, lifelong expression of transfecting genomes can detect neuroblastoma cells presenting the tumor associated antigens (TAAs). This is of utmost importance in cases of minimal residual disease. Vaccines have been directed against TAAs such as survivin, MYCN, and GD2 have been somewhat effective for this purpose. Retroviruses have been used to transfer IL-2 genes to neuroblastoma cells in mice and have resulted in sustained production of IL-2 and tumor growth control in these animal models, and neuroblastoma cells transfected with IL-1β and TNF-α by means of retroviral vectors showed tumor growth arrest in vitro (Jabbari,2019).

Neuroblastoma evades the attack of the mAbs by escaping to the CNS, which is not accessible to circulating antibodies (Cheung,2013). Studies are working to create an anti GD2 CAR T treatment to redirect T cells against GD2 (Prapa,2015). Though CAR T treatment has a long way to go until it will be effective; therefore, there is not sufficient evidence to abandon the advantages of passive immunotherapy with anti-GD2 monoclonals (Ugo Rovigatti.2021). Scientists used an anti-GD2 single-chain variable fragment (scFv) derived from a murine antibody of IgM class that was linked to the signaling domains of the costimulatory molecules 4-1BB (CD137) and CD3-ζ. The receptor was expressed in T lymphocytes. Then transduced T cells expressed high levels of anti-GD2 CAR into cultures that infiltrated the tumors and persisted into blood circulation inducing massive apoptosis of neuroblastoma cells and destroying the tumor growth. Since the preclinical had positive results, more clinical testing with this approach will be tested in neuroblastoma and other GD2-positive malignancies (Prapa et. al., 2015).

Conclusion

Cancer treatments have improved greatly throughout the years, but researchers are still looking for the best way to eliminate high risk neuroblastoma. The current treatment plans employ the use of an immunotherapy using mAbs that targets the GD2 antigens on the tumor cells. Although this treatment has toxicities while it is administered, there is an overall higher EFS rate in patients receiving it in their maintenance therapy. Sadly, the rate of relapse is still high with the GD2 treatments; therefore, researchers continue to search for better options.

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