

# Cell Based Treatment Approaches in The Headway for Neuroblastoma: An Outline



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## Abstract

Neuroblastoma is specifically the cancer of sympathetic nervous system rarely seen in neonates, hence called embryonal cancer. It usually occurs due to mutations observed in gestation period of child growth. Holistic approaches have been reported for the pharmacological management of the same. Both the approaches focus on the etiopathogenesis of the disease which is seen due to poor functioning of a noncoding mRNA (microRNA) which is regulated by MYCN transcription factor in mutant neuroblastoma cell. Recent approaches being developed are signaling the interactions of signaling proteins with the cellular processes. Pharmacological approach focuses on the nanomolecules targeting the mutant protein of neuroblastoma. Research suggests the MYCN oncogene contributes to high risk of neuroblastoma tumors in neonates which paves a way for advancements in the treatment of the disease both from the therapeutic and pharmacological perspective. Targeting the MYCN oncogene followed by inhibiting the proliferation of residual cells in the haemopoietic compartment can serve as an effective approach for the treatment. Advancements in the myeloablation chemotherapeutic regimen are also under trials. Chimeric anti-GD-2 monoclonal antibody has also shown anti-tumor activity in the initial phases of neuroblastoma which exhibits additive effect when given with granulocyte-macrophage colony stimulating factor (GM-CSF) which is usually considered a combination therapy for the treatment. The article presents the approaches in targeting the MYCN oncogene and the advancements in the myeloablation chemotherapy in combination with GM-CSF.

**Keywords:** Neuroblastoma, MYCN, oncogene, nanomolecules, GM-CSF, myeloablation

## Introduction

Neuroblastoma is a malignancy in children which generally accompanies no symptoms initially, but when the child attains a considerate age of five, symptoms like incompetence in daily activities and vigorous behavior are commonly observed. The extra-cranial tumor in the children is targeted by tumor initiating cells (TIC) to provide durable metastasis cure. The NB TICs withstand various characteristics of cancer stem cells of self-renewal. Though conventional therapies have not been successful in the treatment of NB, systemic study of cell-based treatment targeting SKPs promises to provide potent and less toxic approach for the NB treatment.(1,2,3,4) This article focuses on the approach to target NB TICs for the treatment of NB without injuring normal non-cancerous cells. Neuroblastoma is the cancer of sympathetic nervous system of the body which generally occurs when the fetus is in the development phase during the gestation period. It starts in the neuroblasts which are commonly understood as immature nerve cells that are on the verge of maturity.

The primary site where this disease develops is the supra-renal gland often referred to as adrenal gland.(5,6). The cancerous cells proliferate to other parts of the body like lungs, liver, lymph nodes, etc. Studies suggest that this fatal disease occurs in infants below 5 years of age (Fig.1). Irritability, pain, constipation, swollen belly, dark circles, weakness, fever are the common symptoms of this disease which are often misunderstood as other common diseases, and therefore it is very difficult to diagnose. The neuroblastoma have genetic predisposition. This type of cancer is well understood by classifying into various stages:

L1: In this stage, the tumor cells are confined to one place.

L2: In this stage, the tumor starts its proliferation.

M: Here, the tumor becomes malignant and proliferates to different parts of the body.

MS: This stage, being the last of above all stages, where the tumor reaches bone marrow of the child who is under 18 months of age.

The gene responsible for onset of neuroblastoma is MYCN gene. MYCN class of gene is the master gene that decides the fate of any cell. (6,7)

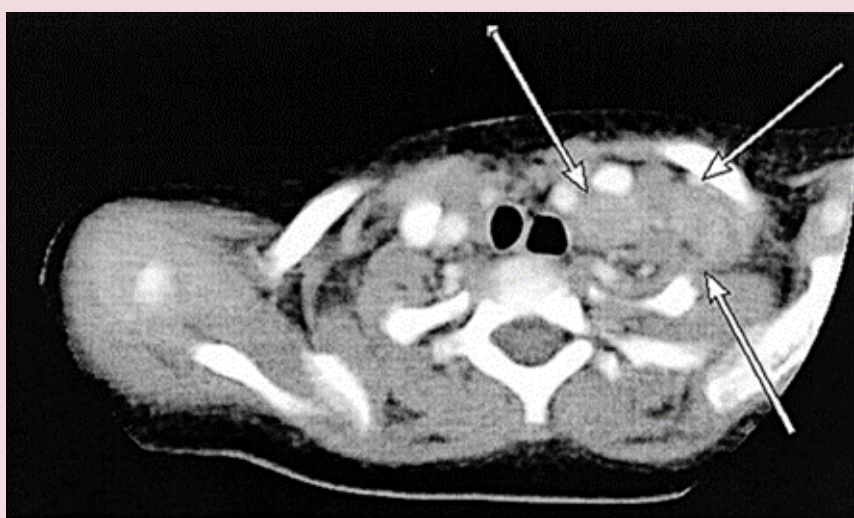


Figure: Computed tomography scan of neuroblastoma in a child

## Cell based approaches in the treatment of neuroblastoma

### 1.1 Anaplastic lymphoma kinase (ALK) targeted therapy in neuroblastoma:

The ALK is a receptor of tyrosine kinase family which is mainly observed in neonatal brains but its expression is least seen after birth. The ALK protein when dimerised, is responsible for the occurrence of neuroblastoma in children. This ALK protein binds with ALK inhibitors to decrease the dimerisation of ALK with each other producing oncogenic response in the infants. The current approach in the control of cancers like neuroblastoma is by binding to the ATP pocket of ALK protein which would block the proliferation of neuroblastoma oncogene.(8,9) Some approved ALK inhibitors are classified under first generation (Crizotinib), second generation (Alectinib) and third generation (Lorlatinib) according to the advancements in the mechanism of action. The fusion of C-terminal region of ALK, aids in the migration and ignorance of apoptosis. The ALK pathway signals a multiple routes of signaling like ALCL, NPM, STAT-3, STAT-5.(Fig 2). Studies show that this ALK protein if given in combination with the MYCN gene approach, creates a co-amplified effect. There are some drugs that are still under clinical trials like- PLB1003, Belizatinib, Fortinib, etc. Crizotinib is found to have a semi-inhibitory effect on the action of ALK protein, thus cannot be considered as a potent drug. (8,9,10) Drugs like Belizatinib and Fortinib are more potent as compared to Crizotinib.

### 1.2 Expression of EZH2:

Enhancer of Zeste homolog-2 is an enzyme which is encoded by EZH2 gene. This EZH2 locus encodes gene silencing via histones. Intratumoral EZH2 inhibits the adaptive response of the immune system. In addition to the EZH2 gene, MHC-1 screening revealed that these two in coordination can be a head start in the advancement of cell based therapy for neuroblastoma. This gene and proteins are responsible for healthy embryonic development of the fetus. This EZH2 protein is responsible for the methylation of H3K27 variety of tumor suppressor gene. EZH2 gene is seen to cause oncogenic activity leading to neuroblastoma like other cancerous diseases.(10) .Over expression of the gene while coding for the protein shows onset of oncogenic activity. EZH2 is made to suppress to diminish the oncogenic activity which leads to neuroblastoma. EZH2 is made to suppress tumorigenesis thereby decreasing proliferation and motility of neuroblastoma. Also, a protein called Focal Adhesion Kinase (FAK) is also responsible in neuroblastoma tumorigenesis. Inhibition of EZH2 also helps to affect FAK expression. It helps in the suppression of MHC-1 pathway in turn inhibiting the T- cell activation thereby treating cancer to a great level. EZH2 also controls the adaptive response to the Treg activity. These are considered as the subtype of T-cells that induce immunotolerance to the cancerous cells. Treg cells are known for the activation of cytotoxic and helper T-effector cells, which triggers potent anti-tumor response. (10,11)

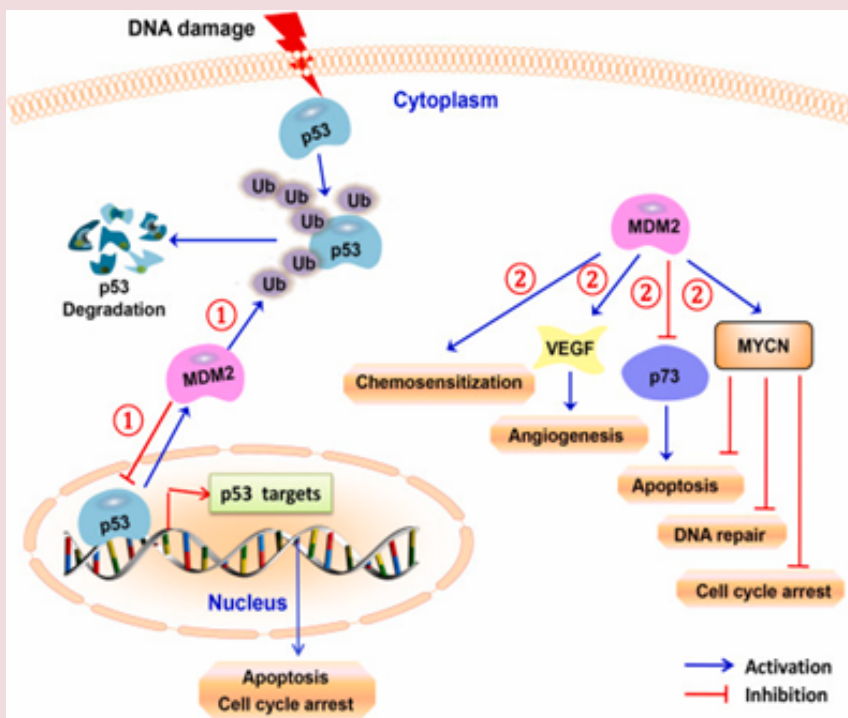


Figure 2: ALK Pathway in Neuroblastoma

### 1.3 Inhibiting anti-apoptotic protein:

Apoptosis is referred to as programmed cell death. The cell death is delayed as in case when the anti-apoptosis proteins are given to treat neuroblastoma. To escape apoptosis, cancer cells possess high level of anti-apoptotic proteins. To get through cancer cells growth, inhibition of apoptotic protein helps to a great extent. IAP (inhibition of apoptic protein) and Bcl-2 (B- cell lymphoma-2) are very good family of proteins that help in the treatment of neuroblastoma to a great extent. These two approaches have additive effect in chemotherapy. The apoptotic protein follows a TGF-beta signaling pathway and acts in double shielding against cancer

especially in neuroblastoma, B-cell lymphoma results in the changing of the mitochondria membrane which leads to cascade of protein activation like Bcl-2, Mcl-1, etc. which classically reduces apoptosis.(11) There are various mechanisms responsible for the same. The first approach being the direction of anti-sense oligonucleotide against the mRNA of targeted protein and second being that of BH-3 proteins which mimics the anti-cancerous activity of Bcl-2 proteins, inducing programmed cell death or apoptosis. Some drugs like Abatoclox, Navitoclox are under clinical trials for the same activity. (12) (Fig 3).

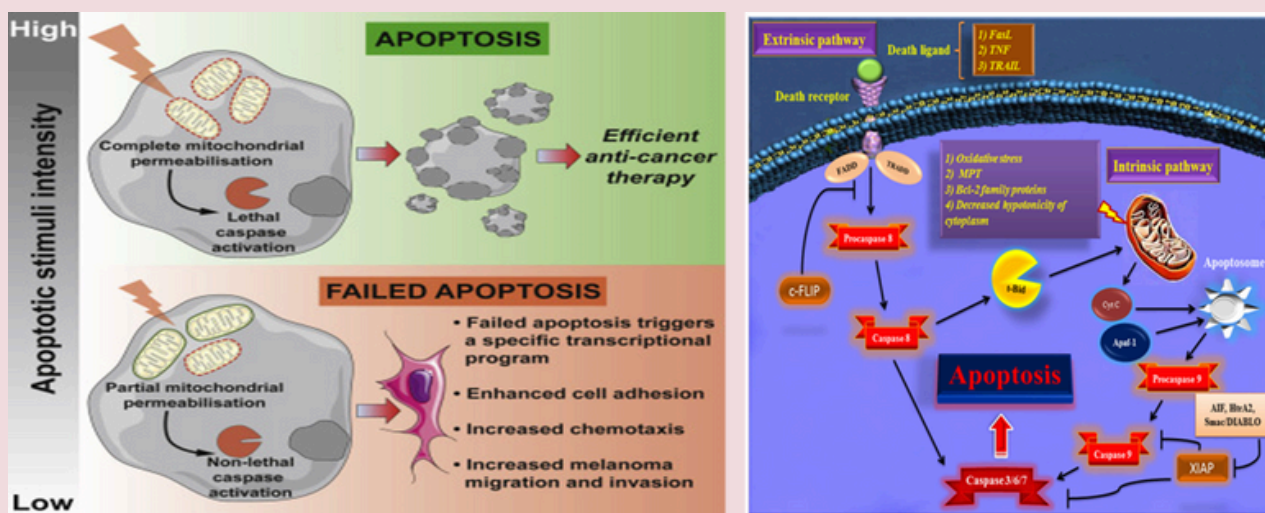


Figure 3: Apoptosis: Programmed cell death

## Conclusion:

Therapeutics including different strategies have made advancements in the treatment of fatal disease like neuroblastoma. One way to improve treatment strategies is having a better understanding of the cancerous cells. If the cancerous cells can be specifically targeted for a certain therapy, with the non-cancerous cells remaining unharmed, better prospects for targeted and personalized treatments can be created. EZH2 expression of gene has led to the advancements in the science of treatment of neuroblastoma. The MYCN gene is responsible for the proliferation and the self-destruction of cancer-causing cells, nearly similar to apoptosis. Both of these approaches can be used for the effective treatment of neuroblastic cells.

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## Fun & frolic – Wordsearch

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