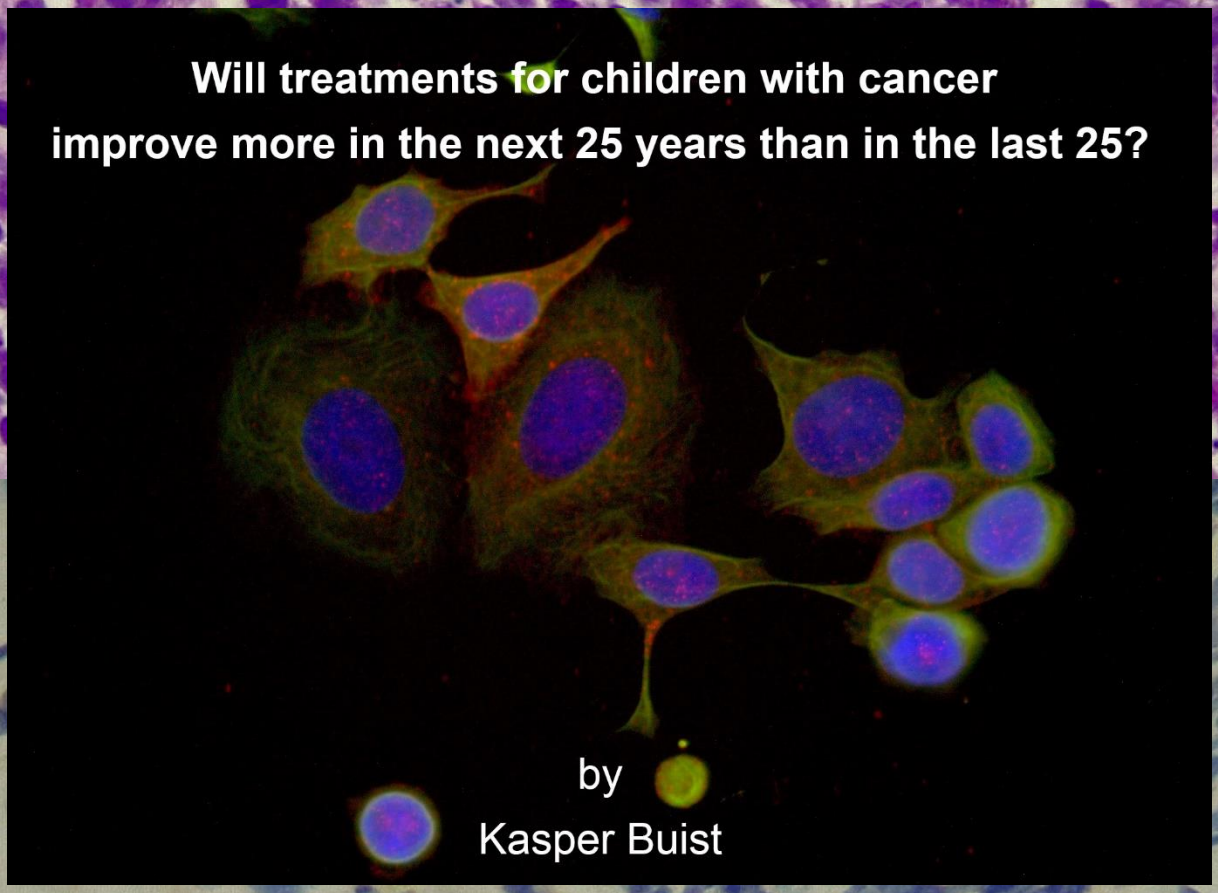
Will treatments for children with cancer improve more in the next 25 years than in the last 25?



by
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INTRODUCTION

This is a circulating rhabdomyosarcoma cancer cell from a teenage child. Rhabdomyosarcoma is the most common soft tissue sarcoma in children and accounts for around 6% of childhood cancers.[1]

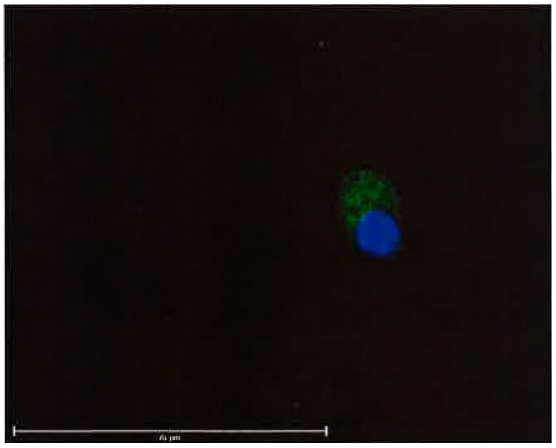

Figure 1 - Circulating tumour cell

In the UK, 1 in every 450 children under 15 develops a cancer.[2] About 1 in 5 of these children will die within 10 years of diagnosis. [3] Although there are treatments, they are still failing many children.

Figure 2 - Facebook RMS post

EFFICACY OF CURRENT TREATMENTS

Azra Raza uses the phrase 'slash, poison, burn' to describe the standard of care treatment protocol currently used in the treatment of children with cancer - surgery, chemotherapy and radiotherapy.

The rhabdomyosarcoma pictured in Figure 1 above was treated with a year of chemotherapy using the following highly toxic drugs. They are listed below in order of the year in which they were approved.

Year approved	Drug name	Type of agent
1963	Vincristine	plant alkaloid
1964	Actinomycin	antitumour antibiotic
1959	Cyclophosphamide	alkylating agent of the nitrogen mustards group
1987	Ifosfamide	alkylating agent of the nitrogen mustards group
1989	Vinorelbine	plant alkaloid

Table 1 - chemotherapy drugs used to treat rhabdomyosarcoma

Some of the chemotherapy drugs being used in 2022 were approved around 60 years ago. The newest one is 33 years old. Nitrogen mustards were banned in warfare by the Geneva protocol of 1925 as their use is considered inhumane.[4]

This project will explore whether there has there been any progress in the treatment of children with cancer in the last 25 years and what the next 25 years may hold. In evaluating this, we look at three criteria: the number of children who survive, number of years of survival and quality of survival and examine whether the gap between survival and healthy survival can be closed.

Number of Children Who Survive

	2001	2018	2022
> 5-year survival rate	75.2%	81.1%	84.0%

Table 2 - as Office of National Statistics and Cancer Research UK[5] Cancer Research UK[6]

The 8.6 percentage point rise in survival rates between 2001 and 2022 is due mostly not to new treatments but to refinements in the protocols. For example, more accurate staging derived from accumulated experience, facilitates better judgements of how much chemo to give for how long and whether a patient requires additional radiotherapy or not. An increasing willingness to use adult drugs on teenagers and the success of trials using combinations of chemotherapy simultaneously has also improved treatment efficacy. Life expectancy and quality of survival, however, remain highly compromised.

Number of Years of Survival

According to JAMA Oncology in their 2020 study over 3 decades, life expectancy of survivors of childhood cancer remains compromised for decades (especially where radiotherapy is given). The report highlights the need "to manage late mortality risks and … for new therapeutic approaches to minimize early mortality risks."[7]

Quality of Survival

According to the Lancet Regional Health – Europe study (January 2022), 95% of childhood cancer survivors will experience a major health issue by the age of 45.[8]
About 2 out of 3 cancer survivors will develop at least one late effect at some point in their lives which range from handicaps such as learning problems, hearing and vision loss, to fertility issues and

the increased risk of future cancers. The British Childhood Cancer Survivor Study found that the 'absolute excess risk of a second malignancy' was 19%.[9]

Recent Momentum for Childhood Cancer Research

The Human Genome Project which was completed in 2003 has led to a far greater understanding of the molecular features of cancer and which gene alterations are implicated yielding new potential treatment targets. It has also ignited an explosion of new research. In addition, favourable legislation earmarking funds for childhood cancer research in the US has directed resources towards childhood cancers many of which might otherwise be too rare to attract investment in cures.

In the US, in the last 2-3 years there were 8 new treatments developed for children with cancer compared to just 47 over the previous 70 years.[10]

However, to keep this in perspective, the stark reality for many areas of childhood cancer is that progress is at best slow and often non-existent. To take our example of rhabdomyosarcoma, according to this 'Frontiers in Oncology' paper:

> *"Over the last four decades, there have been no significant improvements in clinical outcomes for advanced and metastatic rhabdomyosarcoma patients, underscoring a need for new treatment options for these groups."*[11]

THE BIOLOGY OF CANCER

What is Cancer?

NHS definition: "Cancer is a condition where cells in a specific part of the body grow and reproduce uncontrollably." [12] The cancerous cells can invade and destroy surrounding healthy tissue, including organs. Cancer begins in one part of the body, before sometimes spreading to other areas, a process known as metastasis.

What Biological Mechanisms Create and Nurture Cancer?

There are 6 biological capabilities driven by genetic mutations occurring through the multistep development of the tumour that are necessary for cancer growth and progression shown in Figure 3 - The Hallmarks of Cancer below. The following diagram was created to rationalise the complex conditions that cause tumour growth.

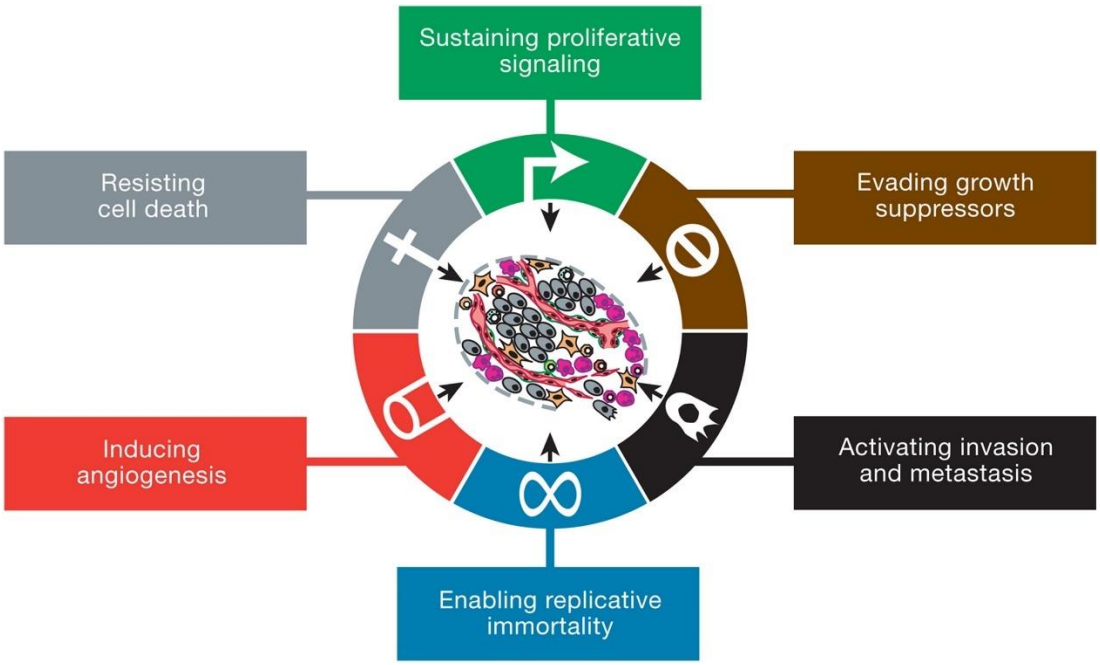

Figure 3 - The Hallmarks of Cancer[13]

Uncontrolled Cell Growth ⇨ *the malignancy of cancer*

Enabling replicative immortality: the in-built replication limit is breached thus enabling limitless cellular replication.
Inducing angiogenesis: through vascularization – the ability to generate new blood vessels required to sustain growth.
Resisting cell death: the evasion of the mechanism that programmes cell death (apoptosis) once cells become damaged.
Sustain proliferative signaling: through stimulation of the tumour cell's own growth making it self-sufficient in growth signals.
Evading growth suppressors: resisting signals that might otherwise inhibit growth.

Inappropriate cell motility and migration ⇨ *metastatic cancer*

Activating invasions and metastasis: the ability to grow into nearby environments and to migrate and establish in distant environments.

In addition to these core hallmarks, two further hallmarks have been determined ("Hallmarks of Cancer: The Next Generation"; Hanahan & Weinberg[14]):

Deregulating cellular energetics: the metabolic rewiring to fuel the additional energy requirements of proliferative growth.
Avoiding immune destruction: the mechanism through which the immune system can be disabled or manipulated.

Genetic Alterations Make Normal Cells Cancer Cells

Cancer cells have combinations of gene mutations, gains (amplifications) or losses and/or over/under expressions. When certain alterations occur in certain oncogenes these genes can transform cells into tumour cells carrying the hallmarks of cancer shown in Figure 3. This means that the hallmarks are the end result of a cascade of genetic alterations to somatic cells, which accrue and reach a tipping point, when tumorigenesis can occur.

Oncogenes are genes with the potential to cause cells to be cancerous if they alter. Tumour cells frequently show mutations in several oncogenes. In most cases *over-activity* in oncogenes makes the cells possessing them cancerous. For example, oncogenes can encode modified proteins called **transcription factors** which then turn gene expression up or down like volume control. This can, for example, cause cells to multiply uncontrollably.

Tumour suppressor genes act to prevent oncogenic activity. They are sometimes called anti-oncogenes. When tumour suppressor genes develop genetic mutations, they can no longer ensure that the hallmarks of cancer are not established in the cell. It is *under-activity* in anti-oncogenes that usually make the cell cancerous. For example, **Cell cycle checkpoint genes** are genes which control potential termination points along the cell cycle. If the cell DNA is not faithfully replicated during cell division, these genes are responsible for arresting replication. If the checkpoints are defective, the hallmarks of cancer can become established.[15,16]

Figure 4 below shows the cell cycle: the Growth, DNA synthesis and Mitotic (cell division) phases and the check points which regulate the process. Combinations of genes provide instructions to encode the proteins which direct each stage of the process and also the checkpoints. It is the failure to control the cell cycle properly that leads to **uncontrolled growth**.

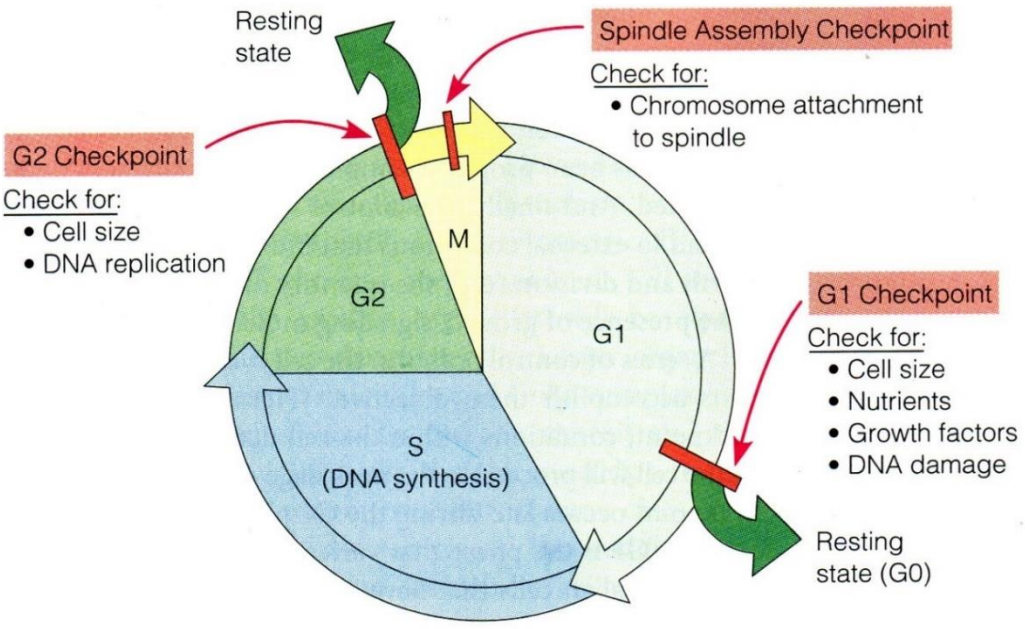

Figure 4 - Checkpoints of the cell cycle (Quora) [17]

Metastatic Cancer

The process by which metastasis occurs is called the metastatic cascade and broadly comprises invasion, intravasation, extravasation and metastatic colonisation.

Invasion into surrounding tissues occurs via a complex set of cellular changes including loss of cell-cell adhesion (ie ability to break away), the secretion of substances that degrade the extracellular matrix and basement membranes (permitting passage into different tissue) and at the same time the inhibition of the expression of proteins controlling motility.

Phenotypic plasticity (the ability of cells to change their type) is a key feature of metastasis.
Sometimes this can involve epithelial-mesenchymal transition (EMT) which results in "the acquisition of a migratory mesenchymal phenotype."[18]

In **intravasation**, cells penetrate through the endothelial barrier to travel via the lymphatic or vascular circulation systems.

The metastatic cells, having mobilised via hematogenous routes, invade the vascular basement membrane and extracellular matrix in the process of **extravasation**. Ultimately, cells that survive 'anchorage-independent' conditions in the bloodstream will thus attach at a new location and proliferate to produce the secondary tumour resulting in **metastatic colonisation**.

However, there is increasing evidence to suggest that the process described above may not be a linear cascade and that primary tumour cells can enter the circulation even *before* stromal invasion. This may also explain why liquid biopsies can reveal circulating tumour cells such as the rhabdomyosarcoma cell pictured on page 1, in the absence of any discernible tumour mass being in evidence on MRI or CT PET scans.[19]

Metastasis may be one of the first crucial events to occur rather than a late-stage development and it is the most life-threatening event in the progression of a cancer. The complexity of the process and the uncertainty even as to how and when it occurs, further defines the sophisticated, multiplex nature of the disease.

References[20,21]

TREATMENTS CURRENTLY AVAILABLE FOR CHILDREN WITH CANCER

The cancer cell	The immune system	Intra and inter cellular signaling pathways
Surgery Radiotherapy Chemotherapy	Immunotherapy	Molecular targeting

Table 3

Current standard of care treatments are likely to comprise a combination of surgery, chemotherapy and radiotherapy known as 'slash, poison, burn' protocols.

Surgery

Most childhood cancer patients will experience surgery whether before or after drug protocols or radiotherapy. It has an important place in the treatment of cancer as it allows the debulking or removal of the tumour tissue. The extent to which this is possible and whether clear margins are achieved will influence treatment intensity. In addition, the tumour tissue can undergo molecular analysis which will allow for exact diagnosis and increasingly provides molecular information that can steer treatment protocols.

The Limitations of Surgery

In addition to surgical risk, there is often a quality-of-life impact – body parts may be removed and bodily functions compromised. Some tumours are inaccessible which means that surgery cannot be used in all circumstances.

Chemotherapy

How Chemotherapy Works

Chemotherapy targets cells that are in the process of splitting into two new cells. Body tissues are made of billions of individual cells. Once we are fully grown, most of the body's cells don't often divide except to repair damage. This means that more destruction is done by chemotherapy to cancer cells than to healthy cells. However, the gut and stomach lining, hair follicles and bone marrow producing blood cells are also always dividing, hence common side effects of chemo such as nausea, hair loss and neutropenia.

Figure 5 describes cell division. It is this process that is targeted by chemotherapy.

G0 phase (resting stage): Cells that are not dividing or preparing to divide carry out the bodily functions they are designed for. Cells remain in this phase for a large portion of their life. Once a signal is received to divide, the cells move into G1 phase.
G1 phase: The cell grows in size and then prepares to divide by making more proteins, RNA and organelles needed to copy DNA, and stores nutrients to fuel the process.
S phase: A duplicate set of chromosomes is created with two identical sets of DNA.
G2 phase: In this rapid growth phase, the cell prepares to split.
M phase (mitosis): The cell splits into 2 identical cells.

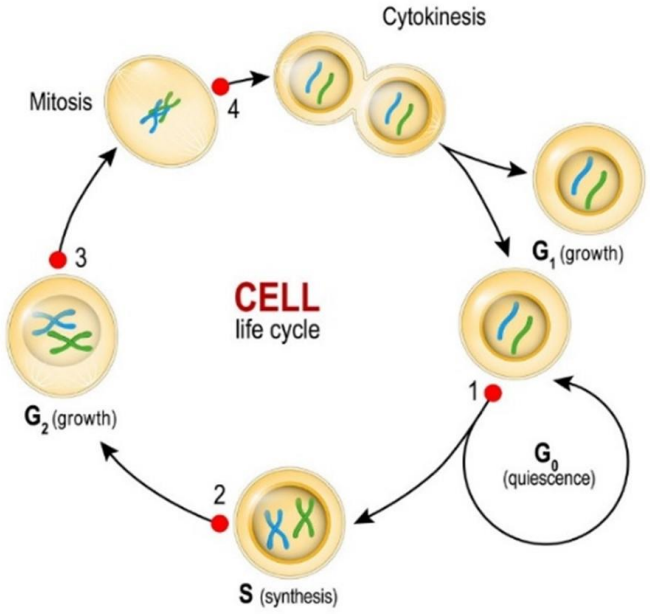

Figure 5 - The cell cycle (Vector Stock image)

Chemotherapy and the Cell Cycle

There are broadly 4 types of chemotherapy which interfere with steps in the cell growth cycle:
Alkylating agents such as nitrogen mustards bond covalently to guanine bases in the DNA and thereby eventually break the DNA chains, preventing DNA replication
Antimetabolites which are inhibitors of DNA synthesis
Anti-tumour antibiotics which bind to DNA
Plant alkaloids are spindle poisons which are antimicrotubule agents that inhibit mitosis

The Limitations of Chemotherapy

There is no way of targeting only cancer cells. Cancer cells do not divide faster than normal cells, it is the fact that they keep on growing (no matter how slowly) without stopping, that makes them cancer. Furthermore, senescent cancer cells, cancer stem cells and or otherwise dormant cancer cells do not replicate frequently making them poor chemotherapy targets.[22]

Cytotoxicity is the major limiting factor for chemotherapy. Doses required for a cure may not be tolerated by the patient – often patients have to choose damage or death by chemo, against destruction or death by cancer. Injury from chemo-toxicity manifests in multiple and debilitating short term side effects including nausea, hair loss, chemo-induced peripheral neuropathy (eg losing the fine motor skills of the hands), neutropenia which compromises the immune system's ability to respond to certain threats, and fatigue, as well as a suite of long effects negatively impacting quality of life both during the treatment and for the rest of life. "The therapeutic efficacies of most chemotherapy drugs are severely hampered by toxicity profiles that limit their life-extending potential."[23]

Radiotherapy / Brachytherapy / Proton therapy

The use of radiation therapy to treat cancer dates back to 1896 soon after the discovery of the x-ray.[24] Radiation works by damaging the genes (DNA) in cells using ionizing radiation delivered by a linear accelerator.

Ionizing radiation forms ions (electrically charged particles) in the cells of the tissues it passes through. It creates ions by removing electrons from atoms and molecules. This can kill cells or change genes, so the cells stop growing.

Different Kinds of Radiotherapy and How They Work

Photon radiotherapy is a therapy using x-rays and gamma rays to control or kill malignant cells and is normally delivered by a linear accelerator.
Proton radiotherapy is a type of particle radiation that uses a beam of protons to irradiate diseased tissue with pinpoint precision but has less general coverage than photon radiotherapy.
Brachytherapy is a form of radiation therapy where a sealed radiation source is placed inside or next to the area requiring treatment.

The limitations of radiotherapy

Radiation toxicity affects general health and normal dividing cells at the time of treatment, but flesh and organs can also show damage later. This includes radiation burns which often reoccur long into the future in the distressed tissues. Moreover, radiation-induced DNA damage, which causes gene mutations, cause future cancers in around 19% of childhood cancer survivors.[25]

The whole body cannot be irradiated; therefore, focus must be on one tumour at a time. If the cancer has metastasised to many locations, the damage of radiotherapy to multiple parts of the body would be prohibitive.

CANCER MECHANISMS OUTSIDE THE SCOPE OF CURRENT TREATMENT PROTOCOLS

Both radiotherapy and chemotherapy target cancer *cells.* They do not tackle **signaling pathways** through which cancer communicates and directs its propagation, the manipulation of the **immune system function** by cancer, **cancer cells which are treatment-resistant** or the cancer-nurturing **tumour microenvironment**.

Current Treatment Protocols:

Do not address: Signaling Pathways

Signaling pathways operate both within a cell and between cells, including those of the immune system. Cancer hijacks or changes signaling pathways to promote its own growth and progress.

During **Signal transduction** a chemical or physical signal is transmitted through a cell. Proteins called receptors are responsible for detecting stimuli in the form of ligands which bind to the protein. The changes elicited by ligand binding in a receptor give rise to a biochemical cascade. This chain of events is known as a signaling pathway.

When signaling pathways interact with one another they form networks, which allow cellular responses to be coordinated as shown in Figure 6 below:

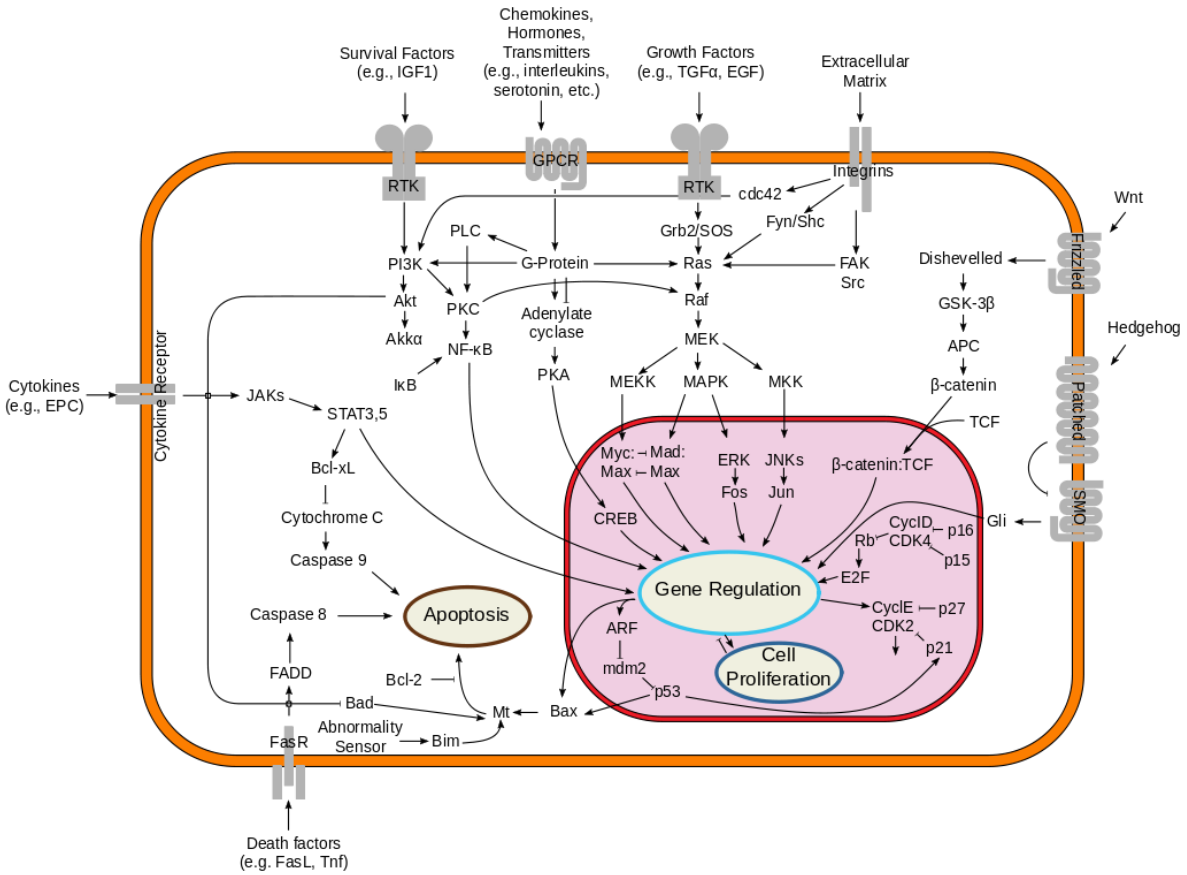

Figure 6 - Signal transduction (Wikipedia) [26]

Genes provide instructions for making proteins in the signaling pathways. Gene mutations that result in the failure to produce the protein, or the production of an altered protein, or more protein, will disrupt the signaling pathways which can result in any of the hallmarks of cancer being established.

Most oncogenes are part of a “chain of command” by which external signals, especially protein growth factors, normally stimulate cell growth and division.

Chemotherapy and radiotherapy do not seek to address any cancer-promoting signaling.

Do not support and can impede: Immune System Function

For the body to get rid of cancer, with or without the help of treatment protocols, the adequate and timely actions of the immune system are critical.

Immune System Overview

The human genome is more than three billion letters long and our body acquires trillions of new mutations every day[27] with increasing frequency as we age. The rate of mutations is also affected by epigenetic factors such as environmental exposure to toxins or UV light. However, most of these mutations are immaterial to the successful functioning of our bodies.

The immune system can recognize proteins and glycoproteins, which are expressed on pathogens or unhealthy cells, including virally-infected or cancer cells, and kill these "stressed" cells by orchestrating a coordinated immune response using both innate and adaptive systems. Cancer occurs when the mutation cascade culminates in cells with the hallmarks of cancer and with immune evasion capabilities. Cancer cells typically have at least 60 mutations.[28]

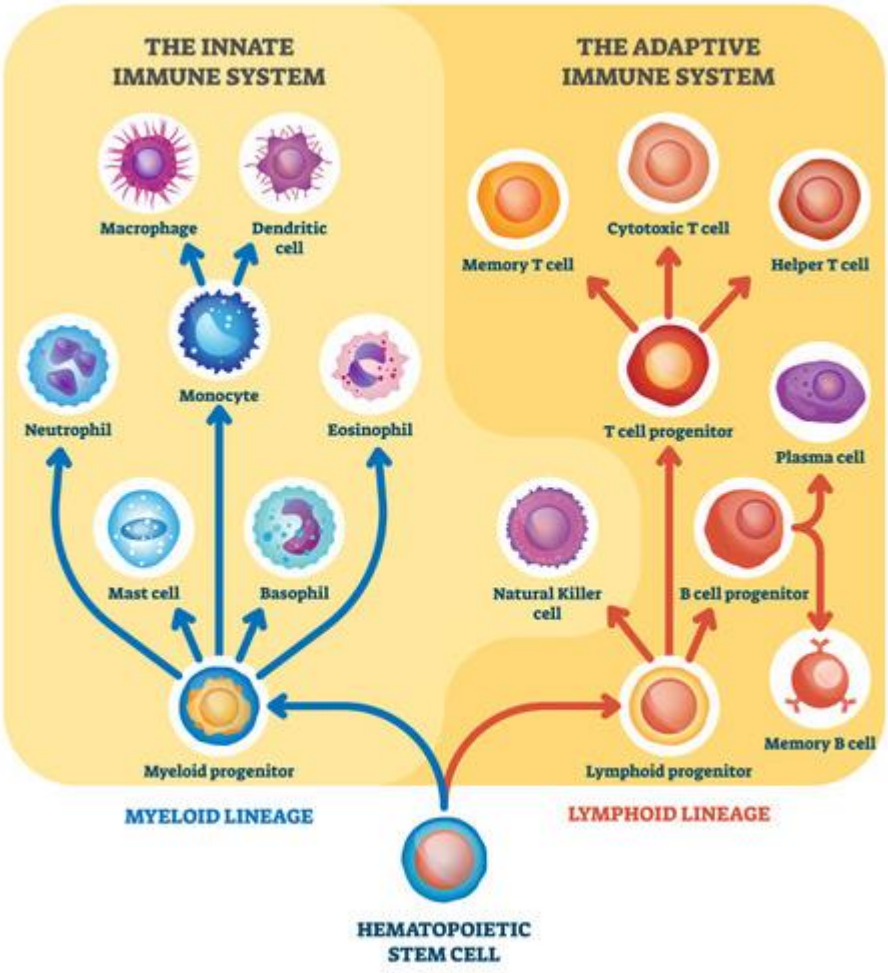

Figure 7 - Cells of the Immune System (Shutterstock image)

Innate immunity is the body's first and fastest immune response. Only around 10 percent of the lymphocytes in the blood are activated by the innate immune system and almost all of these are natural killer cells. These cells recognise proteins which are expressed on tumour cells and target and kill them.

Adaptive immunity is a response to specific antigens on the tumour cell. The response, mostly comprised of T and B lymphocytes, develops antibodies that kill the cancer cells. An immunological memory of each tumour antigen is retained. This leads to a faster innate response if these antigens are recognised again.

Most immunotherapies focus on adaptive immunity. [29]

Immunoediting Capabilities of Cancer

Although someone with a weak immune system may be more susceptible to cancer, the disease is by no means dependant on an immune-compromised host. Cancer, by definition, defeats the immune system using remarkably complex interconnected strategies.

For example, tumour cells can: **disguise themselves** (through the type of proteins they present) to go undetected and evade the immune system; **mutate quickly and extensively** so the adaptive immune system can't keep up or T-cell exhaustion occurs; **appropriate the immune system**, directly signaling to **create a tumour microenvironment that is immune restrictive** by, for example, emitting immunosuppressive cytokines (proteins) that cool down the immune system; **sedate or deactivate attacking immune cells** such as T cells by, for example, removing their mitochondria or blocking checkpoint proteins on T cells to prevent them from taking action.

Illustration of checkpoint blocking action:

The immune system cells first need to check if a cell is normal or unhealthy. Tumour cells present abnormal antigens that trigger the following anticancer response which is called **the cancer immunity cycle**.

> "
> 1. Tumor cells produce mutated antigens that are captured by dendritic cells
> 2. *The dendritic cells prime T cells with tumour antigen, and [these then] stimulate the activation of cytotoxic T cells*
> 3. *The [antigen-specific] T cells then travel to the tumor and infiltrate the tumor environment*
> 4. *The activated T cells recognize and bind to the cancer cells*
> 5. *The bound effector T cells release cytotoxins, which induce apoptosis in their target cancer cells."* [30]

Checkpoints ensure that healthy cells are not attacked (if the checkpoints fail, autoimmune disease occurs). This is done by the expression of immune checkpoint proteins such as PD-L1 which inhibit T cell activity. Cancer can exploit these checkpoints to evade immune detection by overexpressing PD-L1 which then binds to PD-1 receptors on activated T cells. This deactivates the T cell.

Interactions between cancer cells are elaborate and complex. Perfectly timed, primed, and located immunity is essential to fight cancer. **Chemotherapy and radiotherapy treatments not only do not use or help the immune system to tackle cancer, they themselves cause systemic immuno-compromise.**

Do not tackle: <u>Cancer Cells which are Treatment Resistent</u>

Certain types of cancer cell, such as cancer stem cells, senescent, and dormant cancer cells can elude treatments. According to Cancer Research UK

> *"…it is unlikely that any chemotherapy treatment kills every single cancer cell in the body…"* [31]

Cancer Stem Cells

Figure 8 below illustrates how annihilating the minority cancer stem cells (CSCs) has a greater effect on disabling tumour progression than killing a much larger number of non-stem tumour cells.

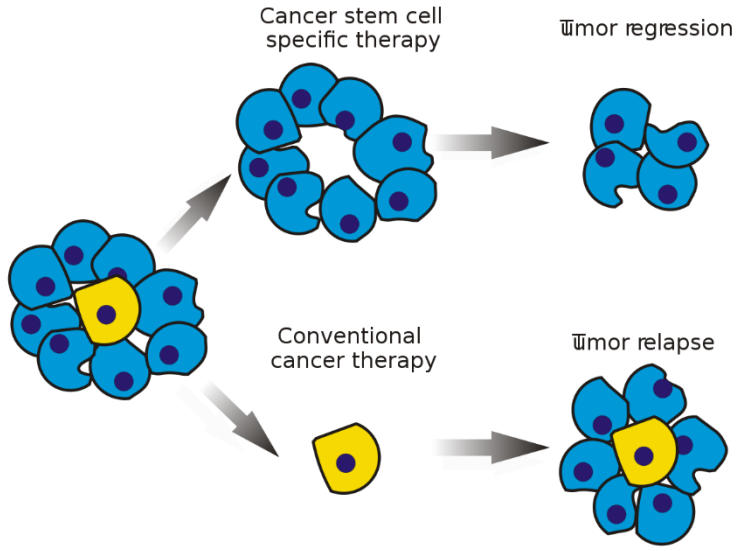

Figure 8 - Cancer Stem Cell (Wikipedia) [32]

Like normal stem cells, cancer stem cells can both self-renew and also generate differentiated cells, and, likewise, they demonstrate:

- relative quiescence (infrequent cell-division)
- expression of multidrug resistance transporters
- DNA damage repair enzymes
- modified metabolism
- resistance to oxidative stress.

On account of these features, chemotherapy and radiotherapy may be effective against the bulk of the tumour but typically fail to eliminate cancer stem cells and worse, can actually create them.

> *"radiation can induce the generation of fresh CSCs [cancer stem cells] from non-stem cancer cells and… the novel CSCs exhibit radioresistant traits"*[33]

According to a 'Frontiers in Oncology' report, "The failure to eradicate CSCs during the course of therapy is postulated to be the driving force for tumour recurrence and metastasis."[34]

It has also been found that cancer stem cells possess the phenotypic plasticity to undergo lineage reprogramming (transdifferentiation) enabling them to substitute normal host cells.

> "certain tumours may acquire stromal support by inducing some of their own cancer cells to undergo various types of metamorphosis to produce stromal cell types rather than relying on recruited host cells to provide their functions."[35]

Transdifferentiation of CSCs again highlights the limitations of treatments aimed at simply killing cancer cells.

Dormancy

Dormancy occurs when cells remain viable but stop proliferating. When a single cancer cell stops proliferating but remains viable it is called quiescence. When the majority of tumour cells are quiescent, tumour dormancy occurs. Dormant cancer cells are implicated in therapy resistance.

> "Cancer stem cells (CSCs) share several overlapping characteristics and signaling pathways with dormant cancer cells, including therapy resistance, and an ability to metastasize and evade the immune system."[36]

Senescence

Senescent cancer cells have irretrievably lost the ability to grow and divide. If this is due to chemotherapy it is called therapy-induced senescence (TIS). Senescent cancer cells are nevertheless, highly problematic in causing a tumour promoting effect in the tumour microenvironment due to the senescence-associated secretory phenotype.[37]

Current Protocols do not eliminate CSCs, senescent or dormant cancer cells, which are implicated in cancer's resurgence and therapeutic resistance.

Do not address the cancer-nurturing: Tumour Micro Environment (TME)

The tumour microenvironment describes the molecules, cells and blood vessels in the immedidate vicinity of the tumour. Chemotherapy and radiotherapy do not address the tumour microenvironment which provides perfect conditions for tumour progression and recurrence.

We have already highlighted that cancer modifies the components of the immune system in the TME creating a tumour-supportive environment.

> *"Cancer stem cells not only adapt to changes in the tumour microenvironment but also affect the TME. Concurrently, the microenvironment also promotes the self-renewal of cancer stem cells, induces angiogenesis, recruits immune and stromal cells, and promotes tumor invasion and metastasis"*[38]

A very graphic example is illustrated in the article "Intercellular nanotubes mediate mitochondrial trafficking between cancer and immune cells" Nature Nanotechnology[39]

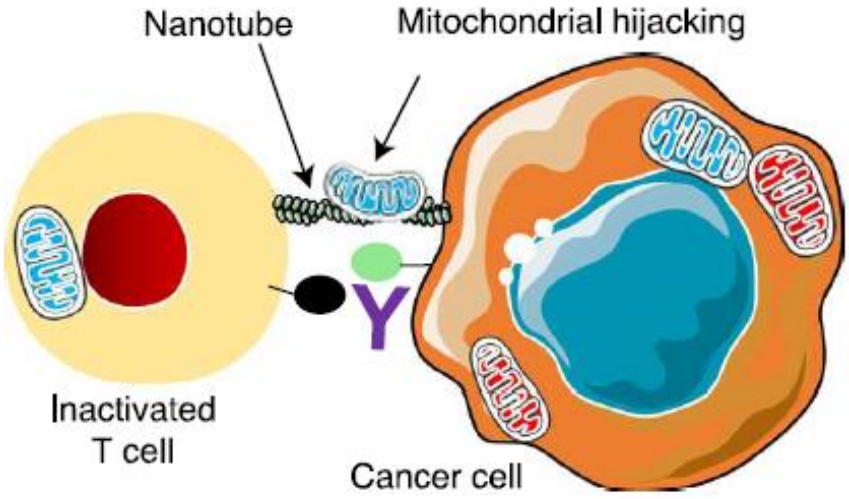

Figure 9 - Proposed model for immune evasion

Figure 9 above illustrates how T cells that are critical to the immune response to cancer are deactivated in the tumour microenvironment by cancer cells infiltrating them with nanotubes and extracting their mitochondria which removes the immune cell's capability for respiration and energy production.

Recent research suggests that cancer cells can switch between viable and non-viable cells depending on cues in the tumour microenvironment. [40] This can be instigated by treatments:

> *"It is well-known that cancer stem cells are resistant to treatment and can cause tumor relapses. However, under the therapeutic pressure and changed microenvironment* ***cancer stem cells can be newly generated****. In this case, these cells do originate from non-cancer stem cells or from therapy-induced senescent tumor cells"*[41]

Current Protocols Demonstrate Benefit with Limitations

As well as the physical mutilation from surgery, and treatments that damage healthy cells, current standard of care protocols also compromise the immune system – the body's own defence against cancer - promote a tumour supportive tumour microenvironment, and can even induce the creation of therapy resistant cancer stem cells.

The slash / poison / burn protocol is therefore at once immediately and in the future damaging, and yet unlikely to eradicate all cancer cells from the body. Those remaining are, by definition, immortal.

Meanwhile we have seen how tumour cells can direct or misdirect the immune system, hijack immune resources, control intra and inter cellular signaling pathways, potentially metastasise even before the first tumour is visible to the naked eye, create or maintain forms that are elusive, and even change format. Crude treatment options appear to be no match for cancer's complexity, and its pervasive and invasive sophistication.

WHAT NEW TREATMENTS ARE BEING DEVELOPED?

The cancer cell	The immune system	Intra and inter cellular signaling pathways
Surgery Radiotherapy Chemotherapy	Immunotherapy	Molecular targeting

Table 4

Immunotherapy

New avenues have been explored by approaching cancer treatment from an immunological point of view rather than simply aiming to resect, poison or burn the cancer cells directly.

Types of Immunotherapy

- Immune Checkpoint Inhibitors
- Adoptive Cell Therapies. CAR T cell therapies, TCR, TIL, NK
- Monoclonal Antibodies (mAbs)
- Cancer Vaccines
- Oncolytic Virus Therapy
- Immune System Modulators such as IL-1

CAR T-cell Therapies

In CAR T-cell therapies, T cells are taken from the patient's blood and are changed in the lab by adding a gene for a man-made receptor (called a chimeric antigen receptor or CAR). This helps them better identify specific cancer cell antigens. The CAR T cells are then given back to the patient.

Natural killer cells can also be engineered with chimeric antigen receptors.[42]

CAR T cell therapy is showing promising results in blood cancers such as leukaemia but is not currently working well for solid tumours. The production of the CAR T cells is time-consuming, expensive and challenging to carry out. It can be difficult to get enough T cells and production-time can be too long for patients with fast progression. In addition, there is a high incidence of adverse effects such as cytokine release syndrome (CRS) or neurotoxicity. If the tumour cells mutate and loose the target antigen, relapse of the underlying disease can occur.[43]

Monoclonal Antibody Therapy (mAbs)

Monoclonal antibody therapy uses monoclonal antibodies to bind to certain target cancer cells or proteins on the cancer cells. This treatment should stimulate the patient's immune system to attack those cells.[44]

Monoclonal antibodies are very expensive and difficult to produce and their large molecular size (150 kDa) limits their tissue and tumour penetration which can limit their efficacy.[45]

MRNA Vaccines

The abnormal molecular features of cancer cells cause them to produce abnormal proteins or neoantigens. MRNA vaccines seek to activate T cells against these specific features.

> *"The manufacturing process starts with the identification of genetic **mutations** in a patient's tumor cells that could give rise to neoantigens. Computer algorithms then predict which neoantigens are most likely to bind to **receptors** on T cells and*

stimulate an immune response. The vaccine can include genetic sequences for up to 34 different neoantigens." [46]

Delivery of mRNA vaccines in vivo, however, is inefficient and tumours frequently express an array of antigens that may not be unique to the tumour. In addition, cancer cells create a tumour microenvironment that is specifically immune-suppressive with the added issue that T cells may be immunologically exhausted. [47]

Immune Checkpoint Inhibitors

As discussed under the section Immunoediting Capabilities of Cancer on page 12 above, one of the methods used by cancer to manipulate the immune system is through PD-L1 over expression. The PD-1 receptor of the T cell binds with the PD-L1 protein as would be the case to prevent an autoimmune reaction with a healthy cell.

Immunotherapy drugs called immune checkpoint inhibitors work by **blocking checkpoint proteins from binding with their partner proteins**. This prevents the "off" signal from being sent, allowing the T cells to kill cancer cells.

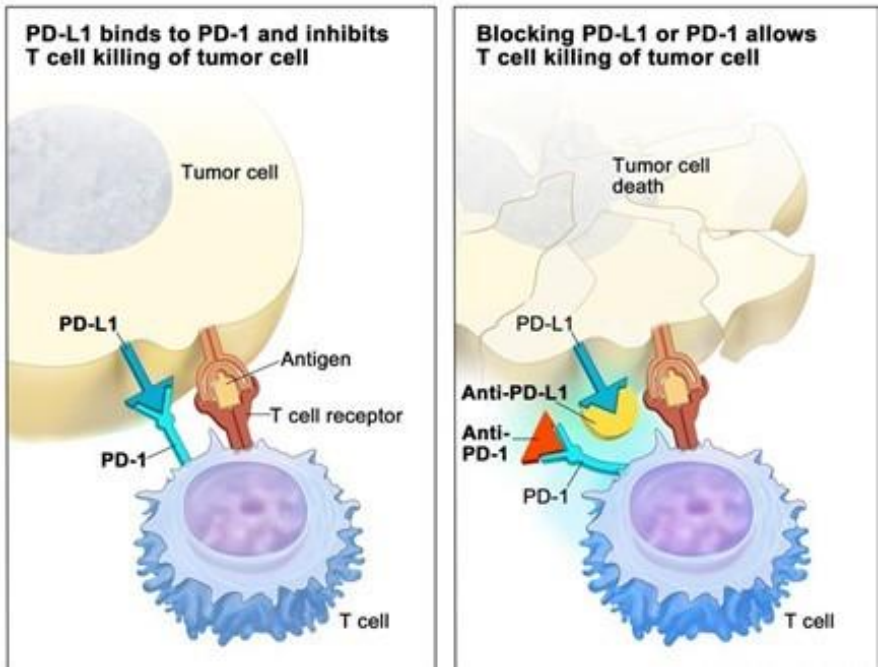

Figure 10 - PDL function of a tumour cell

In Figure 11 "Checkpoint proteins, such as PD-L1 on tumor cells and PD-1 on T cells, help keep immune responses in check. The binding of PD-L1 to PD-1 keeps T cells from killing tumor cells in the body (left panel). Blocking the binding of PD-L1 to PD-1 with an immune checkpoint inhibitor (anti-PD-L1 or anti-PD-1) allows the T cells to kill tumor cells (right panel)." [48]

The treatment, however, comes with several adverse side effects such as fatigue, nausea, vomiting, diarrhoea, and dermatological reactions. Severe immune-related adverse events have also been reported which can lead to permanent health effects, such as diabetes and neurological issues. [49]

Immunotherapy is only effective in some cases and can have lasting side effects

Not all cancers have targetable biomarkers for immunotherapy and if they do, for example with PD-1/PD-L1 immunotherapy, a considerable number of cancer patients currently have relatively low response rates and drug resistance issues. [50]

Cancer Stem Cell (CSC) Therapy

Cancer stem cells are the powerhouse of tumour cell production and metastasis and not responsive to conventional treatments so they are obvious targets for new therapies.

An emerging technology for cancer stem cell therapy is Monoclonal antibodies (mAbs) which target specific surface biomarkers on the cancer stem cell and bond to them, thereby flagging the cell for destruction by the immune system. [51]

The difficulty with this is that the **heterogeneity** of cancer stem cells is so complex that it is difficult to identify cancer stem cell biomarkers.

> *"Developing cancer-stem-cell-specific drugs is complicated by the genotypic variability of cancer stem cells and genomic instability of hyperplastic progeny that makes karyotyping of tumor cell populations enormously challenging"*[52]

The **proportion of cancer stem cells** in tumour tissues is very low and generally accounts for only 0.01–2% of the total tumour mass which makes CSCs difficult to isolate and test.[53] Even where therapy may be successfully engineered, if only individual cancer stem cells are targeted and not signaling pathways and the tumour microenvironment, **more cancer stem cells can be created** from normal stem cells.

> *"under the therapeutic pressure and changed microenvironment cancer stem cells can be newly generated. In this case, these cells do originate from non-cancer stem cells or from therapy-induced senescent tumor cells"*[54]

Targeted Therapy

Chemotherapy mostly uses **cytotoxic** agents which kill tumour cells. Targeted therapies are often **cytostatic** with the intention of blocking tumour cell proliferation.[55]

Targeted molecular therapy may use monoclonal antibodies but, whereas the goal of immunotherapy is to stimulate a host immune response that results in tumour destruction, targeted approaches are intended to inhibit molecular pathways used by tumour cells for growth and maintenance.[56]

Targeted therapies use information from the host's genes, the genes and proteins of the tumour and its microenvironment to treat disease. It is a key element of **precision medicine**.[57]

Drugs Targeting Pathway Signaling – Molecular Targeting

Molecular profiling of an individual's tumour will highlight genetic mutations resulting in cancer hallmark features. The effects of some of these mutations and the proteins they produce can be targeted as illustrated in Figure 11 below.

> *"These work by blocking proliferation through the use of small molecule tyrosine kinase inhibitors which causes [the] impeding [of] the activation of the signaling pathway by blocking the action of an abnormal protein"*[58]

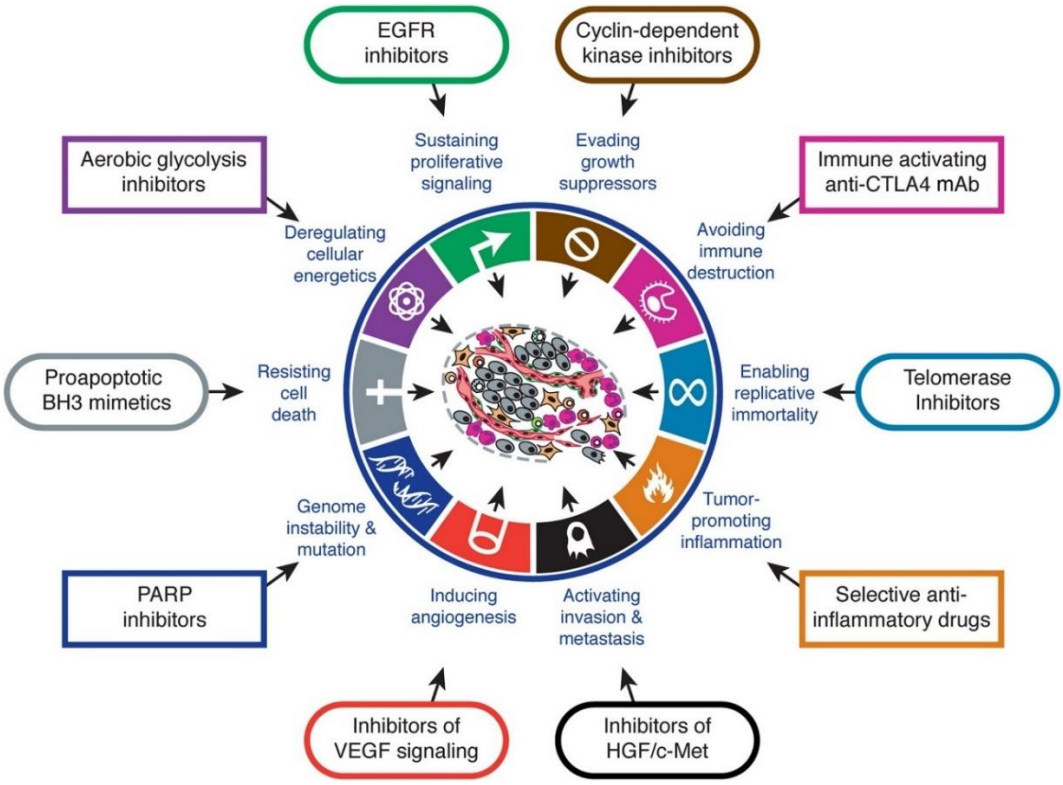

Figure 11 - Hallmarks of Cancer[59]

Figure 11 above shows the hallmark functions necessary for cancer growth and progression and targeting agents that can counter them and illustrates the multi-target challenge of treating cancer. In addition, **dynamic heterogeneity** (ever mutating tumour molecular profiles) allow tumour cells to develop resistance to a particular inhibitor. Also often restrictive are low levels of **pathognomonic mutations.** Even within one type of cancer there may be few molecular features in common as is illustrated in the graph of embryonal rhabdomyosarcoma mutations in Figure 12 below. This means therapy must be individual and personalised. Target drugs may not be available and may never be developed for many rare childhood cancers as no economies of scale can be leveraged.

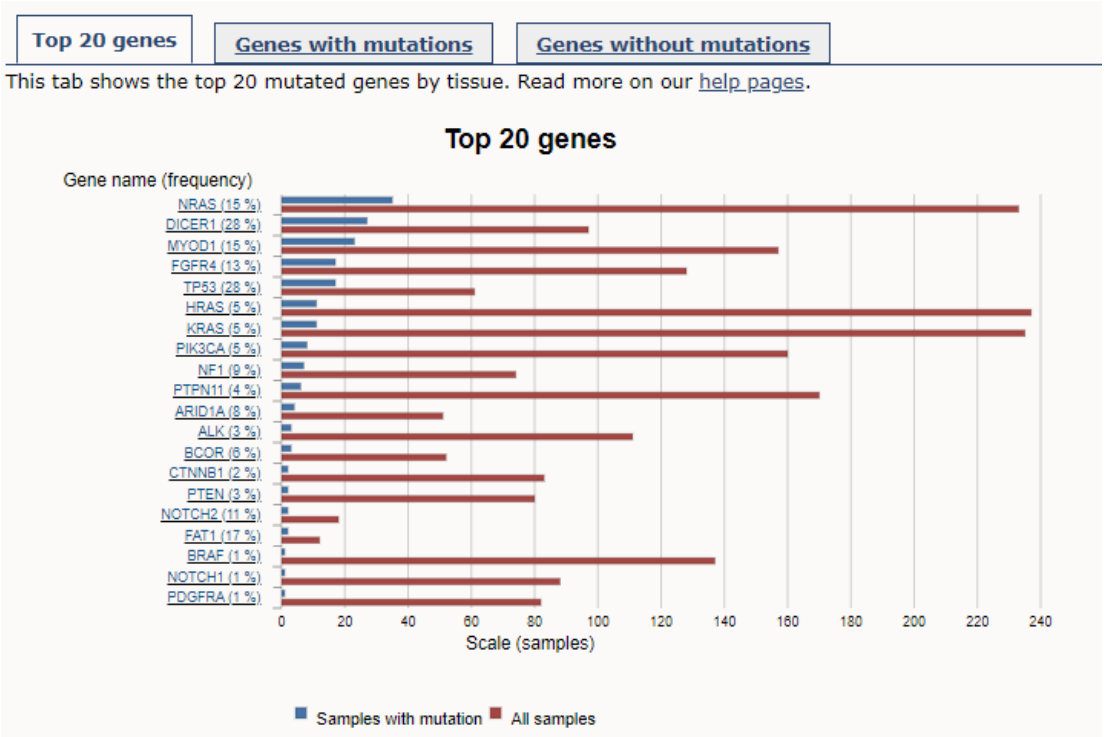

Figure 12 - Rhabdomyosarcoma gene mutations in COSMIC Catalogue of Somatic Mutations in Cancer

Toxicity is one of the major limitations of targeted therapies particularly as a multiple target approach works best. Most of the inhibitor drugs used in targeted therapies can have serious side effects. Many have a **single directional effect**. In other words, by manipulating one element to inhibit the cancer, other processes along the pathway required for underlying health, are disturbed.

Targeted therapies for children with cancer are still mostly at the trial stage and are often not standalone treatments but administered in addition to frontline surgery, chemotherapy and radiotherapy[60] further invoking the dilemma of the tug of war between maximum toxicity against cancer and minimum toxicity for underlying health.

ARE THESE NEW AVENUES OF RESEARCH LIKELY TO REVISE THE OUTCOMES FOR CHILDREN WITH CANCER?

> *"Over the past decade, tumors have increasingly been recognized as organs whose complexity approaches and may even exceed that of normal healthy tissues."*[61]

Chemotherapy and radiotherapy are cell directed strategies. Many of the emerging therapies including immunotherapy and cancer stem cell therapy, also target cancer cell destruction. However, the ability of the tumour microenvironment to turn normal cells into cancer stem cells and disable or even recruit the immune system suggests that this approach could never be entirely successful.

> *"The viewpoint of cancer as fundamentally a stem cell disease represents an important paradigm shift in our conceptual understanding of carcinogenesis and tumor biology and has ushered in a new era that challenges the dogmatic approaches to cancer cell destruction."*[62]

Targeted molecular therapy has developed on the back of exponential growth in molecular analysis availability and knowledge. It moves away from cell-oriented approaches into the arena of signaling pathway manipulation. Personalised targeted medicine seeks to carry out molecular analysis to target a person's own individual tumour features and genetic mutations, amplifications, gains or losses. The difficulty in achieving this lies with the **dynamic heterogeneity** of tumour cells whose molecular features are ever changing.

It is well recognised that targeting more than one feature at a time will significantly increase the chances of arresting the cancer. A multi-targeted approach, however, brings with it often intolerable levels of **cytotoxicity** which can be more damaging to the host than to the cancer.

The Datar Genomics laboratory isolate cancer clusters by applying pressure to the cells in the blood sample. Over a 5-day period, healthy cells die in the extreme, hostile environment. The cells that survive are cancer cells.

Adverse side-effects are even more problematic for children because if they survive, damage to their bodies has the potential to cause disease and new cancers over a period of multiple years. Prolonging the life of a 70-year-old by 10 years is admirable but exposing a child to toxicities at the age of 7 such that they die from the long-term side effects of their treatments aged 17 cannot be viewed in the same way.

Although targeted molecular therapy is directed to the pathways involved in the genetic mutations of an individual's tumour, which expands the scope of treatment from a cellular to a pathway system level, cytotoxicity makes it impossible to address all pathways and so targeted therapy is predominantly precision medicine. It is a reductionist solution to a pervasive problem.

Immunotherapies have had great success in some areas such as childhood leukaemia and lymphoma however they are **not available** for the majority of childhood cancers. The investment required to create these and targeted therapies is significant but most childhood cancers are rare and the numbers of children too small for the cost of drug development to be recouped.

Consequently, it will be difficult for these strategies to produce sufficient improvements in the treatments for children with cancer because they cannot address all the complex aspects of the disease. In other words, they cannot address ALL possible pathways rather than just cell cycle functions or a narrow shopping list of molecular features.

The Problem

Currently available treatments for rhabdomyosarcoma are surgery, chemotherapy and radiotherapy. There are no existing immunotherapy treatments for rhabdomyosarcoma. For the teenage child whose tumour cell is pictured on page 1, there are no molecular targets for available targeted therapy and no way of targeting rhabdomyosarcoma cancer stem cells.

Pharmaceutical drugs clearly have an important role to play in the treatment of children with cancer but there remains a compelling requirement for treatments which can promote quality of survival and life expectancy, including for the rare cancers found in children, as well as deal with refractory or untreatable disease.

A new paradigm in the treatment of children with cancer would need to expose the child's normal cells to minimum levels of toxicity and offer long term protection from cancer recurrence both from the original tumour and as a result of chemo and radiotherapy. At the same time, it would need to address cancer as a complex system, controlling multiple pathways including immune system function and as a disease with the ability not only to sustain immortality but self-renew when eliminated.

THE FUTURE

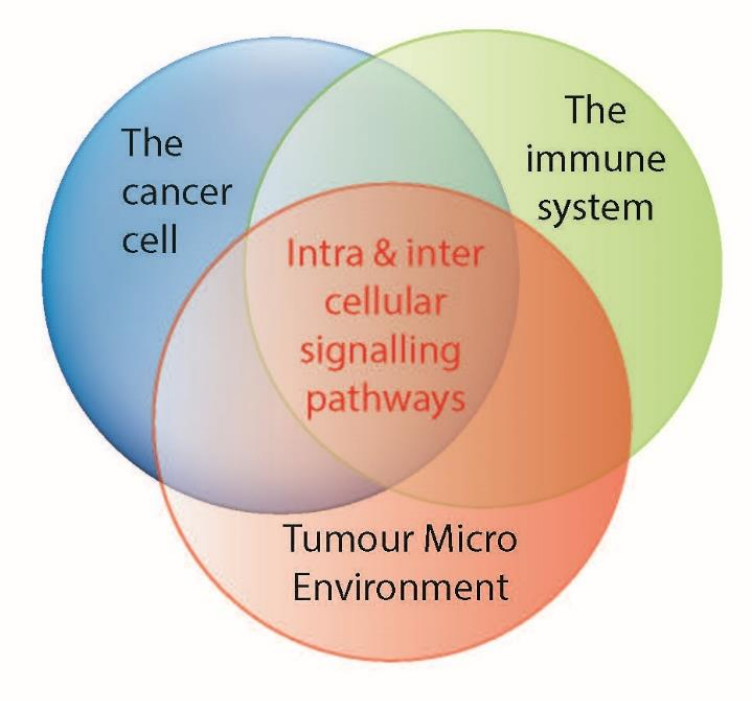

Figure 13 - Intra & inter cellular signaling pathways

Cancer does not progress in isolation, in a cell autonomous manner. Advancements in treatments for children with cancer can only be made by understanding that cancer cells mastermind their survival through a myriad of heterotypic interactions that occur between tumour cells and the tumour micro-environment that they create to support them. They use this platform to infiltrate and direct their proliferation and metastasis via signaling pathways.

Although there is clearly merit in killing the cell that has the hallmarks of cancer, this comes at a high price nor is it enough. It is necessary to target the pathways through which that cell is operating intra and inter cellularly and the means through which it can regenerate when seemingly obliterated.

Indeed, the signaling pathways that regulate the maintenance and survival of cancer cells have become targets for cancer treatment.

> *"Even for the most successful single-agent targeted therapies, however, drug resistance eventually emerges leading to rapid progression of metastatic disease"*[63]

Nor have multi-target strategies been able to change the landscape of outcomes for the majority of children with cancer due to the cost of innovation and toxicity of application.

Figure 14 below shows the cancer pathways that have been identified.

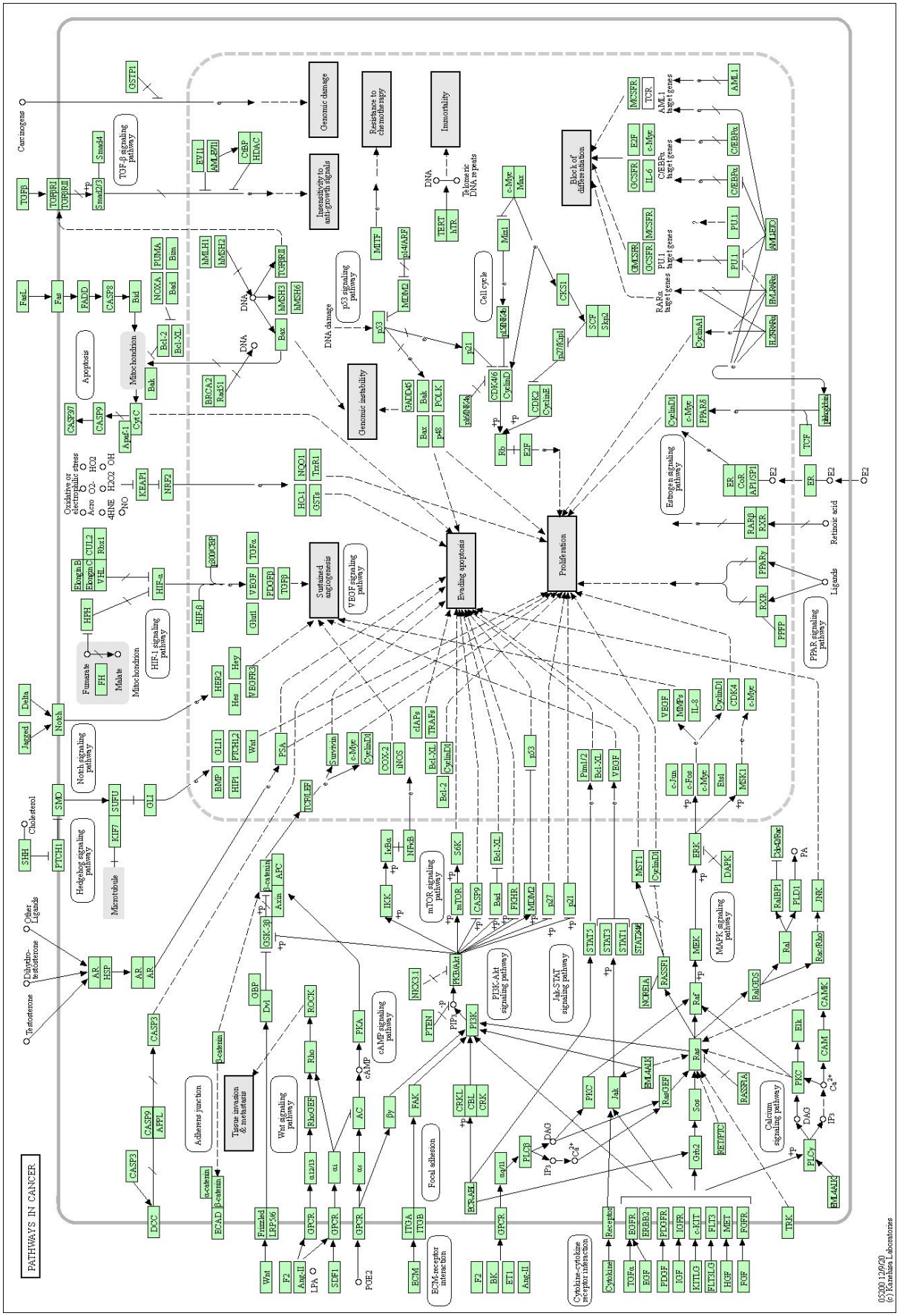

Figure 14 - Cancer signaling pathways (Kegg)

Natural Compounds

> *"interest in plant-derived drugs has progressively increased and a "New Golden Age" for the drug discovery of nature-derived products is emerging"*[64]

When we apply drugs to inhibit or activate elements in the tumorigenic process it can have a negative effect on essential processes. Natural compounds tend to be modulatory in a way that drugs are usually not.

The difference between modulators and inhibitors or activators is that modulators restore BALANCE. This is what is difficult to achieve with many conventional drugs which have a single directional effect.

This means natural compounds can overcome the toxicity barrier to multi-target therapy.

> "*Cellular signaling perturbation by natural products:*
>
> *some natural products…have inhibitory effects on humans … through targeting multiple cellular signaling pathways and thus these 'natural agents' could be classified as multi-targeted agents."*[65]

Are Natural Compounds Effective?

The Human Genome Project drove the advent of molecular genome sequencing laboratories making the molecular profiling of individuals, their tumours and the tumour microenvironment available. This information is pivotal to targeted treatments and has given rise to an explosion of research on the effects of natural compounds on gene expression and the associated cancer signaling pathways. Natural compounds can be used for any or all pathways providing the expertise is there to select and combine evidenced natural compounds into a comprehensive treatment plan.

Example 1 – Cancer Stem Cells

> *"Aberrant gene-expression profiles long considered hallmarks of malignancy have been re-evaluated in the context of the cancer stem cell with* ***emphasis on genes that regulate self-renewal*** *processes and differentiation programs which are normally tightly regulated in the non-cancerous somatic stem cell. As a result, there is an* ***urgent need to identify compounds that strike targets involved in CSC [cancer stem cell] self-renewal and differentiation programs****, respectively."*[66]

Figure 15 below illustrates the effects of a selection of natural compounds on the signaling pathways utilised by cancer stem cells indicating that natural compounds can address the cancer stem cell issue outlined above.

> *"Natural compounds derived from botanicals and food sources may modulate vital signaling pathways involved in the maintenance of the cancer stem cell phenotype."*[67]

The major mechanistic routes exploited by cancer stem cells for these pro-survival signaling and self-renewal are the WNT/β-catenin, Hedgehog, Notch and PI3K/AKT/mTOR pathways (Figure 14 - Cancer signaling pathways (Kegg) above)

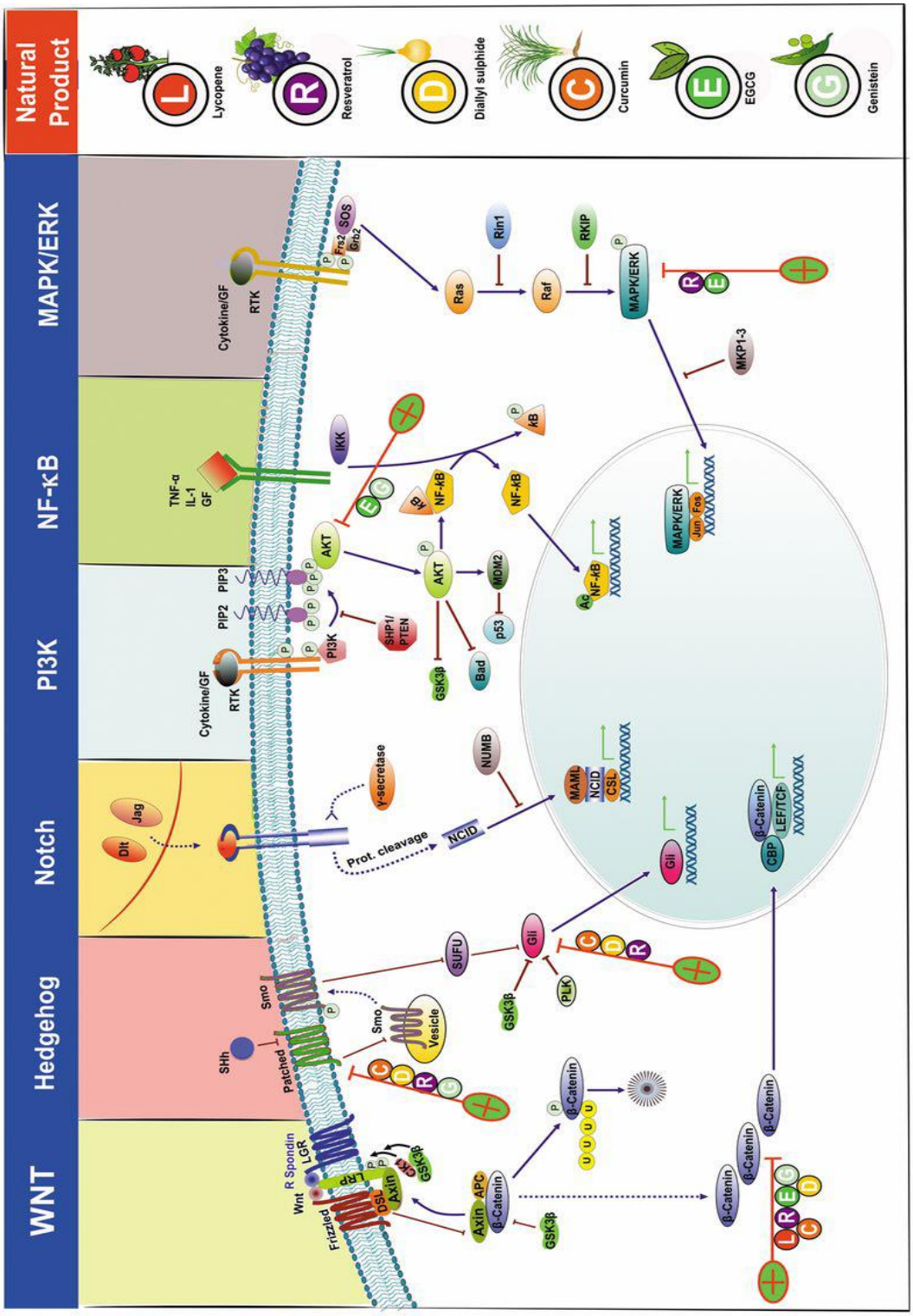

Figure 15 - Effect of natural compounds on cancer stem cell signaling pathways[68]

> *"Conventional therapeutics including chemotherapy and radiation therapy have demonstrated efficacy against many differentiated tumor cell types, but exhibit poor performance against cancer stem cell-specific targets, leading to tumor regrowth and metastasis. Many Natural Products have demonstrated ability to modulate pathways responsible for cancer stem cell function and inhibition. As knowledge of molecular biology and properties of CSCs [cancer stem cells] is gleaned for various tumor types, more Natural product inhibitors of CSCs may be identified and tested in combination with each other and in formulations with conventional chemotherapy drugs to form more potent therapeutic treatment strategies than those currently available"*[69]

Example 2

Some therapies such as Tumour Dormancy Therapy are *only* currently addressed with natural compounds as there are no approved drugs for this kind of treatment despite significant benefits in refractory disease.

> *"Standard cancer therapy prolongs survival, but can be detrimental to the quality of life, compromise the immune system, and leave residual disease that can cause recurrence years or decades in the future. Tumor dormancy therapy is a novel therapeutic approach that may improve these shortcomings, promote quality of life, and prolong survival."*[70]

Natural compounds can address some of the key shortcomings of current treatments by providing the benefits of multi-target therapy without increased toxicity, maintaining underlying health during toxic chemotherapy protocols and improving outcomes by reducing interruptions in chemotherapy cycles due to infection or poor blood counts.[71]

Natural compounds are multi-target agents that can be combined safely, verified by vast resources of peer-reviewed research and used in conjunction with new and existing therapies. Natural compounds can benefit all children including outliers on the normal distribution curve with refractory or untreatable cancer disease. They are safer for growing children. If life expectancy is reduced by 10% in someone who is 85 years old, the loss of life is not nearly as great as a 10% reduction in life expectancy of a 7-year-old. Given the financial pressure on healthcare systems, it is important that natural compound treatments are, from a cost perspective, realistically realisable.

Science has made significant leaps forward over the last 25 years. However, as the oncologist Azra Raza says in her book 'The First Cell',

> *"the gaping disconnect between knowledge about cancer biology and the capacity to use this knowledge to benefit patients is staggering".*

Professor Albert K Harris (UNC) believes

> *"What is lacking is someone with … the imagination to figure out how to put these facts to use, the energy to develop methods for using these facts, all combined with the stubbornness not to give up along the way."*[72]

TABLE OF FIGURES

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