

Tumor Proliferation Through Sialic Acid Dynamics and Anti-Siglec-Sialoglycans Preventive Strategies

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ABSTRACT: Glycans, carbohydrates on cell membranes, are often overexpressed in cancer cells. Sialic acid, a sugar found at the terminal branch of glycans, plays a vital role in carbohydrate-protein interactions, intercellular communication, and bacterial or viral infections. However, hypersialylation of glycans has been linked to hallmarks of cancer such as tumor growth, angiogenesis, metastasis, and resistance to immune cells. Upregulation of sialylation promotes evasion of immune surveillance and survival of malignant cells. Given this role of sialic acid, inhibiting the sialylation of glycans could provide quantum leaps in treating cancer. Over-sialylation is significant to cancer mechanisms (growth, metastasis, and immune evasion); therefore, a high therapeutic value could be found in preventing sialic acid's role in cancer. This paper reviews how sialic acid plays a key role in assisting the growth and survival of cancer cells, despite human immune cells' attacks; it also summarizes findings that encourage removing hypersialated glycans as a strategy against multiple cancerous cell types.

KEYWORDS: Biomedical and Health Sciences; Genetic and Molecular Biology of Disease; Cancer Progression; Resistance; Sialic Acid; Siglec.

■ Introduction

Upon every cell's surface are carbohydrates called glycans, many of which can form a layer called a glycocalyx.¹ Glycoprotein, glycolipids, and proteoglycans are all different forms of glycans, which consists of a single unit that makes cellular communication possible.²

Sialic acids are attached to the terminal end of glycans through various glycosidic linkages such as $\alpha 2,3$, $\alpha 2,6$, $\alpha 2,8$, and $\alpha 2,9$.³

Approximately, fifty derivatives of neuraminic acids can be found under the category of sialic acid.⁴ N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) is among the most widely-known sialic acid derivatives in mammals.⁴ Whereas Neu5Ac has an acetyl group, Neu5Gc has a glycolyl group.⁴ Both of these groups are placed on the fifth carbon atom (C5).⁴ Since human DNA does not contain a functional cytidine monophosphate N-acetylneuraminic acid hydroxylase (CMAH) gene, which would encode the enzyme forming CMP-Neu5Gc from CMP-Neu5Ac, our cells lack the derivative Neu5Gc.⁴ Fortunately, Neu5Gc still exists in the cellular membrane's glycome due to daily nutrition intake.⁴

Cancer cells have been discovered to be coated with hyper-glycosylated glycans, and even the tiniest modification to these glycomes can result in a significant transformation from normal to cancerous cells.⁵ Aberrant sialylation, truncated O-glycans, and fucosylation is examples of such changes that could lead to the development of cancer tumors.⁵ Over-glycosylation is correlated with all of the cancer hallmarks, from cancer cell growth to metastasis to immune evasion to angiogenesis.⁶ An upregulation of tumor sialoglycans like SLA, SLS, STn, and GM2 are significant promoters in the advancement of cancer.⁷

■ Discussion

In the human body, the synthesis of sialic acid begins at UDP-GlcNAc in the cytosol after the glucose has penetrated.⁸ The enzyme UDP-GlcNAc 2-epimerase/ManNAc-6-kinase (GNE) is the first to catalyze UDP-GlcNAc, transforming UDP-GlcNAc to ManNAc-6-P.⁹ Then, through the help of enzymes Neu5Ac 9-phosphate synthase (NANS) and Neu5Ac-9-phosphate phosphatase (NANP), ManNAc-6-P turns into Neu5Ac.¹⁰ Lastly, Neu5Ac is transported to the nucleus, where it interacts with the cytosine 5'-monophosphate N-acetylneuraminic acid synthetase (CMAS) to construct CMP-Neu5Ac.¹¹

From here, the transportation of CMP-Neu5Ac to the Golgi apparatus's glycoconjugates occurs through the help of SLC35A1 sialyltransferases.¹¹ Through this process, approximately 20 different sialyltransferases, including ST3GAL1-5, ST6GAL1, 2 and ST6GALNAC1-6, and ST8SIA1-6, are supplied with CMP-Neu5Ac as a substrate.⁴ The ST8SIA1-6 sialyltransferases generate $\alpha 2,3$ -, $\alpha 2,6$ -, $\alpha 2,8$, or $\alpha 2,9$ -linked sialic acids through connecting Neu5Ac's second carbon to its third carbon, sixth carbon to eighth carbon.⁴ Lastly, sialylated glycoconjugates, packaged in vesicles, are secreted out of the membrane.⁴

Alternatively, sialidases (also called neuraminidases) can release sialic acid glycoconjugates.¹² There are four distinct types of sialidases: NEU1, NEU2, NEU3, and NEU4.¹³ The sialidase NEU1, which is lysosomal, enables the breakdown of sialoglyco conjugates.¹⁴ The sialidase NEU2, which is cytosolic, prevents interactions with gangliosides.¹⁵ The sialidase NEU3, often found on the plasma membrane, works specifically with gangliosides.¹⁶ The NEU2 and NEU3 function quite differently. And, lastly, the sialidase NEU4, often found in the outer mitochondrial membranes or the lysosomal lumen, is an enzyme whose substrate varies widely; most gly-

coproteins, gangliosides, and oligosaccharides can be processed by sialidase NEU4.¹⁷

After exocytosis outside the cell, the sialic acids are filtered back into the cytoplasm, where they restart their cycle to produce more sialyl glycoconjugates.¹⁸ Otherwise, they would be degraded by N-acetylneuraminase lyase (NAL) to ManNAc and then eventually pyruvate.¹⁸ Since there is a lack of protein transporter on the membrane that specifically works for Neu5Ac or ManNAc, Neu5Ac or ManNAc penetrate the cell mainly through pinocytotic or endocytic pathways.¹⁹

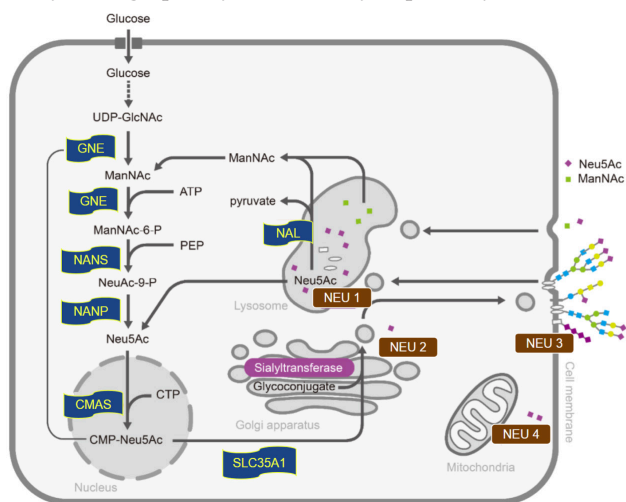


Figure 1: The Life Cycle of Sialic Acid. Zhou, Xiaoman, *et al.* "Biological Functions and Analytical Strategies of Sialic Acids in Tumor." *Cells* vol. 9, 2 273. 22 Jan. 2020, doi:10.3390/cells9020273

Sialic Acid in Tumor Growth:

Overproduction of sialic acids is a distinct characteristic of cancerous cells during tumor formation and malignant advancement.²⁰ Hyper sialylation is more seen in tumor tissues than in any other healthy functioning tissue.²⁰ The total amount of sialic acid present in serum or glycolipid-bound membranes is noticeably high in leukemia, ovarian, breast, colorectal, and pancreatic cancers.²¹ In more deadly cancer such as glioma, neuroblastoma, and lung cancer, polysialic acid is overexpressed.²² Hypersialylation plays major assistance in the acceleration of cancer progression, which eventually results in a poor prognosis.²³ The rise of sialic acids in tumors is a direct consequence of a metabolic flux of sialyltransferases/sialidases.⁴ Tumor cells initiate their hypersialylation by increasing their uptake of glucose, the first raw material entering the cell through endocytosis.⁴

Inactivation of the CMAS gene, which codes for a key enzyme that finalizes the production of sialic acid in the nucleus, has been found to inhibit the production and activation of sialic acid.²³ Sialic acid encourages cancer progression through the promotion of tumor expansion, evasion of apoptosis and immune attack, and development of metastases through bloodstream extravasation.²⁴ Unsurprisingly, sialylation (growth of sialic acid as a terminal sugar on glycans) of proteins can trigger multiple signaling molecules and pathways to further cancer advancement.²⁴

For instance, when α 2-3-linked glycan is hypersialylated in gastric carcinoma, the activation of receptor tyrosine kinases MET and RON occurs, which results in a more invasive phenotype.²⁵ Accordingly, N-glycans, when terminally sialylated, can help tumor cells resist hypoxia which confers malignancy to the cell phenotypes.²⁶ Specifically, the α 2-6 sialic acid growing on N-glycans causes the upregulation of E-cadherin, which then promotes the impairment of cancer cell adhesion and aggrandizer metastasis.²⁷

Drugs targeting sialic acids are developing to further the prevention of cancer metastasis.²⁸ *In vivo*, a sialic acid called glycomimetic (P-3Fax-Neu5Ac) has been defined as the promoter of tumor growth.²⁸ So, delivery of anti-sialic-acid nanoparticles has been performed to stop the metastasis of melanomas to lung cancer in a murine model.²⁹ Drugs such as Ac53FaxNeu5Ac, when intratumorally injected, can thwart sialic acid expression and therefore suppress tumor growth in many *in vivo* tumor models.³⁰ These advances in clinical treatment have shown how important sialic acids are in the progression and development of cancer, ultimately indicating that a sialic-acid-blocking drug would be of high therapeutic and clinical treatment value.³¹

Tumor Proliferation and Metastasis:

Expression of sialyl glycans correlated with the aggressive epithelial-mesenchymal transition (EMT), a transition critical in forming metastasis.³² The EMT process, triggered by growth factor- β (TGF- β), caused the upregulation of numerous sialyltransferases, including ST3GAL1, ST6GAL1, ST8SIA4, ST6GAL2, ST8SIA1, ST8SIA2, and ST3GAL2.³³ When these sialyltransferases upregulate, accumulation of sialoglycans on the cell surface membranes occurs, greatly helping tumor cells to survive and metastasize.³⁴ In hepatocellular carcinoma, the specific α 2,6-sialylation activates the Wnt/ β -catenin signaling pathway, which results in proliferation, migration, and invasion of cancerous cells.³⁴

Through the Akt and ERK pathways, this same α 2,6-sialylation, when overexpressed on the human epidermal growth factor receptor 2 (HER2), boosts gastric cancer growth.³⁵ ST-6GAL1 causes hyper-sialylation on the endothelial growth factors receptor (EGFR) through the PI3K/Akt pathway.³⁶ Likewise, prevention of ST6GAL1's functions can cause induced EGFR to be desialylated and therefore decrease tumor proliferation.³⁷ The integrin α 5 β 1, modified by α 2,6-sialylation, attunes the FAK signaling pathways and cell adhesion.³⁸ By interrupting the NCAM signaling at the cell-cell communication site, polysialic acid ensures tumor growth and mutations.³⁹ To slow the rate of metastasis in a xenograft rhabdomyosarcoma tumor in a mouse model, scientists blocked the poly sialyltransferases ST8SIA2 and ST8SIA4 from creating polysialylated NCAM.⁴⁰

As an important observation, sialyl-glycans thickening the surface of tumor cells also helps their colonization and speeds up metastasis.⁴¹ As an example, sialic acid, when liganded with selectins, which are vascular adhesion molecules, magnifies cancer progression by strengthening the adhesion and extravasation processes in metastasis.⁴²

Tumor Angiogenesis:

Angiogenesis is the creation of more blood vessels using old ones.⁴⁹ In the fast-paced environment suitable for metastasis, new blood vessel growth, to provide oxygen and nutrients, is omnipresent.⁴⁹ After being synthesized by inflammatory cells, angiogenic growth factors (AGFs) go on to initiate angiogenesis.⁴⁹ Compared to other types of AGF, the vascular endothelial growth factor (VEGF) family of VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E plays the most significant role.⁴³ This is because the VEGF undergoes cell-cell communication with polysialic acid.⁴⁴ In an oxygen-deprived environment, sialic acids assist tumor angiogenesis by upregulating growth factor–receptor interactions.⁴⁵

Gangliosides are a sialyl-glycosphingolipid that can be placed on the endothelial cells' membrane and promote angiogenesis through upregulating responsiveness to AGFs.⁴⁶ However, through the transportation of the sialyltransferases ST6GAL1 aided by exosomes, tumor cells can also overexpress the surrounding cells' sialic acid levels.⁴⁷ Sialyl Glycans such as N-glycans need to be α 2,6-sialylated to engage with VEGF and activate angiogenesis in endothelial cells.⁴⁸ The platelet endothelial cell adhesion molecule (PECAM) communicates through homophilic interaction, supported by α 2,6-sialylation.⁴⁹ While the sialylated PECAM communicates and binds with the VEGFR2 receptor and integrin β 3 receptor, the prevention of PECAM-VEGFR2 interaction could be induced by the desialylation of ST6GAL1.⁴⁹ This ultimately results in the inhibition of apoptosis- and angiogenesis.⁴⁹

Resistance to Apoptosis and Cancer Cell Therapy:

Cell apoptosis could be prevented by sialic acid through the inhibition of two main processes: the Fas receptor–Fas ligand (FasR–FasL) pathway and the anoikis pathway.^{50,53} The FasR–FasL interaction, triggered by T cells and promoted through caspase activation, plays an important role in immune cell homeostasis and the human body's immune maintenance.⁵⁰ However, when the FasR is sialylated, it no longer allows the binding of FasL to its binding site, which ultimately inhibits apoptosis.⁵¹ For example, the elevation of α 2,6-sialylation on Fas, caused by sialyltransferase ST6GAL1, inhibits the colon carcinoma cells' apoptotic signaling.⁵¹ Furthermore, α 2,6-sialylation inhibits the process in which FasR internalizes and cancels the positive feedback loops for FasR–FasL interaction apoptosis.⁵²

Another apoptosis pathway is anoikis, which is the detachment of cells off of an extracellular matrix (ECM).⁵³ Anoikis is a vital pathway that ensures the growth of an adherent-independent cell and the cell's attachment to a mismatched matrix.⁵³ Resistance to anoikis is often more prominent in cancer cells compared to normal epithelial cells.⁵³ Attachments of cells to ECM, a process mediated by integrin, is the direct root cause of the upregulation of tumor angiogenic response, which speeds up cancer metastasis.⁵⁴ The absence of cell–ECM interactions, due to loss of mediation from integrin, downregulates the phosphorylation (the addition of a phosphate group) of downstream effectors, including FAK, ERK1, PI3-K, and MAP kinases.⁵⁵ As a result, this makes the cell susceptible to anoikis.⁵⁵ However, the α 2,6-hypersialylation in

the fibronectin receptor integrin α 5 β 1 could block the binding of galectin-1 and therefore prevent anoikis.⁵⁶ Unsurprisingly, α 2,6-sialylation showed therapeutic drugs ineffective in many cancers, most likely through the sialylation of the EGFR receptors, which was found to reduce the dimerization of EGFR receptors and increase phosphorylation by them.^{57,58} All of this reduces the effectiveness of the tyrosine kinase inhibitors.⁵⁹

Glycosyltransferase and Glycosidase enzymes:

The culmination of multiple glycosylation enzymes, whose role is to assist the distribution of specific glycans on a cell's surface, generates the tumor glyco-code.⁶⁰ These additions or removals of a glycan completed by glycosylation enzymes are often the consequence of DNA mutations in the genes responsible for glycan synthesis.⁶⁰

Altered regulation of either sialyltransferase or sialidase enzymes has been linked to hyper-sialylation.⁶¹ Sialyltransferases are a specific type of enzyme that sialylate the termini of glycoconjugates.⁶² These enzymes consist of 20 different versions that can all catalyze the attachment of sialic acid to a glycan chain, utilizing different glycosidic linkages including α 2-3, α 2-6, or α 2-8.⁶² Sialyltransferases, categorized into four types of ST3Gal, ST6Gal, ST6GalNAc, and ST8SIA, are expressed in a tissue-specific manner.⁶³

Sialyltransferases, usually upregulated in cancer, have been correlated with the formation of cancerous antigens.⁶⁴ Key sialyltransferases playing a significant role in cancer progression include ST3GAL6, ST6GAL1, ST3GAL4, and ST6GalNAc1/2.⁶⁵ ST6GAL1 is responsible for many cancer types and has been implicated as the cause of all the cancer hallmarks.⁶⁵ Clinically, ST6GAL1 expression can be used as reliable data to predict responses to EGFR/HER2 inhibitors in cancer cells, specifically ovarian.⁶⁵

In the same sialyltransferases family, ST3GAL4 is also overexpressed in cancer cells, making prognosis less accurate and giving way to the formation of Sialyl Lewis X (sLeX) in gastric carcinoma, which will ultimately end in a metastasis.⁶⁶ Besides, ST3GAL6 is also responsible for the synthesis of sLeX and E-selectin ligands.⁶⁷ Lately, the homing of bone marrow and the ineffectiveness of multiple myeloma therapy is found to be the direct results of ST3GAL6 activity.⁶⁷ Luckily, a study has discovered that this can be inhibited using an E-selectin antagonist called GMI-1271.⁶⁸ The sialyltransferases ST6GalNAc1, which catalyzes the sTn antigen responsible in cancer development, is associated with the growth of metastasis.⁶⁹ The ST6GALNAC2 has been identified by researchers as a suppressor of metastasis in breast cancer and could be potentially used for treatment with galectin-3 inhibitors.⁷⁰

The loss of terminal sialylation in glycoconjugates is catalyzed by sialidase enzymes, which moderate certain functional molecules' binding sites and play an important role in numerous biological processes.⁷¹ The sialidase enzymes can be found in four specific forms: NEU1, NEU2, NEU3, and NEU4.⁷² Sialidase can modify the glycocalyx of cancer cells and, by doing this, might render them more vulnerable to the immune system.^{73, 86} Sialidase enzymes, therefore, appear as a promising area for novel therapeutic innovations.⁷⁴ For instance, oseltamivir phosphate can target NEU1 in pancreatic, breast,

and ovarian cancers.⁷⁵ This method can be used to treat chemoresistant cells which are also drug-sensitive.

Siglecs and Cancer treatment:

The atypical glycosylation found in tumor cells' membranes can lead to novel cell-cell communication with immune cells that leads to the suppression of immune response.⁷⁶ The thick layer of sialic acids on the surface of cancer cells has long been recognized for its ability to protect tumors from our body's immune system.⁷⁷ With scientists believing that sialic acids play a vital role in the evasion of cancer cells from immune response, recent advances in glyco-tools have opened up new possibilities in the curing of cancer through the immuno-effective pathways.³⁰ Sialylated glycans on glycoproteins and glycolipids are the key to fitting with the lock of Siglecs, a family of lectins that exist on the membrane of many immune cells.⁷⁸ In the tumor microenvironment, this interaction between cancer cell sialic acid and Siglecs can mediate intracellular immune cell response that allows for the evasion of sialic acid from the immune cells.⁷⁹ Cancer-promoting glycans, specifically the STn and sialyl T, are a classic example of this.⁸⁰ The STn and sialyl T compromise the healthy functions of macrophages and dendritic cells, deaden the natural killer (NK) cells, and diminish the creation of guarding T cells.⁸¹ Therefore, determining the glycoside, which is the distinct aspect of glycan on cancer cells, is pivotal to grasping the mechanism through which glycans aid the evasion of cancer cells from the body's immune system.⁸²

Impairing sialic acid's role in communication with Siglecs has become a promising strategy for cancer immunotherapy.³⁰ Therefore, a reduction of sialic acid poses a huge benefit for our immune system.⁸² Emerging research has suggested that loss of tumor sialic acid is positively linked to the loss of Siglecs-sialic acid interaction, which can lead to proficient tumor immunity.⁸² If we can block sialic acid from forming interactions with our immune cells, we can allow for more CD8+ T cells, filter out myeloid and regulatory T-cells, and promote cytotoxic T-cells which can kill cancer cells.³⁰ As discovered previously, the interaction between Siglecs-9, Siglecs-7, Siglecs-10, or Siglecs-15 and their specific ligands has shown how bypassing anti-tumor immunity occurs.⁸²

When Siglec-9 matches up with sialylated MUC1, the increase in transformation of monocytes into cancer-promoting macrophages and expression of the checkpoint ligand PD-L1 occurs.⁸³ Siglec-9 is overexpressed on tumor-eliminating T cells in non-small lung cancer and colorectal and ovarian cancer.⁸³ Thus, diminishing the sialoglyco-Siglec-9 interaction proves to be a potential enhancement of T-cells activation.⁸³ Siglec-15 can often be found in tumor-infiltrating myeloid cells and is an important player in cancer immune suppression.⁸⁴ Siglec-15 inhibits antigen-specific T-cell responses at the tumor key-and-lock site, which compromises T-cell attacks on cancer cells.⁸⁴ Therefore, an anti-Siglec-15 antibody can realow T-cell effectiveness to carry out tumor immunity.⁸⁴ CD24, a type of sialoglycoprotein, is an anti-phagocytic signal that cancer cells show in response to attack from Siglec-10-expressing macrophages.⁸⁵ So, blocking CD24-Si-

glec10 communication can boost the clearance of CD24+ tumors and is a potential immunotherapy plan.⁸⁵

Precise glycocalyx addition in cooperation with antibody-sialidase conjugates has been reported as a potentially highly-valuable avenue for cancer immunotherapy.⁸⁶ In this strategy, an antibody induces the sialidase to remove specifically harmful sialic acid, those that are overexpressed, from tumor cell membranes and so open up ways for termination of desialylated cancer cells.⁸⁶ Other methods include externally delivering a sialidase enzyme to the tumor and using anti-glycan vaccines to block cancerous glycan-lectin interactions.

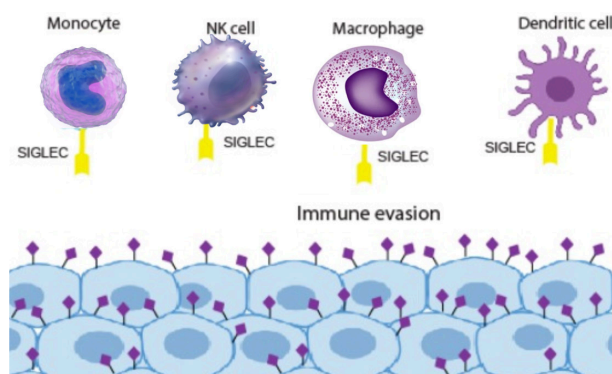


Figure 2: Cell-cell interactions between sialic acid and siglecs that prevent immunity and promote immune suppression.

Munkley, Jennifer, and Emma Scott. "Targeting Aberrant Sialylation to Treat Cancer." *Medicines (Basel, Switzerland)* vol. 6,4 102. 14 Oct. 2019, doi:10.3390/medicines6040102

Conclusion

In this review, I covered the key roles sialic acids play in cancer development and progression. A key feature of cancer is hyper-glycosylation, which is the overexpression of sialic acid as terminal sugar on glycans. Sialic acid, whose charge is negative and whose property is hydrophilic, can give the cancer cell many altered traits that help it survive. Over-expressed sialylation is a hallmark of tumors and produces many challenges in curing cancer. As shown above, sialylation is fundamental to the growth, evasion of immunity, progression, and metastasis of cancer cells. Therefore, removing extra sialic acids or inhibiting their functions will likely lead to improvement in the treatment.

Clinical approaches to compromise sialic acids' interactions with Siglecs, thereby canceling the evasion of immunity, proves to be a potentially effective method against cancer development.⁸⁷ Playing on the fact that sialic acids hide the glycoproteins' antigenic sites, researchers have been using sialidases to remove sialic acids, so sialic acid can no longer be used as a mask.⁸⁷ For instance, when sialidase was present in solid tumor cells, the body's chimeric antigen receptor T-cells (CAR-T cells) successfully removed cancerous cells.⁸⁸

Furthermore, the use of an antibody that titrates siglecs-15, a protein that binds with sialic acid to suppress T-cells, is expected to be effective in cancer immunotherapy, opening up new advanced treatments for patients whose cells have acquired resistance to the anti-PD-1/PD-L1 therapy.⁸⁸ Advances in technologies have led to further study of glycos-

ylation, which can ultimately emphasize glycan targeting as the main focus of cancer therapeutics, especially regarding cancer immunotherapy.⁸⁸ Shortly, it is probable that the energization of cancer immunotherapy through sialoglycan blocking and traditional chemotherapy/radiation will improve cancer treatment outcomes.⁸⁹ Besides being effective drug targets, sialic acids can also be studied to gather more information about cancer cells' sensitivity and resistance to other emerging therapies, which will conclusively improve the fight against cancer.⁸⁹

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