REVIEW ARTICLE

Exploring Neonatal NaV1.5 Voltage-Gated Sodium Channel as a Therapeutic Target in Cancer

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ABSTRACT

Voltage-gated sodium channels (VGSCs) play pivotal roles in cancer progression and have emerged as promising therapeutic targets and biomarkers. VGSCs comprise multiple subtypes with distinct tissue distributions, influencing tumour characteristics in different ways. Among these, the tetrodotoxin-sensitive α-subunits and the β1 subunit, commonly found in breast cancer, have been implicated in metastasis and tumour aggressiveness. The NaV1.5 channel and its neonatal variant (nNaV1.5) are overexpressed in aggressive cancers such as breast, prostate, colorectal, and lung cancers, thereby enhancing their invasive capacity. nNaV1.5 is particularly significant due to its tumour-specific expression and strong association with poor prognosis, especially in breast cancer, where it regulates cell proliferation, invasion, and tumour microenvironment remodelling. This review highlights nNaV1.5 as a critical ion channel that drives metastasis through ion regulation, extracellular acidification, and cytoskeletal remodelling. We further evaluate current therapeutic strategies, including siRNA, monoclonal antibodies, and small-molecule inhibitors, while addressing translational challenges such as tumour heterogeneity, drug delivery limitations, and off-target cardiotoxicity due to its similarity with the adult isoform. In addition, we explore the potential of nNaV1.5 as a biomarker subject to epigenetic regulations by factors including RE1-silencing transcription factor (REST) and histone deacetylase 2 (HDAC2), which may facilitate patient stratification and treatment optimization. By integrating mechanistic insights, therapeutic opportunities, and translational challenges, this review goes beyond descriptive summaries to provide a framework for advancing nNaV1.5 research from preclinical studies toward clinical application in cancer therapy.

Keywords:
Voltage-gated sodium channel, neonatal NaV1.5, metastasis, targeted therapy, biomarker.

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INTRODUCTION TO VOLTAGE-GATED SODIUM CHANNELS (VGSCS) AND CANCER

Voltage-gated sodium channels (VGSCs) are essential for the generation and conduction of electrical signals in excitable cells, including neurons and muscle cells. These channels consist of pore-forming α-subunits and auxiliary β-subunits, which regulate channel activity. VGSCs belong to a wider superfamily of ion channels that also includes voltage-gated potassium and calcium channels. The α-subunits of VGSCs are classified according to their tetrodotoxin (TTX) sensitivity and tissue-specific characteristics.[1] The β1 subunit, highly expressed in breast cancer tissues, can be a significant modulator of tumour cell behaviour and interactions within the tumour microenvironment.[2] VGSCs exist in three conformational states: open, closed, and inactivated, which control Na^+ ion conductance and enable fast inactivation within milliseconds.[3]

Structural research has significantly improved our knowledge of VGSCs, demonstrating how voltage sensors regulate gating charge movements, while the selectivity filter mediates Na^+ conductance through a water-lined channel.[4] Furthermore, it was unravelled that slow

inactivation mechanisms are controlled by conformational changes on the intracellular side of VGSC, and are crucial in cellular excitability and physiological activities.[5] VGSCs possess multiple toxin- and drug-binding sites, which differently influence the channel function.[6] This structural and functional diversity highlights the therapeutic value of VGSCs. To date, nine functional α-subunits and four β-subunits have been characterized in mammals.[7] The subtypes of α-subunits are shown in Table 1.

Table 1: The list of Subtypes of VGSCs6

α-Subunits	Gene Symbol	Chromosomal Location	TTX-S/R	Predominant Location	Expression in DRG	Effect of Mutation
$Na_{V1.1}$	SCN1A	M:2 H:2q24	TTX-S	PNS	+++	Epilepsy
$Na_{V1.2}$	SCN2A	M:2 H:2q23-24	TTX-S	CNS	+	Epilepsy
$Na_{V1.3}$	SCN3A	M:2, H:2q24	TTX-S	CNS (embryonic)	Upregulated after axotomy	None reported
$Na_{V1.4}$	SCN4A	M:11, H:17q23-25	TTX-S	Skeletal muscle	-	Myotonia, periodic paralysis
$Na_{V1.5}$	SCN5A	M:9, H:3p21	TTX-R	Heart muscle	-	Long-QT, Brugada 'syndrome, Progressive familial heart block
$Na_{V1.6}$	SCN8A	M:15, H:12q13	TTX-S	CNS, PNS, glia nodes of Ranvier	+++	Cerebellar atrophy
$Na_{V1.7}$	SCN9A	M:2, H:2q24	TTX-S	PNS Schwann cell	+++	Increased and decreased pain sensitivity
$Na_{V1.8}$	SCN10A	M:9, H:3p22-24	TTX-R	PNS (sensory neurons)	+++	None reported
$Na_{V1.9}$	SCN11A	M:9, H:3p21-24	TTX-R	PNS	+++	None reported

PNS: Peripheral Nervous System, CNS: Central Nervous System, Tetrodotoxin-Sensitive: TTX-S, Tetrodotoxin-Resistant: TTX-R, DRG: Dorsal Root Ganglion

In the last two decades, VGSCs have been a focus of attention as therapeutic targets because of their involvement in cancer metastasis. According to a study, VGSC expression was found to be greater in tumours than in normal tissues.[8] The tumour microenvironment also showed higher levels of K^+ and Na^+ and a reduced pH.[9] Hence, VGSCs have been reported to be upregulated in multiple studies concerning numerous carcinomas, including prostate, breast, lung, colon, cervical, brain, and ovarian cancers .[10-16] VGSC expression has also been identified in gliomas, with specific subtypes that are prevalent in tumours, such as $Na_V1.1$, 1.2, and 1.3.[17] In general, high-grade gliomas exhibit fewer Na^+ channel subtypes. $Na_V1.6$, which is strongly expressed in pilocytic astrocytomas, is almost absent in glioblastomas, highlighting the potential of VGSCs as diagnostic markers.

Additionally, VGSCs have been linked to the metastatic potential of various cancers. Studies have demonstrated that VGSC activity promotes cancer cell migration and invadopodia formation, which are critical metastatic processes.[18,19] Several studies have associated VGSCs with poor prognosis in breast cancer.[1] The overexpression of $Na_V1.5$ in breast cancer has also been linked to poor prognosis and increased invasiveness.[20] Moreover, VGSCs enhance the invasiveness of lung cancer cells, and highly metastatic cell lines express functional sodium channels, where sodium influx disrupts sodium homeostasis and its signalling pathways.[12] In another analysis, VGSC activity was found to facilitate prostate cancer metastasis *in vivo*[14] via Na^+ channel proteins expressed in malignant prostate cells. Hence, elevated Na^+ channel expression is thought to correlate with increased invasiveness.[21]

Different VGSC subtypes are overexpressed in different cancer cells, with $Na_V1.5$ and 1.7 being the dominant isoforms. The upregulation of these subtypes influences the migration and invasion of cells, hence increasing metastatic potential.[22] VGSCs are also functionally expressed in cervical cancer with differential expression of Na_V 1.2 and 1.7,[11] suggesting their utility as prognostic markers for cervical cancer.

VGSC Mechanism of Invasiveness in Cancer

VGSC activity promotes cancer cell migration and invasion by increasing the influx of Na^+, which leads to the formation of invadopodia, which are cellular protrusions that secrete proteolytic enzymes, such as metalloproteases and cathepsins, to break down the extracellular matrix (ECM) and enable metastasis (refer to Figure 1).[16,19,23] In breast cancer, the association between neonatal $Na_V1.5$ ($nNa_V1.5$) and sodium/hydrogen exchanger-1 (NHE-1) escalate this invasive mechanism. Additionally, VGSC expression enhances endocytic membrane activity, as observed in small-cell lung cancer (SCLC), where uptake of horseradish peroxidase (HRP) is more than four times higher than in normal cells and correlates strongly with metastatic potential.[24] VGSCs are

controlled by hormones and growth factors, such as the epidermal growth factor (EGF), which increases the channel expression and metastatic behaviour at both transcriptional and post-translational levels.[25] Moreover, VGSCs affect signalling pathways via abnormal ion transport mechanisms and cause depolarization of cancer cells.[26] This, in turn, activates Rac1 and initiates cytoskeletal reorganization.[27] Rac1 is intracellularly anchored to the plasma membrane and functions as a protein that controls cell shape and movement.[26] As a result, VGSCs promote cancer by regulating ion flux, membrane dynamics, and intracellular signalling, which ultimately leads to metastasis.

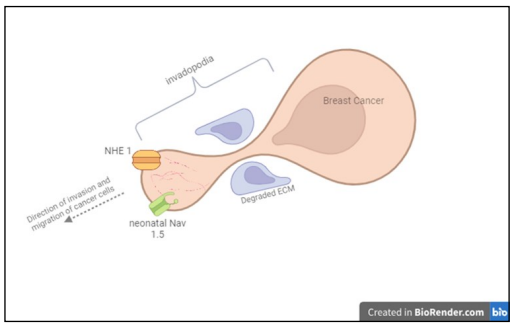

Figure 1: Schematic of invadopodia formation in breast cancer cells. Neonatal NaV1.5 (nNaV1.5) regulates Na+ influx, which activates sodium/hydrogen exchanger-1 (NHE-1), leading to extracellular acidification and protease activity that degrades the extracellular matrix.[20]

VGSC Inhibitors and Blockers in Cancer Therapy

Ion channel inhibitors, especially VGSC blockers, are gaining traction in oncology for their potential to suppress tumour progression. *In vitro* studies have demonstrated that inhibiting VGSC expression reduces cancer cell invasiveness. For example, phenytoin and riluzole significantly suppressed proliferation in prostate cancer cell lines.[27] Some VGSC inhibitors not only suppress tumour growth but also enhance immune responses.[9] Specific subtypes like $Na_V1.5$ and its neonatal variant ($nNa_V1.5$) have been targeted in breast cancer, where NP siRNA and monoclonal antibody mAb-$nNa_V1.5$ effectively reduced invasion and metastasis.[28,29]

However, achieving tumour specificity remains a challenge. Combination therapies such as tamoxifen with VGSC inhibitors or si-$Na_V1.6$ to block TNF-α, have shown promise in targeting both hormonal and ion channel pathways.[30-32] Nonetheless, not all combinations are effective; for instance, both propranolol and ranolazine failed to demonstrate synergistic effects.[33]

Other VGSC blockers include tramadol, which reversibly inhibits $Na_V1.7$ and $Na_V1.5$;[34] lidocaine, which induces apoptosis in ovarian cancer cells; and DHA,[35] which reduced migration in MDA-MB-231 breast cancer cells by 26%.[10] Agents like phenytoin and ranolazine have shown anti-metastatic properties, with the latter also providing cardio-neuroprotection.[26,37] Additionally, naringenin reduces $Na_V1.7$ expression and suppresses cancer cell motility.[38]

In conclusion, VGSC inhibitors hold significant therapeutic promise, but their roles in modulating chemoresistance and driving tumour progression warrant further studies.[8]

Exploring the Uniqueness of Neonatal $Na_V1.5$

$nNa_V1.5$ Structure and Expression

The $nNa_V1.5$ is a splice isoform identified in humans, rats, and mice, which features a sequence distinct from the adult $Na_V1.5$ primarily found in cardiac tissues.[39] Unlike the adult form, $nNa_V1.5$ is selectively expressed in various cancers but not in normal tissues, making it a promising candidate for early cancer diagnosis and targeted anti-metastatic therapies.[23] This cancer-specific expression pattern sets $nNa_V1.5$ apart from other sodium channels, whose splice variants differ only slightly, limiting their therapeutic selectivity. Although there may be apparent homogeneity between the adult and neonatal forms, they are pharmacologically different, allowing for the design of drugs that specifically target $nNa_V1.5$ without affecting the adult form.[28]

The transition from the adult to the neonatal form results from alternative splicing of exon ^{6}, leading to the substitution of seven amino acids within the S3–S4 segment of the voltage sensor domain I (VSDI).[40] Notably, this includes the replacement of a negatively charged aspartate at position 211 with a positively charged lysine in $nNa_V1.5$. This lysine is located in a helix -turn-helix motif in the voltage-sensing region, which is exposed on the cell surface. This particular site is a

potential target for peptide toxins from animal venoms, which can selectively bind with and modify gating behaviour in a subtype-specific manner,[41] thus adding to the therapeutic benefit of nNa$_V$1.5.

nNa$_V$1.5 Roles

The nNa$_V$1.5 is highly expressed in neonates and is re-expressed in various cancers, especially breast cancer, where it is associated with metastasis. In contrast to the adult variant, nNa$_V$1.5 is capable of promoting cancer cell invasion, particularly in acidic tumour microenvironments, because of its higher acid resistance.[23,42] nNa$_V$1.5 activity is regulated by Protein Kinase A (PKA); inhibition with KT5720 has been shown to reduce nNa$_V$1.5-mediated expression and metastasis.[2] In breast cancer, nNa$_V$1.5 expression is associated with oestrogen receptor (ER) status and increased levels of glutamate, which further promote metastasis.[28,43]

Epigenetic control by RE1-silencing transcription factor (REST) and histone deacetylase 2 (HDAC2) also has been established, where they repress nNa$_V$1.5 expression at low levels. In MCF-7 cells, and histone deacetylase (HDAC) inhibitors such as Trichostatin A (TSA) decrease the level of REST and HDAC2, thereby increasing the expression of nNa$_V$1.5, making it more aggressive.[44] In colorectal cancer, the levels of nNa$_V$1.5 correlate with the progression-free survival and the Tumour-Node-Metastasis (TNM) breast cancer stage.[45]

Outside cancer, nNa$_V$1.5 is also present in dorsal root ganglia (DRG) neurons where it is responsible for causing neuropathic pain.[46] Compared to the adult isoform, nNa$_V$1.5 has a 50% greater Na^+ influx, activates at more positive voltages, and exhibits slower activation and recovery kinetics.[40] These properties reinforce its value as a cancer-specific therapeutic target.[28,47] Figure 2 shows a comparison of the protein sequences, illustrating the functional architecture of the general α-subunit of the voltage-gated sodium channel and the VSDI S3–S4 region of adult and neonatal Na$_V$1.5.

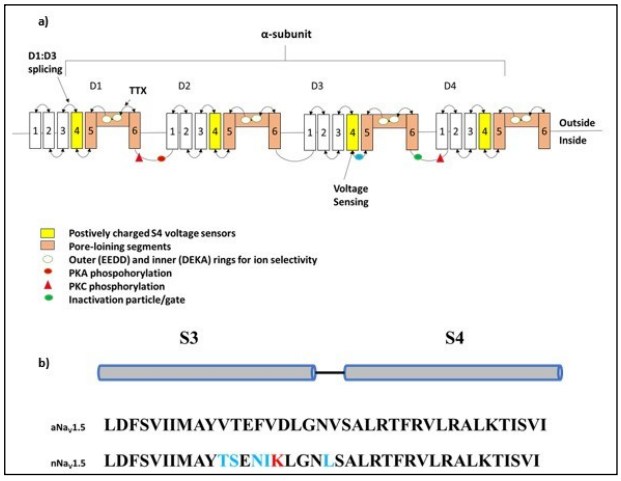

Figure 2: Protein sequence comparison of (a) functional architecture of the general α-subunit voltage-gated sodium channel; (b) Adult (aNa$_V$1.5) and Neonatal (nNa$_V$1.5) isoforms in the voltage-sensing domain (VSDI): S3-S4 region with important residues highlighted in blue and red[23,40]

Expression Patterns of nNa$_V$1.5 in Cancers

The nNa$_V$1.5 has emerged as a critical contributor to cancer progression, particularly in breast, colorectal, and brain cancers. In triple-negative breast cancer (TNBC), nNa$_V$1.5 is significantly upregulated and strongly associated with metastasis.[28,29,42] Elevated anti-nNa$_V$1.5 antibody levels in advanced breast cancer patients suggest its potential as a biomarker.[20] These antibodies may influence immune responses, possibly contributing to tumour progression. Functionally, inhibition of nNa$_V$1.5 reduces lymph node metastasis, reinforcing its role in invasiveness.[39] To date, most supporting evidence comes from *in vitro* studies using models such as MDA-MB-231 cells and limited serum antibody analyses. While these findings indicate strong clinical relevance, validation in larger patient cohorts is still lacking. In particular, the prognostic value of circulating anti-nNa$_V$1.5 antibodies needs to be tested against established clinical markers such as ER, progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Future research should focus on integrating nNa$_V$1.5 expression data with clinical outcomes in well-annotated cohorts to determine its reliability as a prognostic and predictive biomarker.

In colorectal cancer, nNa$_V$1.5 expression is significantly elevated in advanced conditions and correlates with poor prognosis, highlighting its potential as a biomarker and

therapeutic target in resistant cases.[45] However, much of the data relies on immunohistochemistry with relatively small sample sizes, and mechanistic insights into how nNa$_V$1.5 drives invasion under hypoxia or chemotherapy resistance remain incomplete. Expanding studies to multi-center cohorts and integrating transcriptomic analyses is crucial for validating nNa$_V$1.5 as a clinically useful biomarker in colorectal cancer. Meanwhile, in brain cancer, nNa$_V$1.5 was initially identified via exon 6A,[48] with later studies confirming its high expression in astrocytomas and correlation with tumour grade.[15,49-51] Several Na$_V$1.5 isoforms have been identified in the human brain.[46,52] Additionally, Na$_V$1.6 expression in gliomas offers broader therapeutic avenues.[32] Using ion channel gene (iCG) signatures, including Na$_V$1.5 and Na$_V$1.6, studies have shown that higher expression levels predicted poorer glioma survival and aided risk stratification.[53]

In addition, Na$_V$1.5 increases NHE-1 pH sensitivity, which increases Li^+ uptake in acidic tumour microenvironments.[54] nNa$_V$1.5-driven extracellular acidification may modulate immune evasion by increasing regulatory T cell (Treg) activity and suppressing cytotoxic responses.[55] Additionally, nNa$_V$1.5 was associated with enhanced glutamate secretion in TNBC cells, which facilitates a pro-metastatic state. This effect was later abolished by TTX treatment, supporting the functional role of nNa$_V$1.5 in metastasis.[28] Evidence for nNa$_V$1.5 in gliomas highlights its association with higher tumour grade and poorer survival, but these studies are largely observational and often lack functional validation in vivo. A major barrier to translation is drug delivery across the blood-brain barrier, which remains poorly addressed in current preclinical work. Innovative delivery systems such as nanobody- or nanoparticle-based approaches should be tested in animal models to establish the feasibility of targeting nNa$_V$1.5 in brain cancers. Table 2 presents a summary of the expression patterns and clinical associations of nNa$_V$1.5 across various cancer types.

Table 2: Expression patterns and clinical associations of nNaV1.5 across cancer types

Cancer Type	Expression Pattern of nNa$_V$1.5	References
Breast Cancer (esp. Triple-Negative)	Strong upregulation in invasive and metastatic cells; detectable circulating antibodies in advanced disease	Brisson et al., 2013; Azahar et al., 2022; Rajaratinam et al., 2022; Sharudin et al., 2022
Colorectal Cancer	Elevated in advanced disease stages; expression correlates with TNM stage progression	Lastraioli et al., 2021; Guzel et al., 2019
Brain Tumours (Gliomas, Astrocytomas, Glioblastoma)	Overexpression in high-grade gliomas and astrocytomas; exon 6A isoform/nNa$_V$1.5 confirmed in brain tissue.	Xing et al., 2014; Wang et al., 2015; Schrey et al., 2002
Prostate Cancer	Na$_V$1.5 and nNa$_V$1.5 functionally expressed in malignant prostate cells.	Yildirim et al., 2012; Smith et al., 1998
Ovarian Cancer	Functional contribution of Na$_V$1.5/ nNa$_V$1.5 in tumour cells.	Liu et al., 2021
Cervical Cancer	Functional VGSC expression, including Na$_V$1.5 and nNa$_V$1.5	Díaz et al., 2007
Lung Cancer (non-small cell, small cell)	Functional Na$_V$1.5 expression in highly metastatic cell lines	Roger et al., 2007; Onganer & Djamgoz, 2005

Therapeutic Targeting of nNa$_V$1.5

Targeting nNa$_V$1.5 to Prevent Breast Cancer Invasion

In breast cancer, particularly in aggressive subtypes such TNBC, nNa$_V$1.5 contributes to a distinct set of pro-metastatic processes. Its activity enhances glycolysis, drives extracellular acidification that activates proteases, and alters ion dynamics to promote cell migration. Inhibition of nNa$_V$1.5 disrupts these pathways, thereby reducing the invasive behaviour of breast cancer cells and offering a rationale for therapeutic intervention.[1] Moreover, antibodies specifically targeting nNa$_V$1.5 have shown promise as therapeutic agents and have been demonstrated to effectively inhibit its function, thereby limiting metastatic behaviour.[56] Their efficacy has also been tested using 3D spheroid invasion assays, providing more physiologically relevant models of breast cancer metastasis.

Preclinical studies support the anti-metastatic potential of targeting nNa$_V$1.5. For instance, siRNA-mediated knockdown of the SCN5A gene, which encodes nNa$_V$1.5, significantly reduced breast cancer cell migration and invasion.[47] Pharmacological agents like ranolazine, originally developed to treat angina, have demonstrated strong inhibitory effects on VGSC activity, particularly against Na$_V$1.5 and its neonatal variant. In addition,

ranolazine significantly reduced invasiveness in both 2D and 3D models of breast cancer, especially within acidic tumour microenvironments, where nNa$_V$1.5 expression is elevated.[42] Combination therapies have also shown potential; for example propranolol, a β-blocker, inhibited lateral motility and invasion in MDA-MB-231 cells and exhibited enhanced anti-metastatic effects when combined with ranolazine.[57]

Epigenetic mechanisms also regulate nNa$_V$1.5 expression. HDAC inhibitors such as TSA have been found to modulate Na$_V$1.5 expression, reducing its oncogenic activity in metastatic breast cancer.[44] Since treatment with TSA led to a reduction in nNa$_V$1.5 levels and metastatic potential in cancer cells, this suggests that epigenetic therapies may represent a viable strategy for controlling metastasis.

Other inhibitors with distinct pharmacological characteristics, such as phenytoin and DAPT (N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine-t-butyl ester), have been found to inhibit the proliferation, migration, and invasion of MDA-MB-231 breast cancer cells.[33,58] These results strengthen the therapeutic potential of nNa$_V$1.5. However, more research is needed to fully understand the genetic and epigenetic regulation of nNa$_V$1.5 and to translate these findings into targeted clinical approaches for metastatic breast cancer.

Targeting nNa$_V$1.5 to Inhibit Brain Tumour Invasion

Different research works have emphasised the significance of nNa$_V$1.5 in cancer cell motility, highlighting its role in regulating ion exchange that drives migration and invasion within the tumour microenvironment.[31] In brain tumours such as glioblastoma, nNa$_V$1.5 overexpression is strongly associated with extracellular matrix degradation and is correlated with higher tumour grade and aggressiveness. This close link to malignancy underscores its value as a therapeutic target.[15] Thus, blocking this channel can potentially reduce cancer cell invasion and metastasis.

Inhibition of nNa$_V$1.5 and other ion channels as a therapeutic strategy in glioblastoma has been explored.[31] A study has demonstrated that inhibition of nNa$_V$1.5 reduced the motility of glioblastoma cells, a key characteristic of this very invasive cancer. Drug delivery systems that employ nanobodies have also shown increased specificity and efficacy. Nanobodies are small single-domain antibody fragments derived from camelid antibodies, noted for their ability to penetrate tissues and cross the blood-brain barrier (BBB), making them promising for targeting CNS tumours.[59] Nanobodies present a new therapeutic approach by delivering drugs directly to tumour cells that express nNa$_V$1.5, thereby attenuating off-target effects and enhancing therapeutic precision. This strategy can be particularly valuable in the management of aggressive cancers like glioblastoma, where the BBB is a limiting factor to effective drug delivery.

Potential Role of nNa$_V$1.5 as a Diagnostic Marker

Beyond its therapeutic potential, nNa$_V$1.5 can also serve as an early diagnostic marker of aggressive cancers. Upregulation of nNa$_V$1.5 has been associated with poor prognosis and high metastatic potential in breast and colorectal cancers.[23] Screening of nNa$_V$1.5 expression may allow early detection of metastatic disease and the timely initiation of treatments targeting VGSC activity. In addition, the search for anti-metastatic agents that selectively block nNa$_V$1.5 function is a potentially rewarding approach for developing novel therapeutics.

The possibility of using anti-nNa$_V$1.5 antibodies as biomarkers of breast cancer progression has been investigated.[55] In the study, the detection of neonatal Na$_V$1.5 antigens in the blood samples enabled the identification of antibodies that correlated with breast cancer metastasis. Pro-inflammatory cytokines (IL-6) and anti-nNa$_V$1.5 antibodies could also be used as biomarkers for monitoring tumour development and immune system functioning. Furthermore, it was suggested that the immunogenicity of nNa$_V$1.5 may be used as a marker of immune surveillance, associating nNa$_V$1.5 with the triad interplay between breast cancer, metastasis, and the immune system.[20] These findings highlight the potential of nNa$_V$1.5 as a cancer immunotherapy target and a promising direction for further studies.

LIMITATIONS AND FUTURE PERSPECTIVES

Despite the promising therapeutic potential of targeting nNa$_V$1.5 in cancer metastasis, several significant challenges remain. First, most studies on nNa$_V$1.5-targeted therapies have been conducted in preclinical models, and only a few clinical trials have evaluated their effectiveness in cancer patients. Currently, nNa$_V$1.5-targeted strategies remain at the preclinical stage. Monoclonal antibodies (mAb-nNa$_V$1.5) and siRNA approaches have shown promise *in vitro* and animal models, but no registered clinical trials have yet advanced beyond exploratory preclinical work. The translation of these preclinical findings into clinical application is challenging due to the heterogeneity of tumour biology and patient-specific responses. Moreover, considering that anti-nNa$_V$1.5 antibodies and VGSC inhibitors (including TTX and ranolazine) primarily demonstrate *in vitro* activity, their off-target effects and potential toxicity in humans are of concern because of the essential roles of VGSCs in cardiac and neural function.

Another major limitation is the heterogeneity of VGSC expression in various types of cancers, as well as the variability of nNa$_V$1.5 expression within tumours. Such diversity complicates the development of therapeutic approaches that are broadly applicable. Furthermore, the mechanisms underlying epigenetic regulation of nNa$_V$1.5 expression is poorly understood, and thus, more studies are required to clarify how HDACs and other epigenetic therapies can be leveraged in cancer treatment.

Future research should address these limitations by conducting well-designed clinical trials to demonstrate the safety and efficacy of Na$_V$1.5–targeted therapies. A key obstacle that needs to be overcome is drug delivery, especially in brain cancers such as glioblastoma, where the BBB restricts access of large molecules like antibodies. In addition, the high homology between the neonatal and adult isoforms poses a risk of off-target cardiac toxicity, underscoring the urgent need for highly selective therapeutic agents. Tumour heterogeneity represents another challenge, as intra-tumoural differences in nNa$_V$1.5 expression and inter-patient variability may reduce therapeutic consistency and complicate biomarker validation.

The development of more specific VGSC inhibitors and improved delivery systems, such as nanobody- or nanoparticle-based platforms, could enhance therapeutic precision and minimize systemic side effects. Furthermore, exploring combination strategies that integrate nNa$_V$1.5-targeted therapies with immunotherapy or chemotherapy may provide synergistic benefits in metastatic cancers. Finally, advancing this field will require defining the role of nNa$_V$1.5 across cancer types and establishing its application as a clinically reliable biomarker for early diagnosis, prognosis, and treatment stratification.

CONCLUSION

The neonatal isoform of Na$_V$1.5 plays a critical role in cancer metastasis, particularly in breast, colorectal, and brain tumours. With its ability to promote cancer cell migration, invasion, and survival, it represents a unique and highly promising therapeutic target. Preclinical studies have demonstrated that inhibition of nNa$_V$1.5 through specific antibodies, VGSC inhibitors, or epigenetic regulators can reduce invasiveness and metastatic potential, underscoring its value as both a therapeutic target and a biomarker of aggressive cancers. Nonetheless, major challenges remain in advancing this approach to the clinical stage. Future research should focus on developing highly selective inhibitors, validating their efficacy in clinical trials, and investigating strategic combination therapies. Advancing the understanding of nNa$_V$1.5's role in cancer biology may ultimately enable new and effective treatment strategies for metastatic tumours and improve survival outcomes for cancer patients.

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REFERENCES

1. Leslie TK, Brackenbury WJ. Sodium channels and the ionic microenvironment of breast tumours. *J Physiol* 2023; 601:1543-53. Doi: 10.1113/JP282306
2. Chioni AM, Shao D, Grose R, Djamgoz MBA. Protein kinase A and regulation of neonatal $Na_V1.5$ expression in human breast cancer cells: Activity-dependent positive feedback and cellular migration. *Int J Biochem Cell Biol* 2010; 42(2):346-58. Doi: 10.1016/j.biocel.2009.11.021
3. Errington AC, Madison K, Kumar A, Lees G. Voltage-gated sodium channels and their roles in drug action. *Curr Anaesth Crit Care* 2005; 16(2):81-7. Doi: 10.1016/j.cacc.2005.03.001
4. Payandeh J, Scheuer T, Zheng N, Catterall WA. The crystal structure of a voltage-gated sodium channel. *Nature* 2011; 475(7356):353-9. Doi: 10.1038/nature10238
5. Chen H, Xia Z, Dong J, et al. Structural mechanism of voltage-gated sodium channel slow inactivation. *Nat Commun* 2024; 15(1):3691. Doi: 10.1038/s41467-024-48125-3
6. Zhang F, Xu X, Li T, Liu Z. Shellfish toxins targeting voltage-gated sodium channels. *Mar Drugs* 2013; 11(12):4698-723. Doi: 10.3390/md11124698
7. Catterall WA. Voltage-gated sodium channels at 60: Structure, function and pathophysiology. *J Physiol* 2012; 590(11):2577-89. Doi: 10.1113/jphysiol.2011.224204
8. Mao W, Zhang J, Körner H, Jiang Y, Ying S. The emerging role of voltage-gated sodium channels in tumour biology. *Front Oncol* 2019; 9:124. Doi: 10.3389/fonc.2019.00124
9. Capatina AL, Lagos D, Brackenbury WJ. Targeting ion channels for cancer treatment: Current progress and future challenges. *Rev Physiol Biochem Pharmacol* 2022; 183:1-43. Doi: 10.1007/112_2020_46
10. Isbilen B, Fraser SP, Djamgoz MBA. Docosahexaenoic acid (omega - 3) blocks voltage-gated sodium channel activity and migration of MDA-MB-231 human breast cancer cells. *Int J Biochem Cell Biol* 2006; 38(12):2173-82. Doi: 10.1016/j.biocel.2006.06.014
11. Diaz D, Delgadillo DM, Hernández-Gallegos E, et al. Functional expression of voltage-gated sodium channels in primary cultures of human cervical cancer. *J Cell Physiol* 2007; 210(2):469-78. Doi: 10.1002/jcp.20871
12. Roger S, Rollin J, Barascu A, et al. Voltage-gated sodium channels potentiate the invasive capacities of human non-small-cell lung cancer cell lines. *Int J Biochem Cell Biol* 2007; 39(4):774-86. Doi: 10.1016/j.biocel.2006.12.007
13. House CD, Vaske CJ, Schwartz AM, et al. Voltage-gated Na+ channel SCN5A is a key regulator of a gene transcriptional network that controls colon cancer invasion. *Cancer Res* 2010; 70(17):6957-67. Doi: 10.1158/0008-5472.CAN-10-1169
14. Yildirim S, Altun S, Gumushan H, Patel A, Djamgoz MBA. Voltage-gated sodium channel activity promotes prostate cancer metastasis *in vivo*. *Cancer Lett* 2012; 323(1):58-61. Doi: 10.1016/j.canlet.2012.03.036
15. Xing D, Wang J, Ou S, et al. Expression of neonatal $Na_V1.5$ in human brain astrocytoma and its effect on proliferation, invasion and apoptosis of astrocytoma cells. *Oncol Rep* 2014; 31(6):2692-700. Doi: 10.3892/or.2014.3143
16. Besson P, Driffort V, Bon É, Gradek F, Chevalier S, Roger S. How do voltage-gated sodium channels enhance migration and invasiveness in cancer cells? *Biochim Biophys Acta* 2015; 1848(10): 2493-501. Doi: 10.1016/j.bbamem.2015.04.013
17. Schrey M, Cdoina C, Kraft R, et al. Molecular characterization of voltage-gated sodium channels in human gliomas. *Neuroreport* 2002; 13(18):2493-8. Doi: 10.1097/00001756-200212200-00023
18. Brisson L, Driffort V, Benoist L, et al. $Na_V1.5$ Na^+ channels allosterically regulate the NHE-1 exchanger and promote the activity of breast cancer cell invadopodia. *J Cell Sci* 2013; 126(Pt 21):4835-42. Doi: 10.1242/jcs.123901
19. Brackenbury WJ. Voltage-gated sodium channels and metastatic disease. *Channels* 2012; 6(5):352-61. Doi: 10.4161/chan.21910
20. Rajaratinam, H., Rasudin, N. S., Yahya, M. M., et al. Circulating Neonatal $Na_V1.5$ (n$Na_V1.5$) antigen and anti-n$Na_V1.5$ antibodies as potential biomarkers for

breast cancer metastasis. *Sains Malaysiana* 2022; 51 (9):2967-84. Doi: 10.17576/jsm-2022-5109-18
21. Smith P, Rhodes, N. P., Shortland, A. P., et al. Sodium channel protein expression enhances the invasiveness of rat and human prostate cancer cells. *FEBS Letters* 1998; 423(1):19-24. Doi: 10.1016/S0014-5793(98)00050-7
22. Horne J, Mansur S, Bao Y. Sodium ion channels as potential therapeutic targets for cancer metastasis. *Drug Discov Today* 2021; 26(5):1136-47. Doi: 10.1016/j.drudis.2021.01.026
23. Fraser SP, Onkal R, Theys M, Bosmans F, Djamgoz MBA. Neonatal $Na_V1.5$ channels: pharmacological distinctiveness of a cancer-related voltage-gated sodium channel splice variant. *Br J Pharmacol* 2022; 179(3):473-86. Doi: 10.1111/bph.15668
24. Onganer PU, Djamgoz MBA. Small-cell lung cancer (human): Potentiation of endocytic membrane activity by voltage-gated Na+ channel expression *in vitro*. *J Membrane Biol* 2005; 204(2):67–75. Doi: 10.1007/s00232-005-0747-6
25. Fraser SP, Ozerlat-Gunduz I, Brackenbury WJ, et al. Regulation of voltage-gated sodium channel expression in cancer: Hormones, growth factors and auto-regulation. *Philos Trans R Soc Lond B Biol Sci* 2014; 369(1638):20130105. Doi: 10.1098/rstb.2013.0105
26. Djamgoz MBA, Coombes RC, Schwab A. Ion transport and cancer: From initiation to metastasis. *Philos Trans R Soc Lond B Biol Sci* 2014; 369 (1638):20130092. Doi: 10.1098/rstb.2013.0092
27. Sanchez-Sandoval AL, Hernández-Plata E, Gomora JC. Voltage-gated sodium channels: from roles and mechanisms in the metastatic cell behaviour to clinical potential as therapeutic targets. *Front Pharmacol* 2023*;* 14:1206136. Doi: 10.3389/fphar.2023.1206136
28. Azahar II, Sharudin NA, Noor Din AHM, et al. n$Na_V1.5$ expression is associated with glutamate level in breast cancer cells. *Biol Res* 2022; 55(1):18. Doi: 10.1186/s40659-022-00387-1
29. Erdogan MA, Yuca E, Ashour A, Gurbuz N, Sencan S, Ozpolat B. SCN5A promotes the growth and lung metastasis of triple-negative breast cancer through EF2-kinase signaling. *Life Sci* 2023; 313:121282. Doi: 10.1016/j.lfs.2022.121282
30. Ning J, Wakimoto H, Rabkin SD. Immunovirotherapy for glioblastoma. *Cell Cycle* 2013; 13(2):175-6. Doi: 10.4161/cc.27039
31. Varricchio A, Ramesh SA, Yool AJ. Novel ion channel targets and drug delivery tools for controlling glioblastoma cell invasiveness. *Int J Mol Sci* 2021; 22:11909. Doi: 10.3390/ijms222111909
32. Ai Y, Zhang X, Hu X, et al. Role of the voltage-gated sodium channel $Na_V1.6$ in glioma and candidate drugs screening. *Int J Mol Med* 2023; 51 (6):46. Doi: 10.3892/ijmm.2023.5249
33. Aktas CC, Zeybek ND, Piskin AK. *In vitro* effects of phenytoin and DAPT on MDA-MB-231 breast cancer cells. *Acta Biochim Biophys Sin* 2015; 47(9):680-6. Doi: 10.1093/abbs/gmv066
34. Bok CS, Kim RE, Cho YY, Choi JS. Tramadol as a voltage-gated sodium channel blocker of peripheral sodium Channels $Na_V1.7$ and $Na_V1.5$. *Biomol Ther* 2023; 31(2):168-75. Doi: 10.4062/biomolther.2023.002
35. Liu C, Yu M, Li Y, et al. Lidocaine inhibits the metastatic potential of ovarian cancer by blocking $Na_V1.5$-mediated EMT and FAK/Paxillin signaling pathway. *Cancer Med* 2021; 10(1):337-49. Doi: 10.1002/cam4.3621
36. De M, Ruiz L, Kraus RL, De Lera Ruiz M, Paragon A, Environment P. Voltage-gated sodium channels: structure, function, pharmacology and clinical indications. *J Med Chem* 2015; 58(18):7093-118. Doi: 10.1021/jm501981g
37. Djamgoz MBA. Ranolazine: a potential anti-metastatic drug targeting voltage-gated sodium channels. *Br J Cancer* 2024; 130(9):1415-9. Doi: 10.1038/s41416-024-02622-w
38. Lopez-Charcas O, Pukkanasut P, Velu SE, et al. Pharmacological and nutritional targeting of voltage-gated sodium channels in the treatment of cancers. *iScience* 2021; 24(4):102270. Doi: 10.1016/j.isci.2021.102270
39. Chioni AM, Fraser SP, Pani F, et al. A novel polyclonal antibody specific for the $Na_V1.5$ voltage-gated Na+ channel "neonatal" splice form. *J Neurosci Methods* 2005; 147(2):88-98. Doi: 10.1016/j.jneumeth.2005.03.010

40. Onkal R, Mattis JH, Fraser SP, et al. Alternative splicing of $Na_V1.5$: An electrophysiological comparison of "neonatal" and "adult" isoforms and critical involvement of a lysine residue. *J Cell Physiol* 2008; 216(3):716-26. Doi: 10.1002/jcp.21451
41. Ahern CA, Payandeh J, Bosmans F, Chanda B. The hitchhiker's guide to the voltage-gated sodium channel galaxy. *J Gen Physiol* 2016; 147(1):1-24. Doi: 10.1085/jgp.201511492
42. Onkal R, Fraser SP, Djamgoz MBA. Cationic modulation of voltage-gated sodium channel ($Na_V1.5$): Neonatal versus adult splice variants - 1. monovalent (H+) ions. *Bioelectricity* 2019; 1(3):139-47. Doi: 10.1089/bioe.2019.0012
43. Yamaci RF, Fraser SP, Battaloglu E, et al. Neonatal $Na_V1.5$ protein expression in normal adult human tissues and breast cancer. *Pathol Res Pract* 2017; 213 (8):900-7. Doi: 10.1016/j.prp.2017.06.003
44. Kamarulzaman NS, Dewadas HD, Leow CY, Yaacob NS, Mokhtar NF. The role of REST and HDAC2 in epigenetic dysregulation of $Na_V1.5$ and n$Na_V1.5$ expression in breast cancer. *Cancer Cell Int* 2017; 17(1):74. Doi: 10.1186/s12935-017-0442-6
45. Lastraioli E, Fraser SP, Guzel RM, et al. Neonatal $Na_V1.5$ protein expression in human colorectal cancer: Immunohistochemical characterization and clinical evaluation. *Cancers* 2021; 13(15):3832. Doi: 10.3390/cancers13153832
46. Wang J, Ou SW, Zhang ZY, Qiu B, Wang YJ. Molecular expression of multiple $Na_V1.5$ splice variants in the frontal lobe of the human brain. *Int J Mol Med* 2018; 41(2):915-23. Doi: 10.3892/ijmm.2017.3286
47. Guzel RM, Ogmen K, Ilieva KM, Fraser SP, Djamgoz MBA. Colorectal cancer invasiveness *in vitro*: Predominant contribution of neonatal $Na_V1.5$ under normoxia and hypoxia. *J Cell Physiol* 2019; 234 (5):6582-93. Doi: 10.1002/jcp.27399
48. Ou SW, Kameyama A, Hao LY, et al. Tetrodotoxin-resistant Na+ channels in human neuroblastoma cells are encoded by new variants of $Na_V1.5$/SCN5A. *Eur J Neurosci* 2005; 22(4):793-801. Doi: 10.1111/j.1460-9568.2005.04280.x
49. Wang J, Ou SW, Wang YJ, et al. New variants of $Na_V1.5$/SCN5A encode Na+ channels in the brain. *J Neurogenet* 2008; 22(1):57-75. Doi: 10.1080/01677060701672077
50. Wang J, Ou SW, Wang YJ, Kameyama M, Kameyama A, Zong ZH. Analysis of four novel variants of $Na_V1.5$/SCN5A cloned from the brain. *Neurosci Res* 2009; 64(4):339-47. Doi: 10.1016/j.neures.2009.04.003
51. Ren CT, Li DM, Ou SW, et al. Cloning and expression of the two new variants of $Na_V1.5$/SCN5A in rat brain. *Mol Cell Biochem* 2012; 365(1–2):139-48. Doi: 10.1007/s11010-012-1253-7
52. Wang J, Ou SW, Wang YJ. Distribution and function of voltage-gated sodium channels in the nervous system. *Channels* 2017; 11(6):534-54. Doi: 10.1080/19336950.2017.1380758
53. Wang R, Gurguis C.I, Gu W, et al. Ion channel gene expression predicts survival in glioma patients. *Sci Rep* 2015; 5:11593. Doi: 10.1038/srep11593
54. Brisson L, Gillet L, Calaghan S, et al. Na_V 1.5 enhances breast cancer cell invasiveness by increasing NHE1-dependent H efflux in caveolae. *Oncogene* 2011; 30(17):2070-6. Doi: 10.1038/onc.2010.574
55. Rajaratinam H, Rasudin NS, Al Astani TAD, et al. Breast cancer therapy affects the expression of antineonatal $Na_V1.5$ antibodies in the serum of patients with breast cancer. *Oncol Lett* 2021; 21 (2):108. Doi: 10.3892/ol.2020.12369
56. Sharudin NA, Din AHMN, Azahar II, et al. Invasion and metastasis suppression by anti-neonatal $Na_V1.5$ antibodies in breast cancer. *Asian Pac J Cancer Prev* 2022; 23(9):2953-64. Doi: 10.31557/APJCP.2022.23.9.2953
57. Lee A, Fraser SP, Djamgoz MBA. Propranolol inhibits neonatal $Na_V1.5$ activity and invasiveness of MDA-MB-231 breast cancer cells: Effects of combination with ranolazine. *J Cell Physiol* 2019; 234 (12):23066-81. Doi: 10.1002/jcp.28868
58. Yang M, Kozminski DJ, Wold LA, et al. Therapeutic potential for phenytoin: Targeting $Na_V1.5$ sodium channels to reduce migration and

invasion in metastatic breast cancer. *Breast Cancer Res Treat* 2012; 143(2):603-15. Doi: 10.1007/s10549-012-2102-9

59. Jin, B.-k.; Odongo, S.; Radwanska, M.; Magez, S.NANOBODIES®: A Review of Generation, Diagnostics and Therapeutics. *Int J Mol Sci* 2023, 24, 5994. Doi: 10.3390/ijms24065994