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## Nothing But NET: A Review of Norepinephrine Transporter Expression and Efficacy of <sup>131</sup>I-mIBG Therapy

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### Abstract

Neuroblastoma is unique amongst common pediatric cancers for its expression of the norepinephrine transporter (NET), enabling tumor-selective imaging and therapy with radioactive analogues of norepinephrine. The majority of neuroblastoma tumors are avid for <sup>123</sup>I-metaiodobenzaguanidine (mIBG) on imaging, yet the therapeutic response to <sup>131</sup>I-mIBG is only 30% in clinical trials, and off-target effects cause short- and long-term morbidity. We review the contemporary understanding of the tumor-selective uptake, retention, and efflux of mIBG and strategies currently in development for improving its efficacy. Combination treatment strategies aimed at enhancing NET are likely necessary to reach the full potential of <sup>131</sup>I-mIBG therapy.

### Keywords

Neuroblastoma; norepinephrine transporter; meta-iodobenzylguanidine

### Introduction

Neuroblastoma is the most common extra-cranial solid tumor of childhood [1]. Less than 50% of patients with high-risk features survive long-term, despite multi-modal therapy with surgery, chemotherapy, antibody therapy, retinoic acid, high-dose chemotherapy and autologous hematopoietic progenitor cell transplant [2,3]. Among the novel therapeutic agents under active investigation to improve outcomes is <sup>131</sup>I-mIBG (meta-iodobenzylguanidine).

mIBG is an analog of the catecholamine norepinephrine, which can be labeled with a radioactive isotope, <sup>123</sup>Iodine or <sup>131</sup>Iodine, to use clinically for imaging or treatment,

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### Conflict of Interest Statement

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respectively, of neuroendocrine tumors. Neuroblastoma and other cancers of sympathetic neuronal precursors express the norepinephrine transporter (NET), a transmembrane protein which functions to shuttle norepinephrine across the cell membrane. 90% of children with neuroblastoma have mIBG-avid tumors by imaging with  $^{123}\text{I}$ -mIBG, but a recent meta-analysis suggests that only 30% of children who receive  $^{131}\text{I}$ -mIBG radiotherapy have any clinical response, which is usually not curative [4–6]. The reasons for this discrepancy are not clear but are likely multifactorial. One possibility is the lower mRNA and protein expression of NET found in children with high-risk neuroblastoma compared to those with low- or intermediate-risk disease, as classified by anatomic, histologic, and genetic factors [6].

In order to improve the utility of  $^{131}\text{I}$ -mIBG therapy and to rationally design combination treatment strategies, it is important to understand the detailed mechanisms of tumor-selective  $^{131}\text{I}$ -mIBG uptake, retention, and efflux, as well as the dynamics of NET expression (Figure 1).

## Norepinephrine Transporter Function

Neuroblastomas arise from the sympathetic neural precursors derived from the embryonic neural crest. As such, 90% of neuroblastomas express the norepinephrine transporter (NET), a 12 domain transmembrane protein encoded by the *SLC6A2* gene with high affinity and specificity for norepinephrine and its analogs [5,7]. NET actively transports norepinephrine primarily into adrenal chromaffin cells and pre-synaptic terminals by an ATP-dependent and specific process known as Uptake-1 [8,9]. Uptake-1 transportation is saturable and dependent upon serum sodium and chloride (co-transported substrates), temperature, pH, oxygen, and vascularity. Norepinephrine transportation is impaired in hypoxic, hyperthermic, and nitric oxide depleted environments [10–12]. Tricyclic antidepressants, such as desmethylimipramine (DMI), specifically inhibit the norepinephrine transporter uptake of norepinephrine and its analogs [13]. DMI most likely binds to the substrate recognition site but may interact with an additional sodium-dependent site of NET as DMI is more lipophilic and bulky than norepinephrine [8]. Uptake-1 can also be inhibited by Na-K-ATPase inhibitors such as ouabain and by competitive inhibition by other catecholamines/analogs [14]. Several non-neuronal cells also uptake norepinephrine by a transportation system similar to Uptake-1 (adrenal medulla, locus ceruleus, lung, heart, endothelial cells of small vessels, dental polyp, myometrial cells, placenta, vas deferens, syncytiotrophoblasts, and in glial cells in the CNS) [15,16].

The majority of NET is freely mobile within the cytoplasm when it is either unoccupied or fully occupied with its substrate and co-substrates; however, once sodium binds to NET, this mobility is lost. Thus, NET localization to the cell membrane is dependent on the presence of an inward sodium gradient created by the  $\text{Na}^+\text{-K}^+$ -ATPase pump. Sodium and chloride binding to NET also decreases the Michaelis Constant ( $K_m$ ) for norepinephrine transportation, increasing the affinity of NET for norepinephrine. NET therefore requires sodium for both membrane localization and active transport of its substrates. NET also requires free disulfide bonds, free disulfide groups, and lipids to function. NET is recruited to the cell membrane via N-linked glycosylation in the extracellular loop between domains 3

and 4 for cell surface expression [16]. This glycosylation is required for cell surface expression, but not for substrate or inhibitor recognition [17,18]. Paradoxically, N-glycosylation may also be partially responsible for the efflux of norepinephrine and its analogs from cells [8].

Norepinephrine is also brought into cells by passive, non-specific diffusion (a process known as Uptake-2) that is energy-independent, unsaturable, and results in low level norepinephrine accumulation in most tissues. After it gains entry, neuronal/adrenal cells store norepinephrine within numerous neurosecretory vesicles via the vesicular monoamine transporter (VMAT). The mechanisms of uptake and retention of mIBG are similar yet significantly differ from endogenous norepinephrine.

## mIBG

In the 1980s, Dr. Wieland and his colleagues at the University of Michigan developed mIBG (meta-iodobenzylguanidine), an analog of norepinephrine, as a scintigraphic agent to allow imaging of the adrenal medulla. They showed that mIBG, accumulates in the neurosecretory granules of adrenal chromaffin cells, similarly to norepinephrine [19]. In 1984, mIBG was also found to accumulate in neuroblastoma [5]. Numerous studies since then have provided a more detailed understanding of the mechanisms of mIBG's uptake, efflux, and retention in neural crest derived tumors.

## mIBG uptake

The majority of mIBG uptake in neuroblastoma cells is by active transport (Uptake-1), which is approximately 50 times more efficient than passive transport [8,20]. DMI and other noradrenaline analogs can inhibit Uptake-1, thus patients undergoing  $^{131}\text{I}$ -mIBG therapy are asked to minimize concomitant medications such as tricyclic antidepressants in the recognition that these and possibly other drugs may affect Uptake-1. Any decrease in the activity of the Na/K-ATPase leads to reduced uptake and increased outward transport of norepinephrine and its analogs. Thus, if the neuroendocrine cells are located in a hypoxic and glucose-depleted microenvironment, there is decreased ATP synthesis, an increase in intracellular sodium concentration, and enhanced outward transport of norepinephrine/mIBG. These features, common to many cancers, may thus limit mIBG uptake [16]. Additionally, norepinephrine and mIBG are stored in cells with large serotonin storing capacities, such as in platelets, which may contribute to the significant thrombocytopenia commonly noted in patients after high-dose mIBG therapy [21].

## mIBG retention

After  $^{131}\text{I}$ -mIBG gains entry, cancer cells store mIBG in neurosecretory vesicles via the VMAT, delivering and retaining targeted radiation within the cancer cells of gastrointestinal stromal cell tumors, pheochromocytomas, and other well-differentiated tumors [22]. Similarly to NET, VMAT expression is thought to be an important tool to select candidates for uptake studies prior to significant radiation exposures [23,24]. However, studies have shown that in neuroblastoma, the majority of mIBG remains in the cytoplasm and/or may be stored in mitochondria, presumably because neuroblastoma cells tend to have fewer storage

vesicles than more mature and/or differentiated cancer cells [25–27]. In neuroblastoma cells, only 7% of mIBG is stored in neurosecretory granules or vesicles as revealed by electron microscopy, while 93% is stored within the cytoplasm [28]. Servidei and his colleagues confirmed this finding when they stimulated vesicular release of mIBG by a calcium-dependent process and found <15% of total mIBG efflux from cells was from the neurosecretory vesicles [29]. Thus, VMAT expression likely does not play as significant a role in mIBG efficacy as NET expression for patients with neuroblastoma. Therefore, blocking VMAT is unlikely to provide a successful strategy for increasing mIBG retention. This theory is further supported by studies showing that the inhibition of catecholamine storage in neurosecretory vesicles by directly inhibiting VMAT with reserpine did not promote loss of mIBG from neuroblastoma [14,27]. mIBG is not degraded by mitochondrial monoamine oxidase (like norepinephrine), but mIBG may be stored (possibly in large amounts) in the mitochondria and the cytoplasm [30].

## mIBG efflux

mIBG often follows a diffusion gradient out of the cells and requires enough NET protein expression for re-uptake of the radioactively labeled mIBG to exert its cytotoxic effects. Efflux is highly temperature-sensitive and is inducible by high extracellular potassium concentrations or the addition of norepinephrine, unlabeled mIBG, or DMI [29]. Efflux may be carrier-mediated by the same NET receptors responsible for mIBG uptake. mIBG efflux is also a saturable process, thus adding more support to the idea that NET reverses directions and transports mIBG out of cells at low intracellular mIBG concentrations. At high intracellular concentrations, mIBG efflux is due to the passive transport across the cell membrane secondary to the diffusion gradient created after mIBG uptake. NET-mediated mIBG efflux is further supported by Servidei's study which showed that DMI likely immobilizes NET outside of the cell membrane; whereas sodium-depleted media, calcium channel blockers, cell depolarization by hyperkalemia, and additional unradiolabeled mIBG all enhanced mIBG efflux [29]. Studies by Lashford et al. show 66% of the injected radioactivity remained in the cells 24 hours later. However, upon adding DMI to block NET, only 10% of injected radioactivity was detected, likely secondary to the inhibition of the reuptake of mIBG by NET. Lashford demonstrated that mIBG typically is rapidly lost from the cells and then undergoes reuptake back into the cells via NET [28]. Therefore, not only is NET important for uptake, but mIBG accumulation likely depends on a dynamic equilibrium generated by the reuptake of the released drug [25]. We can also conclude the main efflux mechanisms of mIBG are likely carrier-mediated efflux and passive diffusion. To improve the cytotoxic effect of mIBG, stimulation/enhancement of mIBG uptake and/or inhibition of mIBG release may be important [29].

## Clinical applications of mIBG

mIBG scintigraphy was first clinically utilized in adult patients with pheochromocytoma, another neuroendocrine tumor secreting catecholamines [31,32]. In the early 1990s, <sup>131</sup>I-mIBG was first found to be safe for use in children and to have a therapeutic benefit in patients with relapsed/refractory neuroblastoma [33–35]. Escalating doses of <sup>131</sup>I-mIBG showed efficacy until the maximum tolerated dose of 18 mCi/kg [35]. A few small clinical

trials increased the number of cycles and/or the cumulative dose of  $^{131}\text{I}$ -mIBG, which also demonstrated feasibility and efficacy [21,36–42]. Today, mIBG remains an investigational therapy and continues to offer palliative pain relief and hope for a cure, mainly in some form of multi-modal therapy.

Current relapsed/refractory neuroblastoma protocols utilizing  $^{131}\text{I}$ -mIBG alongside high-dose chemotherapy and autologous stem cell rescue (ASCR) also demonstrate outcome improvement but not significantly increased long-term survival [30,43–46]. Several recent European studies show efficacy of  $^{131}\text{I}$ -mIBG as an induction monotherapy or in combination with multiple-drug chemotherapy for newly diagnosed high-risk neuroblastoma [47–50]. The Children’s Oncology Group is currently evaluating  $^{131}\text{I}$ -mIBG during induction therapy with 5 cycles of multiple chemotherapy agents, surgical resection and ASCR for high-risk neuroblastoma in the pilot study ANBL-09P1. However, in spite of all of these efforts, the discrepancy between mIBG uptake and therapy response remains.

$^{131}\text{I}$ -mIBG uptake in tumors in children with neuroblastoma is less than 0.1% of the injected dose per gram of tumor [51]. A “no-carrier added” (NCA) preparation of  $^{131}\text{I}$ -mIBG enhances the specific uptake and antitumor efficacy of  $^{131}\text{I}$ -mIBG in preclinical studies [20,52–54]. However, the utility of such preparation in patients is not yet known as the increased treatment efficacy and improved imaging did not translate in the few small Phase I/II clinical trials reported to date [55,56]. Further randomized clinical trials are required to evaluate the clinical utility of NCA-mIBG.

Multiple infusions of  $^{131}\text{I}$ -mIBG, with both higher cumulative and fractionating doses, have yielded mixed results in small Phase I/II clinical trials. While many trials show efficacy, significant improvements in long-term outcomes in children with neuroblastoma remain to be seen [35,41]. Additionally, myelotoxicity, secondary malignancy, and other systemic effects occasionally occur acutely and as late-effects in patients who received mIBG radiotherapy, further demonstrating the need for a comprehensive understanding of  $^{131}\text{I}$ -mIBG therapy to improve its therapeutic window [57–59].

The clinical therapeutic benefit exists due to  $^{131}\text{I}$ -mIBG’s pleomorphic effects on cells with high NET protein expression [35]. Certainly some of the cytotoxic effect on cells is due to the emission of  $\beta$ -radiation, inducing DNA strand breaks and inducing cell death. However, the majority of cytotoxicity is thought to be due to the phenomenon known as the *radiation-induced bystander effect*. In this bystander effect, non-irradiated neighboring cells are affected by soluble factors transmitted from the irradiated cell through direct gap junction intercellular communications and by signaling molecules released from the irradiated cell into the immediate surrounding microenvironment [60–63]. The cytotoxic effects on bystander cancer cells and/or normal tissues largely remain unclear, but studies have shown radiation-induced bystander effects to cause apoptosis and other forms of cell death, oxidative stress, proliferation, and genomic instability. Even non-radioactive mIBG exhibits cytotoxic effects, thought in part to be due to its guanidylated side chain inhibiting neuroendocrine cellular mono ADP-ribosyl transferases [64,65] and possibly due to effects on mitochondria [64,66–69]. However, the clinical significance of unlabeled mIBG’s direct cytotoxicity is minimal [70].

## Enhancing NET expression

According to a recent study by the Children's Oncology Group, children with high-risk neuroblastoma frequently have negative  $^{123}\text{I}$ -mIBG scans and a lower mRNA and protein expression of NET in their tumors by PCR and protein analysis [6], possibly related to the undifferentiated state of high-risk neuroblastomas, since NET expression is a property of relatively mature neuroendocrine/neural cells [71,72]. Low NET protein expression is likely a barrier for efficacy, though given the limited tools available, a correlation between  $^{131}\text{I}$ -mIBG dosimetry and  $^{131}\text{I}$ -mIBG efficacy in clinical trials has not been established [73,74].

It follows from these studies that one strategy to improve clinical responses in patients with neuroblastoma is to either be more selective of eligible patients or to undertake strategies to increase NET protein expression on tumor cells (Figure 1) [7,75,76]. Selecting patients for  $^{131}\text{I}$ -mIBG therapy eligibility is possible by testing one's tumor sample for NET mRNA by PCR analysis [7], but this approach will not help those with low or absent NET protein expression. Early studies found only a subset of neuroblastoma cell lines demonstrate specific uptake of radiolabeled mIBG while the other neuroblastoma lines show only passive diffusion of the drug into the cells [11]. Interestingly, ionizing radiation itself increases mIBG uptake in SK-N-SH neuroblastoma cells *in vitro*, suggesting there could be a positive-feedback effect [77]. Priming doses of radiation may thus show benefit in mIBG therapy, a concept not yet tested clinically.

It is also possible to pharmacologically stimulate NET expression. Neuroblastoma and adult neuroendocrine tumor cells treated with interferon-gamma and/or alpha prior to mIBG show increased mRNA NET expression, increased  $^{131}\text{I}$ -mIBG uptake and increased  $^{131}\text{I}$ -mIBG retention. This enhancement of NET expression and/or function may be due to tumor cell differentiation and maturation [70,72,78]. The effects of retinoic acid are more controversial. An early study showed retinoic acid-induced terminal neuronal differentiation enhanced mIBG uptake and retention *in vitro*, but this was not a direct effect of retinoic acid on the neuroblastoma cells [79]. Retinoic acid had no effect on mIBG uptake in another early study [80]. A third study showed increased mIBG uptake in neuroblastoma cells treated with retinoids combined with gamma-interferon [72]. The effect of retinoic acid on neuroblastoma mIBG uptake remains unclear due to these conflicting studies and requires further investigation.

In another neuroblastoma experiment *in vitro*, treatment with corticosteroids upregulated NET mRNA expression in a dose-dependent manner prior to  $^{131}\text{I}$ -mIBG exposure. Prolonged exposure over 3 weeks increased NET protein expression by almost 250% and enhanced norepinephrine uptake in neuroblastoma cells but downregulated NET mRNA expression *in vitro* [81]. No studies have been done to date showing this same effect *in vivo*.

Pretreatment with cisplatin and doxorubicin, both active chemotherapy agents in treating patients with neuroblastoma, also increases NET mRNA expression and increases Uptake-1 transportation of mIBG *in vitro* and *in vivo* [82,83]. Cisplatin has been studied in combination with  $^{131}\text{I}$ -mIBG in several small clinical trials in children in Italy and showed 2

patients with a complete response and 14 patients had a partial response of 21 total patients [48,84,85].

Several signaling pathways have been implicated in regulating NET expression, suggesting that targeted therapies may have utility in enhancing mIBG therapy. Protein kinase C (PKC) activation causes decreased NET protein surface expression and/or NET transport activity via PKC-dependent and PKC-independent pathways. Decreased norepinephrine uptake was noted in the presence of PKC activation and phorbol esters (which inhibit PKC) increased NET expression and norepinephrine uptake as well [86]. Thus, as PKC inhibitors are developed, they may be useful in upregulating NET. Other intracellular signaling cascades involved in augmenting the activity of membrane-localized NET include mitogen activated protein kinase (MAPK), phosphatidylinositol-3 kinase (PI3K), and calcium/calmodulin dependent protein kinase (CaMK). Brief membrane depolarization of neuroblastoma cells with potassium chloride rapidly enhances mIBG uptake via the calcium/calmodulin pathway. Potassium chloride may act through CaMKII and myosin light chain kinase to upregulate the functional capacity surface NET [87]. Further exploitation of the calcium/calmodulin pathway could thus also lead to increased NET expression. Caution is required for interpreting cell line studies, however, which may not correlate with effects on cells within a tumor microenvironment.

### Radiosensitizers

HDAC inhibitors, such as Vorinostat, are another category of novel anti-cancer therapeutics under clinical investigation that may play a role in enhancing  $^{131}\text{I}$ -mIBG therapy. HDAC inhibitors have radiosensitizing effects in a variety of cancer models [88]. Vorinostat inhibits the expression of double strand DNA break repair enzyme Ku-86 and also prolongs the expression of phosphorylated  $\gamma\text{H2AX}$ ; thus, Vorinostat sensitizes cells to radiation by inhibiting repair of radiation-induced DNA damage. HDAC inhibitors also increase NET protein expression and demonstrate an additive effect with  $^{131}\text{I}$ -mIBG radiotherapy in neuroblastoma cell cytotoxicity [89]. The use of Vorinostat prior to mIBG is being tested in a current clinical trial ([www.clinicaltrials.gov](http://www.clinicaltrials.gov), NCT02035137).

Proteasome inhibitors are currently in preclinical evaluation for use in combination with  $^{131}\text{I}$ -mIBG for neuroblastoma. Proteasome inhibitors downregulate NF- $\kappa\text{B}$  and induce cell cycle arrest in the G2/M phase, thus leading to radiosensitization of cancer cells. Proteasome inhibitors, such as Bortezomib, further enhance targeted radiotherapy by enhancing the effect of HDAC inhibitors and topoisomerase I inhibitors. Early studies suggest the combination of proteasome inhibition and  $^{131}\text{I}$ -mIBG radiation shows promise for enhancing radiation-induced cancer cell kill [90].

Topoisomerase I inhibitors increase mIBG uptake in neuroblastoma cells and disrupt DNA repair, thus also serving as radiosensitizers [91]. Topotecan is active as monotherapy for some patients with refractory neuroblastoma [92]. Irinotecan, in combination with vincristine and escalating doses of  $^{131}\text{I}$ -mIBG, also shows a 25% complete or partial response rate in patients with relapsed/refractory disease [93]. When combined with  $^{131}\text{I}$ -mIBG, topoisomerase inhibitors induce supra-additive levels of cancer cytotoxicity and increased efficacy against neuroblastoma xenografts *in vivo* [94].

Even further efficacy was noted *in vitro* and *in vivo* when PJ34, a poly (ADP-ribose) polymerase (PARP) inhibitor, was added to mIBG and Topotecan. Disruption of PARP activity leads to further disruption in DNA repair pathways, increased formation of double-stranded DNA breaks, and increased G2/M cell cycle arrest (which thus enhances radiosensitivity). Another contribution to this combination's anti-tumor efficacy is the simultaneous inhibition of PARP-1 by PJ34 and PARP-3 by mIBG. Of note, resistance to radiation developed in cancer cells after pretreatment with the PARP-inhibitor PJ34 [94]. Further investigation utilizing a PARP-1/2 inhibitor (MK-4827) together with irradiation in neuroblastoma cells revealed radiosensitization by inhibition of DNA repair, enhanced cytotoxicity, and improved survival in a metastatic murine model compared to either MK-4827 or radiation monotherapy. Preclinically, a PARP inhibitor and <sup>131</sup>I-mIBG combination holds promise for more efficacious targeted radiotherapy [95].

Gene therapy is also effective in increasing both NET expression and mIBG uptake *in vitro* and *in vivo* [96–98]. Neuroblastoma cell lines can be transfected with the NET gene and induced to actively take-up mIBG when they previously only demonstrated passive mIBG diffusion [97]. However, further targeting cytotoxicity to cancer cells by gene therapy is dependent upon achieving selective expression of therapeutic transgenes in tumors. Gene promoters stimulated by ionizing radiation are especially of interest in the field of radiotherapy; promoters regulating early growth response gene 1 (*Egr-1*), the bacterial *RecA* gene, *GADD45a*, the NF-κB binding site of the *c-IAP2* gene, and the promoter *WAF1* for the *p21<sup>WAF1/CIP1</sup>* gene all show promise. The *WAF1* promoter is of particular interest to neuroblastoma as it displays radiation-, tumor- and hypoxia-specificity in addition to increasing NET mRNA expression and increasing <sup>131</sup>I-mIBG uptake with both external beam gamma radiation and with the radionuclide, <sup>211</sup>astatine (At)-mABG [99]. Notably, gene therapy with NET creates the potential for utilizing this targeted radiation therapy for tumors that don't normally express NET.

Further investigation into interferon gamma, HDAC inhibitors, PKC modulation, gamma irradiation, and gene therapy are also necessary to improve the clinical efficacy of <sup>131</sup>I-mIBG radiotherapy. Utilization of other radionuclides, such as 4-methylated <sup>131</sup>I-mIBG (able to retain mIBG within neuroblastoma cells longer), and <sup>211</sup>At-mABG may improve the therapeutic window of radiotherapy as its shorter wavelength may cause less damage to surrounding normal tissue [60,63]. It is likely that curative therapy for neuroblastoma will require combinations of such targeted and/or molecular therapies.

## Conclusion

High-risk and relapsed/refractory neuroblastoma remain challenging and children continue to have unacceptably low survival rates despite contemporary multimodal and intensive therapies. While we have made clear advances in recent years with the addition of retinoic acid and anti-GD2 antibody therapy, pediatric oncologists and families continue to struggle with this disease and urgent novel therapies and/or combinations of therapies are necessary. A promising strategy for improving outcomes is to enhance the expression and function of NET by combining chemotherapy and other agents or treatments with <sup>131</sup>I-mIBG, including ionizing radiation, phorbol esters, retinoids, interferon-gamma, cisplatin, and doxorubicin.



The 30% response rate with mIBG therapy affords hope of a cure if this treatment modality can be fully understood and exploited as a targeted therapy. Limiting toxicities while improving outcomes remains a challenge in oncology but is one that is vitally important in neuroblastoma children who are already at significant risk of multiple toxicities after standard treatment.

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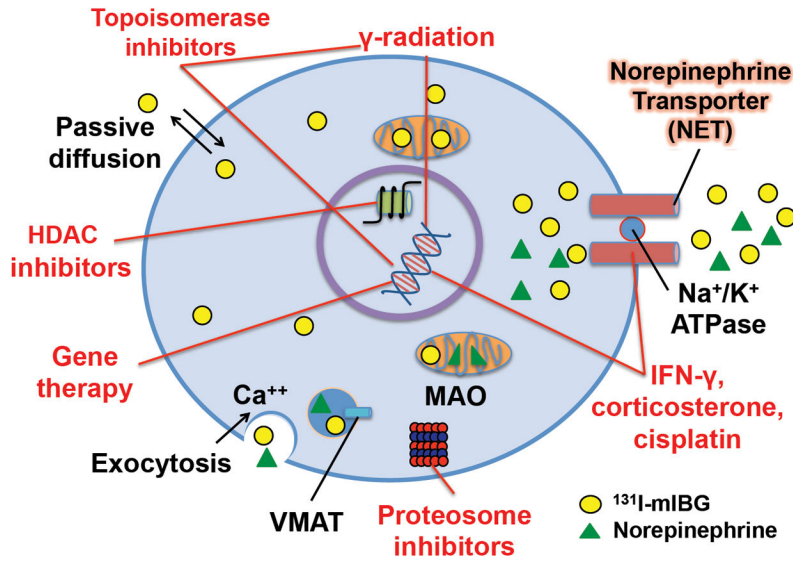
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**Figure 1.** Representation of the mechanisms involved in <sup>131</sup>I-mIBG uptake, retention, and efflux. Along with a passive diffusion phenomenon and exocytosis, <sup>131</sup>I-mIBG may be released by the uptake carrier working in a reverse mode. The latter mechanism can be triggered either by the inversion of the sodium gradient across the cell membrane or by trans-stimulation by a ligand outside the cell membrane. Red text and lines represent the mechanisms being explored to enhance <sup>131</sup>I-mIBG uptake, retention, and cytotoxicity. Some therapies radiosensitize neuroblastomas (HDAC inhibitors, gamma radiation, topoisomerase inhibitors, proteasome inhibitors) while others directly increase NET expression or enhance its function. Circle = <sup>131</sup>I-mIBG, triangle = norepinephrine. HDAC = histone deacetylase; VMAT = vesicular monoamine transporter

**Table 1**

Summary of enhancing NET expression and <sup>131</sup>I-mIBG efficacy in pre-clinical and clinical trials.

	Treatment	Preclinical data	Clinical data	References
Giving more <sup>131</sup> I-mIBG	Escalating doses of <sup>131</sup> I-mIBG	n/a	+	35
	Increasing cycles and/or cumulative doses of <sup>131</sup> I-mIBG	n/a	+	21, 35-42
<sup>131</sup> I-mIBG as first-line therapy	<sup>131</sup> I-mIBG given in induction or consolidation +/- CT +/- ASCR	0	+	47-50
<sup>131</sup> I-mIBG (relapsed/refractory)	<sup>131</sup> I-mIBG + HDCT +/- ASCR	+	+	30, 33-35, 43-46
No-carrier added <sup>131</sup> I-mIBG		+	=	20, 52-57
<sup>211</sup> At-mABG, <sup>125</sup> I-mIBG		+	0	61, 64
Increasing NET expression	Cisplatin/Doxorubicin	+	+	48, 83-86
- Differentiating agents	Retinoic acid	+/-	0	73, 80, 81
	IFN alpha	+	0	73
	IFN gamma	+	0	71-73, 79, 81
- Modulating intracellular cascades	Protein Kinase C, CaMK	+	0	87, 88
- HDAC inhibitors		+	+	89, 90
- Corticosteroids		+	0	82
- Hyperthermia		-	-	12
<b>Radiosensitization</b>	HDAC inhibitors	+	+	89
	Proteasome inhibitors	+	0	91
	Topoisomerase inhibitors	+	P	92-94
	PARP inhibitors	+	0	95, 96
Ionizing radiation	External beam radiation	+	0	78, 100
Gene therapy		+	0	97-99
Hyperbaric O2		+	0	16

(+): increases NET expression/<sup>131</sup>I-mIBG efficacy, (-): ineffective, (=): no change in <sup>131</sup>I-mIBG efficacy or NET, P: pending results, 0: no studies conducted, mIBG: meta-iodobenzylguanidine, mCi: milliCuries, kg: kilogram, ASCR: autologous stem cell rescue, CT: chemotherapy, HDCT: high-dose chemotherapy, NET: norepinephrine transporter, IFN: interferon, CaMK: calcium calmodulin dependent protein kinase, HDAC: histone deacetylase inhibitor, PARP: poly-(ADP-ribose) polymerase