REVIEW

Redifferentiation of radioiodine-refractory thyroid cancers

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Abstract

The management of radioiodine refractory thyroid cancers (RAIR TC) is challenging for the clinician. Tyrosine kinase inhibitors classically prescribed in this setting can fail due to primary or acquired resistance or the necessity of drug withdrawal because of serious or moderate but chronic and deleterious adverse effects. Thus, the concept of redifferentiation strategy, which involves treating patients with one or more drugs capable of restoring radioiodine sensitivity for RAIR TC, has emerged. The area of redifferentiation strategy leads to the creation of new definitions of RAIR TC including persistent non radioiodine-avid patients and 'true' RAIR TC patients. The latter group presents a restored or increased radioiodine uptake in metastatic lesions but with no radiological response on conventional imaging, that is, progression of a metastatic disease, thus proving that they are 'truly' resistant to the radiation delivered by radioiodine. Unlike these patients, metastatic TC patients with restored radioiodine uptake offer the hope of prolonged remission or even cure of the disease as for radioiodine-avid metastatic TC. Here, we review the different redifferentiation strategies based on the underlying molecular mechanism leading to the sodium iodide symporter (NIS) and radioiodine uptake reinduction, that is, by modulating signaling pathways, NIS transcription, NIS trafficking to the plasma membrane, NIS post-transcriptional regulation, by gene therapy and other potential strategies. We discuss clinical trials and promising preclinical data of potential future targets.

Key Words

- NIS
- radioiodine refractory thyroid cancers
- redifferentiation
- MAPK inhibition
- NIS trafficking
- epigenetic regulation

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Introduction

Although the majority of patients with differentiated thyroid cancers (DTC) could be successfully managed by surgery and radioiodine administration, for which the indication is decided according to published guidelines (Haugen *et al*. 2016, Ahuja *et al*. 2019, Luster *et al*. 2019), the evolution of a minority of these DTC will be less favourable and will require personalized management. Distant metastases encountered in less than 10% of DTC (https://seer.cancer.gov/statfacts/html/thyro.html) can lead to serious complications and even decrease the survival of patients (Wassermann *et al*. 2016). Radioiodine therapy is the cornerstone of the treatment of distant metastases from DTC. This therapy is based on the expression at the plasma membrane of normal and tumoral thyroid epithelial cells of a symporter, the sodium iodide symporter (NIS), that transports two sodium ions and one iodide ion into the cytosol. Iodide is then handled by an iodine-metabolizing machinery that concentrates iodine into the thyroid cells which has probably a major impact on radioiodine efficacy. One-third of metastatic DTC patients will be in remission after one or more radioiodine sessions (Durante *et al*. 2006). The others are or will become radioiodine refractory (RAIR), which is correlated with high tumor burden (multiple and macrometastases) along with a less differentiated tumor state and worse prognosis (Wassermann *et al*. 2016, Deandreis *et al*. 2017). Dedifferentiation is related to a decrease in or a loss of NIS expression, and/or targeting to the plasma membrane where NIS is fully effective, which results in the loss of iodine uptake in thyroid cells (Fig. 1). Other histological types are less or not at all sensitive to radioiodine such as poorly DTC (PDTC) or anaplastic thyroid cancer (ATC) as these tumours have also lost the expression of thyroid specific genes, particularly those encoding for the iodine-metabolizing machinery during the dedifferentiation process. Therapeutic strategies for RAIR thyroid cancers include the implementation of local therapy (surgery, external radiotherapy, interventional radiology approach) whenever possible and ,in the case of diffuse significant progression of distant metastatic disease systemic therapy, mainly tyrosine kinase inhibitors (TKI). However, TKI raise various issues such as primary and acquired resistance with tumor escape on the one hand and on the other hand serious and/or moderate but chronic and deleterious adverse effects, especially for antiangiogenic TKI. Thus, the concept of redifferentiation strategy has emerged with a view to finding one or more drugs capable of restoring radioiodine sensitivity for RAIR thyroid cancers (Fig. 1). One of the challenges of this redifferentiation strategy is to find a treatment protocol involving the short-term use of systemic therapy so that, even if side effects occur, they only have to be tolerated temporarily and frequently vanish with the discontinuation of re-differentiation treatment. Numerous compounds have been tested for several years with inconsistent results. A renewed interest for this strategy came from the publication of a pilot trial with a series of 24 patients showing promising results obtained with a pharmacological MEK inhibitor

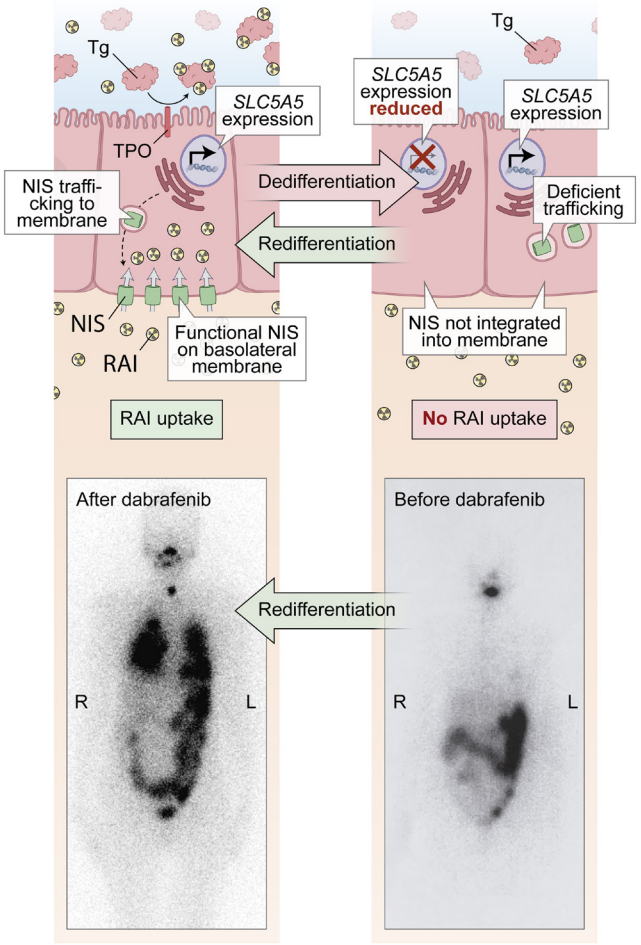

Figure 1
Differentiation and redifferentiation of thyroid cancer schematic. The left panel illustrates a (re-)differentiated state where the sodium iodide symporter (NIS) is normally expressed and localized at the plasma membrane, allowing radioactive iodine (RAI) to be uptaken by tumoral cells. The right panel illustrates a dedifferentiated state where the expression of the *SLC5A5* gene, encoding for the NIS, is reduced and/or not correctly targeted to the plasma membrane and consequently with no RAI uptake. The bottom panels illustrate the case of a patient with metastatic RAI refractory papillary thyroid cancer treated for 8 weeks with dabrafenib. The post-therapeutic WBS after dabrafenib demonstrates a clear restoration of RAI uptake in the metastatic sites (lung and neck lymphadenopathy).

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prescribed for 4 weeks (Ho *et al*. 2013), which was in line with the pre-clinical mouse model validating this strategy (Chakravarty *et al*. 2011).

Here, we discuss the different redifferentiation strategies based on the underlying molecular mechanism leading to NIS and radioiodine uptake reinduction, that is, by modulating signaling pathways, NIS transcription, NIS trafficking to the plasma membrane, NIS post-transcriptional regulation, by gene therapy and other potential strategies. From a pre-clinical point of view, our review focuses on studies with data obtained from at least one cell line of authenticated thyroid cancer origin, as some cell lines tested in several studies were subsequently found to be of non-thyroid origin (Schweppe *et al*. 2008, Landa *et al*. 2019). Finally, promising results of clinical trials on redifferentiation strategies are outlined.

Modulation of signaling pathways

Modulation of the MAPK pathway: preclinical and clinical evidence

Several publications demonstrate that MAPK pathway activation is associated with dedifferentiation and in particular NIS repression (Fig. 2). The comprehensive characterization of 496 papillary thyroid cancers (PTC) published in 2014 by the The Cancer Genome Atlas (TCGA) Research Network highlighted that $BRAF^{V600E}$-mutated PTC, which had the strongest activation of the MAPK pathway, showed the most dedifferentiated state, that is, low expression of some thyroid differentiation genes such as the *SCLC5A5* gene encoding for the NIS, thyroglobulin (Tg) or thyroperoxydase (TPO) (Cancer Genome Atlas Research 2014).

Several *in vitro* studies have demonstrated an increase in NIS expression and/or radioiodine uptake in human thyroid cancer-derived cell lines with various genetic backgrounds or patient-derived tumor tissue of ATC and PDTC with different MAPK pathway inhibitors namely $BRAF^{V600E}$ inhibitors such as vemurafenib (Cheng *et al*. 2016, Zhang & Chen 2018) or dabrafenib (Fu *et al*. 2019), multityrosine kinase inhibitor such as sorafenib (Ruan *et al*. 2015, Wachter *et al*. 2018*a*) or cabozantinib (Ruan *et al*. 2015) and MEK inhibitors such as selumetinib (Wachter *et al*. 2018*a*,*b*, Fu *et al*. 2019), PD98059 (Vadysirisack *et al*. 2007, Hou *et al*. 2010, Zhang & Chen 2018) or refametinib (Hou *et al*. 2010).

The first clinical phase II study testing a pharmacological inhibitor of the MAPK pathway reported the effect of the multityrosine kinase inhibitor sorafenib in 31 patients (Hoftijzer *et al*. 2009). This study was based on *in vitro* data demonstrating that another multityrosine kinase inhibitor namely sunitinib was able to induce NIS expression in PTC cell lines harboring RET/PTC1 rearrangement through inhibition of the MEK/ERK and SAPK/JNK pathway (Fenton *et al*. 2010) and that sunitinib increased iodine uptake in normal rat thyroid cells (Salem *et al*. 2008). Of the 20 patients evaluable for redifferentiation, after 26 weeks of treatment with sorafenib, only one restored a faint uptake of radioiodine in an occipital skeletal metastasis on the diagnostic whole body scan (WBS) performed, which was not confirmed on the therapeutic WBS performed after the administration of 200 mCi (Hoftijzer *et al*. 2009). A renewed interest for redifferentiation strategy with MAPK pathway pharmacological inhibitors came from the publication

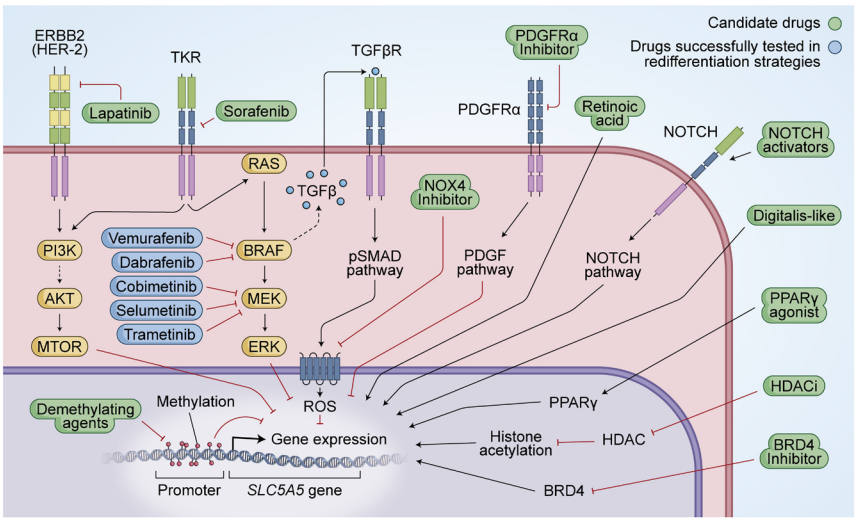

Figure 2
Molecular mechanism controlling NIS expression and current or future actionable targets.

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by the James Fagin laboratory (Memorial Sloan-Kettering Cancer Center, MSKCC) (Chakravarty *et al.* 2011). His team developed an ingenious mouse model expressing the $BRAF^{V600E}$ oncogene specifically in the thyrocytes and with the ability to switch the expression of $BRAF^{V600E}$ on and off with the administration or withdrawal of doxycycline (Chakravarty *et al.* 2011). Switching on $BRAF^{V600E}$ expression induced thyroid cancers in mice that recapitulate most human $BRAF^{V600E}$-tumor characteristics and was associated with a virtual abolition of thyroid-specific gene expression along with radioiodine uptake. Switching off $BRAF^{V600E}$ restored not only thyroid follicular architecture but also thyroid-specific gene expression and radioiodine incorporation. Pharmacological inhibitors of MEK, that is, selumetinib or $BRAF^{V600E}$ inhibitor, that is, dabrafenib for 2 weeks, partially restored thyroid-specific gene expression and radioiodine uptake. Following this preclinical *in vivo* model, Ho *et al.* from the MSKCC performed a clinical study in 24 patients with PTC or follicular thyroid cancer (FTC) or poorly DTC, confirming the ability of selumetinib to restore radioiodine in tumors previously shown to be radioiodine resistant (Ho *et al.* 2013). In this study, patients were treated with selumetinib for 4 weeks and then submitted to an 124-iodine PET-CT under thyrotropin stimulation to estimate the activity of 131-iodine required to deliver an arbitrary dose of 2000 cGy and above to the metastatic lesions. If it appeared that at least one lesion could be treated by an activity of less than 300 mCi, the patient was then treated with a personalized therapeutic dose of 131-iodine. Out of the 20 patients evaluable, selumetinib increased radioiodine uptake in 12. Of these 12 patients, eight reached the dosimetry threshold for radioiodine therapy, including all five patients with NRAS mutation but only one out of nine patients with $BRAF^{V600E}$ mutation. Of the eight patients treated with radioiodine, seven had a confirmed radiological partial response according to the RECIST 1.1 criteria, 6 months after selumetinib withdrawal and radioiodine therapy. The authors attributed their good results in a large part to the dosimetric approach enabled by the 124-iodine PET-CT evaluation of the patients. This publication has opened the field of successful redifferentiation strategy with MAPK inhibitors (Table 1).

To confirm the data of the pilot study conducted by Ho *et al.*, a multicenter UK single arm phase II trial (SEL-I-METRY) has opened (Brown *et al.* 2019). Patients with locally advanced or metastatic RAIR DTC or PDTC treated for 4 weeks with selumetinib are evaluated by 123-iodine SPECT/CT under recombinant human TSH to select patients showing significant increase in 123-iodine uptake and who will subsequently be treated with a fixed activity of 150 mCi of 131-radioiodine.

Despite these encouraging preliminary results on RAIR DTC patients, the results of the ASTRA phase III study (NCT01843062) evaluating MEK inhibitors as an adjuvant therapy in non-proved RAIR DTC were disappointing. This trial included patients with DTC at high risk of recurrence after total thyroidectomy (i.e. pT >4 cm, pT4, N1 with ≥5 lymph nodes or with at least 1 lymph node ≥1 cm). Patients were randomized to receive placebo or selumetinib for 4 weeks prior radioiodine ablation to assess the effect of selumetinib on the complete remission rate at 18 months. The addition of selumetinib to radioiodine did not improve the complete remission rate (40% *vs* 38.5% in the placebo group) in this patient population, in any subgroup of patients, even when genotype was taken into account. However, the placebo group established a 38.5% complete remission rate with standard radioiodine alone in high-risk patients, suggesting the need for improved therapeutic approaches. Subgroup analyses of this trial suggest that treatment compliance and tailoring the targeted therapy approach to the oncogenic driver mutation, especially the absence of a BRAF mutation, may be critical design elements to consider for future trials (unpublished data from J. Fagin's team).

Owing to the relatively poor response of BRAF-mutated thyroid cancers in the study published by Ho *et al.*, others have evaluated the capacity of the selective $BRAF^{V600E}$ inhibitor dabrafenib to specifically redifferentiate $BRAF^{V600E}$ (Rothenberg *et al.* 2015). Out of the ten patients included with a RAIR BRAF-mutated PTC, defined as the absence of radioiodine uptake on a first WBS (diagnostic or therapeutic dose), 6 weeks of dabrafenib restored radioiodine uptake on a diagnostic WBS in six of them. Those six patients were then treated with 150 mCi 131-iodine with a therapeutic WBS confirming the data obtained with the diagnostic scan. Two patients showed partial response and four stable disease on standard radiological imaging obtained 3 months after radioiodine therapy and discontinuation of dabrafenib, while out of the four patients without restoration of radioiodine uptake, one had progressive disease and three stable disease. These results should be put into perspectives considering that, at study entry, five patients out of ten had progression per RECIST v1.1 within the14 months prior to enrolment. Ho and his team published another pilot trial (Dunn *et al.* 2019) evaluating vemurafenib in 12 patients with a BRAF mutated-PTC or PDTC, with a methodology similar to their first study (Ho *et al.* 2013). Out of the ten patients evaluable,

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Table 1 Published trials or clinical cases of redifferentiating strategy with MAPK inhibitors.

Reference	TKI Duration of treatment before RAI	Patients (*n*)	Main results
Ho *et al*. (2013)	**Selumetinib (MEKi)** 4 weeks	24 20 evaluable	– RAI uptake: 60% (12/20) of increase or induction – 40% (8/20) retreated with RAI : 5/5 NRAS – 1/9 BRAF – 1/3 RET/PTC – 1/3 WT (Best responders: RAS mutated patients) – At 6 months: 5 PR and 3 SD
Rothenberg *et al*. (2015)	**Dabrafenib (BRAF^{V600E} i)** 6 weeks	10 BRAF^{V600E}	– 60% (6/10) retreated with RAI – At 3 months: 2 PR and 4 SD
Huillard *et al*. (2017)	**Vemurafenib, and then dabrafenib** Several months, without withdrawal	1 BRAF^{V600E}	– Increase in Tg (redifferentiation effect) while on TKI – Diagnosis of an unsuspected pulmonary miliary on therapeutic WBS – Loss of the redifferentiation effect after short and transitory interruption of the TKI
Jaber *et al*. (2018)	**Dabrafenib ± trametinib** **Vemurafenib (BRAF^{V600E} i)** **Trametinib (MEKi)** **Investigational MEKi** Median duration of treatment: 14 months	13	– RAI uptake: 69% (9/13) of increase or induction (3/3 RAS – 5/9 BRAF – 1/1 WT; Best responders: RAS mutated patients) – Increase in Tg or Tg Ab levels in 6 out of 9 responders while on TKI
Dunn *et al*. (2019)	**Vemurafenib** 4 weeks	12 BRAF^{V600E} 10 evaluable	– RAI uptake: 60% (6/10) of increase or induction – 40% (4/10) retreated with RAI – At 6 months: 2 PR and 2 SD
Iravani *et al*. (2019)	**Trametinib ± dabrafenib** **Vemurafenib + cobimetinib (MEKi)** 4 weeks	6	– 67% (4/6) retreated with RAI (1/3 NRAS – 3/3 BRAF; Best responders: BRAF mutated patients) – At 3 months: 3 PR – 1 SD
Leboulleux *et al*. (2019)	**Dabrafenib + trametinib** 8 weeks	1 BRAF^{K601E}	– Clinical hyperthyroidism and increase in FT4 and FT3 (6.6 and 4.4 N) 8 weeks later – Histological redifferentiation documented – No therapeutic RAI because of unresectable primary thyroid tumor

BRAF^{V600E} i: BRAF^{V600E} inhibitor; MEKi: MEK inhibitor; PR: partial response; RAI: radioiodine; SD: stable disease; Tg Ab: thyroglobuline antibody; Tg: thyroglobuline.

four reached the dosimetry threshold on the 124-iodine PET scan and were retreated with a therapeutic dose of iodine 131. Of those four patients, radiological assessment revealed partial response ($n=2$) and stable disease ($n=2$) 6 months after radioiodine therapy. Interestingly, the serum Tg value among 124-iodine responders was significantly higher than in non-responders. This suggests that cancers with better differentiation at baseline (high serum Tg) have a better chance to respond favourably to the redifferentiation strategy. Molecular tumor biopsy analysis performed before and under vemurafenib revealed that, to some extent, the degree of iodine avidity restoration is linked to the degree of MAPK pathway output inhibition and the induction of thyroid-specific gene expression.

The retrospective analysis of patients with RAIR DTC (PTC, FTC or PDTC) treated with MAPK inhibitors (BRAF^{V600E} and/or MEK inhibitors), for a mean of 14 months, also confirmed the data described previously (Jaber *et al*. 2018, Iravani *et al*. 2019). In the first study (Jaber *et al*. 2018), 9 out of 13 patients were treated with a therapeutic dose of 150–250 mCi of radioiodine based on meaningful uptake of radioiodine on a diagnostic WBS except for one. All nine patients had durable disease control. As previously reported, RAS-mutated tumors were the best responders compared to BRAF-mutated ones. In the second study (Iravani *et al*. 2019), out of six patients treated with MEK inhibitor (NRAS mutated) or BRAF and MEK inhibitors (BRAF mutated) for 4 weeks, four were considered suitable for radioiodine therapy based on the result of I124 PET/CT, with I131 post-therapeutic WBS confirming the restoration of radioiodine uptake. In this study, all BRAF-mutated patients responded to the redifferentiation strategy, while only one NRAS-mutated patient out of three did. Of these four patients,

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three achieved a partial imaging response (while progressing or stable at study entry) and one stable disease (while progressing at study entry) after a median follow-up of 16.6 months. Additional case reports demonstrated the potential redifferentiating effect of pharmacological MAPK inhibition in thyroid cancers (Huillard *et al.* 2017, Leboulleux *et al.* 2019). In one of these case reports clinical hyperthyroidism developed, with free triiodothyronine and free thyroxine levels increasing to 6.6 and 4.4 times their upper reference limit, as ultimate evidence of efficient redifferentiation (Leboulleux *et al.* 2019). In the study of Iravani *et al.*, 4 weeks of thyroid hormone withdrawal increased TSH to only 25.9 mUI/L in one patient submitted to a redifferentiation strategy with MEK and BRAF inhibitors, suggesting the redifferentiation of functional thyroid cancer cells.

In the context of a redifferentiation strategy, some patients in the studies by Huillard *et al.* (2017) and Dunn *et al.* (2019) demonstrated a rise in thyroglobulin, which may indicate the success of redifferentiation rather than disease progression. Interestingly, Huillard *et al.* demonstrated that a $BRAF^{V600E}$ inhibitor administered in a RAIR-PTC patient revealed unsuspected pulmonary miliary metastases on the therapeutic WBS performed. Finally, this case report also illustrated that the redifferentiation effect is transitory and disappears after a short period of discontinuation of the redifferentiation drug, highlighting the importance of radioiodine administration while the patient is under treatment.

However, in all these studies, it is difficult to distinguish between tumor response resulting from a cytotoxic effect of the pharmacological inhibitor and a restoration of radioiodine uptake. Selecting patients with mixed response, that is, patients with lesions showing new or enhanced radioiodine uptake and lesions without any uptake, could create the opportunity to distinguish between both effects (Huillard *et al.* 2015).

Future prospects to improve tumor redifferentiation, especially $BRAF^{V600E}$-mutated thyroid cancers, through MAPK inhibition, might come from compounds able to profoundly inhibit the MAPK pathway, such as CKI a MEK inhibitor that functions as a dominant-negative inhibitor of RAF which reduces the feedback reactivation of ERK signaling. James Fagin's laboratory published data, *in vitro*, in rat thyroid cells expressing $BRAF^{V600E}$ and, *in vivo,* in a mouse model with $BRAF^{V600E}$-induced thyroid cancer demonstrates that a small increase in ERK inhibition translates into a markedly increased expression of thyroid differentiation genes and increased iodide accumulation in cancer (Nagarajah *et al.* 2016). A high-throughput NIS enhancer screening platform also enabled the identification of a new tyrosine kinase inhibitor which increased NIS promoter activity along with the expression of NIS and other thyroid specific proteins (Tg, TPO, TSH-R, Pax 8 and TTF-1) in a $BRAF^{V600E}$-mutated thyroid cancer cell line. Moreover this compound, which decreased the level of phosphorylated ERK, that is, the activation of the MAPK pathway, increased ^{125}I uptake in the same cell line and *in vivo* in xenograft mice models (Oh *et al.* 2018). The combination of $BRAF^{V600E}$ and MEK inhibition may be a promising strategy, as the combination synergistically increased radioiodine uptake in human BRAF-mutated thyroid cancer cell lines possibly through inhibition of the rebound of ERK1/2 activation observed with only one drug (Zhang & Chen 2018). An ongoing clinical trial performed by the French RAIR thyroid cancer TUTHYREF network is testing the association of MEK (Trametinib) alone (for *RAS* mutated tumors) or in combination with $BRAF^{V600E}$ (Dabrafenib) inhibitors (for $BRAF^{V600E}$ mutated tumors) followed by radioiodine therapy for the treatment of RAIR metastatic DTC (NCT 03244956).

Another strategy for $BRAF^{V600E}$-mutated thyroid cancers would be to combine inhibitors of the human EGF receptor (Her) belonging to the EGF receptor family with MAPK inhibitors. In thyroid cancer cells harboring $BRAF^{V600E}$ mutation, inhibition of the MAPK pathway by pharmacological inhibitors were transient due to the release, by a RAF or MEK inhibitor, of a transcriptional repressor from the HER3 promoter and consequently induced HER3 gene overexpression (Montero-Conde *et al.* 2013). An autocrine secretion by thyroid cancer cells of a ligand able to bind to and activate by dimerization the tyrosine kinase receptors HER2/HER3 resulted in the reactivation of the MAPK and PI3K pathway. The Her kinase inhibitor lapatinib prevented MAPK rebound and overcame BRAF-mutated thyroid cancer cell resistance to MAPK inhibitors (Montero-Conde *et al.* 2013). In line with these data, Cheng *et al.* (2017) demonstrated *in vitro* in $BRAF^{V600E}$-mutated human thyroid cancer derived cell lines that the combination of lapatinib with dabrafenib or selumetinib increased radioiodine uptake. A clinical trial (NCT 02456701), performed at the MSKCC and which tests the ability of vemurafenib combined with an anti HER3 MAB to restore iodine incorporation in BRAF mutant RAIR thyroid cancer patients, is ongoing.

Iodide oxidation and tyrosine organification are not restored by MAPK blockade. As PI3K inhibition seems to prolong radioiodine retention in thyroid cells (Lakshmanan *et al.* 2015), the combination of MAPK and

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PI3K inhibitors may be an interesting strategy, but with the disadvantage of potential synergistic side effects.

The potential of combining MAPK inhibitors with HDAC inhibitors is developed in the 'HDAC inhibitors' section of this review.

Concerning undifferentiated thyroid cancers, namely ATC, hope for successful redifferentiation comes from a mouse model of lethally aggressive thyroid cancer, harboring $BRAF^{V600E}$ and $PIK3CA^{H1047R}$ mutations, demonstrating that MEK inhibition for only 10 days could increase radioiodine uptake, which could then translate into a stable and profound reduction in tumor burden (ElMokh *et al.* 2019).

Modulation of other pathways: preclinical evidence

The PI3K pathway

In a non-tumoral context, a central role for PI3K in the repression of NIS gene transcription by IGF1 has been demonstrated in the FRTL-5 rat thyroid cell line (Garcia & Santisteban 2002). Paradoxically, Quercetin, a compound with PI3K inhibitory properties, has been shown to reduce NIS mRNA levels in FRTL-5 cells (Giuliani *et al.* 2008).

However, other publications have shown that PI3K pathway inhibition has the potential to restore radioiodine sensitivity of RAIR DTC (Fig. 2).

Song *et al.* (2018) demonstrated *in vitro* in thyroid cancer cell lines that a mutant RasGRP3 (Ras guanine nucleotide-releasing protein 3), which was frequently found in metastases of RAIR DTC, decreased iodine uptake ability besides promoting cell proliferation, migration and invasiveness. This was associated with PI3K pathway activation and the addition of the PI3K pathway pharmacological inhibitor LY294002 restored radioiodine uptake of the cells at their basal level, that is, without overexpression of RasGRP3 mutant. LY294002 also increased radioiodine uptake in human thyroid cancer cells expressing RET/PTC1 and engineered to constitutively express NIS (Kogai *et al.* 2008) and in the non-cancerous PCCL3 rat thyroid cells (de Souza *et al.* 2010) mainly through NIS upregulation (Liu *et al.* 2012).

Interestingly, pharmacological Akt inhibition increased radioiodine uptake, despite a decrease in NIS protein levels, in PCCL3 rat thyroid cells with doxycycline induced expression of constitutively active MEK. Indeed, this Akt1/2 inhibitor acted on iodine uptake through a decreased iodide efflux and a higher iodide affinity, enhancing the NIS-mediated iodide transport rate (Liu *et al.* 2012). The same research team further demonstrated that, among different pharmacological inhibitors targeting Akt, MEK, PI3K, Hsp90 or $BRAF^{V600E}$ tested in PCCL3 rat thyroid cells conditionally expressing RET/PTC3 or $BRAF^{V600E}$, the PI3K inhibitor GDC-0941 outperformed other inhibitors in radioiodine increase (Lakshmanan *et al.* 2015). Surprisingly, the greatest effect was observed in $BRAF^{V600E}$ expressing thyroid cells, where MEK is the canonical downstream of $BRAF^{V600E}$. Again, a decrease in iodide efflux was the main mechanism of action of this PI3K inhibitor which had limited effect on NIS protein levels. Of note, Lakshmanan *et al.* (2015) demonstrated that TGFβ, present in the thyroid tumor microenvironment, reduces the extent of increase in radioiodine uptake induced by the pharmacological inhibitors tested. They also showed that Apigenin, a plant-derived flavonoid, associated with GDC-0941 provided the highest radioiodine uptake level in both $BRAF^{V600E}$ and RET/PTC3 expressing PCCL3 cells, corroborating previous data obtained with an Akt pharmacological inhibitor in the same cells and in a mouse thyroid tumor model (Lakshmanan *et al.* 2014).

Another PI3K inhibitor, rapamycin, was found to induce NIS protein levels along with radioiodine uptake in $BRAF^{V600E}$ and RET/PTC1 PTC derived cell lines (but not in a FTC derived cell line), possibly through a transcriptional effect dependent on the transcription factor TTF1 (Plantinga *et al.* 2014). Rapamycin and its synthetic analog everolimus led to controversial results in a non-tumoral thyroid *in vitro* model (de Souza *et al.* 2010, Liu *et al.* 2012).

Other pathways

The NOTCH signaling pathway

Notch genes encode receptors for a signaling pathway that controls cell fate by regulating cell proliferation and differentiation, especially in oncogenic contexts. *Notch 1* and *Notch3* have been described as tumor suppressor genes in PTC, FTC and ATC (Xiao *et al.* 2009, Yu *et al.* 2013, Somnay *et al.* 2017). Moreover, *Notch1* and *3* expression are inversely correlated to the degree of differentiation in thyroid cancers with ATC having the strongest decreased expression of both *Notch* (Ferretti *et al.* 2008, Somnay *et al.* 2017). From a clinical point of view, low *Notch3* expression levels correlated with overall survival independently from the classical, other prognostic factors (Somnay *et al.* 2017). *In vitro* Notch3 constitutive activation in a FTC cell line increased the mRNA levels of thyroid specific genes, that is, *NIS, TSH-R, TTF-1* and *TTF-2*. In a non-tumoral context, in the normal FRTL-5 rat thyroid cell line, overexpression of Notch1 increased

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the expression of NIS and TPO (Ferretti *et al.* 2008). Pharmacological Notch1 activating compounds (Fig. 2) such as resveratrol (Yu *et al.* 2013) or Hesperetin (Patel *et al.* 2014) have been shown to induce NIS mRNA levels or other differentiation markers such as TSH-R, TTF-1, TTF-2 and PAX8, *in vitro* in ATC cell lines. The silencing of Notch1 by siRNA abrogated this effect, demonstrating the dependence upon Notch1 signaling (Yu *et al.* 2013). Interestingly other compounds with redifferentiation capacity, such as the HDAC inhibitors valproic acid and SAHA or retinoic acid outlined elsewhere in these reviews have also been reported as Notch activating compounds (Lin *et al.* 2003, Xiao *et al.* 2009).

PDGFRα blockade

Lopez-Campistrous *et al.* (2016) have demonstrated in thyroid cancer cell lines and mouse xenograft models that activation of PDGFRα decreases Tg and NIS expression which promotes a decrease in radioiodine uptake (Fig. 2). Blocking PDGFRα in these same models improved radioiodine uptake and also reduced migration and invasion potentials of the cells as well as tumor volume in animal models. The relocation of the transcription factor TTF1 from the nucleus to the cytoplasm is the main mechanism of action of PDGFRα activation. In human PTC, PDGFRα expression was strongly associated with metastatic disease and radioiodine resistance.

Modulation of NIS transcription

Epigenetic modification is responsible for the regulation of gene expression without involving modification of the genomic DNA sequence. Epigenetic dysregulation is emerging as a contributor to carcinogenesis and tumor progression and as a therapeutic target. The restoration of iodine uptake in thyroid tumors that were considered refractory to radioiodine, suggesting reversible repression of the *SLC5A5* gene encoding for the NIS, is an indirect demonstration of an epigenetic regulation of the NIS gene.

Retinoic acid

Retinoids are chemical compounds related to vitamin A. They act on the nuclear receptors retinoic acid receptor (RAR) and retinoid X receptor (RXR). All-trans-retinoic acid (RA)-RAR or RA-RXR complexes bind to the responsive elements in gene promoter sites and activate the transcription of their target genes. The retinoid pathway is involved in cellular differentiation, proliferation and apoptosis. Retinoic acid is a well-known drug for certain dermatological diseases.

In thyroid carcinoma cell lines, *in vitro* experiments showed that retinoids affect thyroid specific functions (induction of type I 5′-deiodinase activity, NIS gene expression), cell-cell or cell-matrix interaction, differentiation markers, growth and tumorigenicity (Van Herle *et al.* 1990, Schreck *et al.* 1994, Kurebayashi *et al.* 2000, Schmutzler & Kohrle 2000, Schmutzler *et al.* 2002, Jeong *et al.* 2006). Moreover, retinoids partly re-differentiate follicular thyroid carcinoma cell lines (Schmutzler & Kohrle 2000) (Fig. 2).

The first clinical series of ten RAIR-DTC patients treated with isotretinoin (13-cis-retinoid acid, a first-generation retinoid) was published by Simon *et al.* Radioiodine uptake was restored in four patients (Simon *et al.* 1996). Other pilot studies reported an increase in radioiodine uptake in about 20 to 40% of patients (Grunwald *et al.* 1994, Simon *et al.* 2002, Gruning *et al.* 2003, Courbon *et al.* 2006, Fernandez *et al.* 2009, Kim *et al.* 2009, Oh *et al.* 2011). However, tumor response did not always correlate with increased radioiodine uptake and other direct antiproliferative effects of isotretinoin might have been involved (Simon *et al.* 2002). Prospective clinical trials failed to demonstrate a clinical utility of isotretinoin. In an open-label phase II trial, isotretinoin did not significantly increase radioiodine uptake in 16 RAIR-DTC (Short *et al.* 2004). In another published phase II trial, increased radioiodine uptake was observed in 17% (n=9/53) of patients. However, this did not translate into tumor response or clinical benefit (Handkiewicz-Junak *et al.* 2009).

Few clinical data exist with other retinoids. In a series of 11 patients treated with tretinoin (all-trans-retinoic acid (RA)), also a first-generation retinoid, authors reported increased radioiodine uptake in four patients and partial response in five patients (Zhang *et al.* 2007). Another series of 13 patients treated with tretinoin for RAIR-DTC showed a weak radioiodine uptake in six patients, with no tumor response among those patients (Damle *et al.* 2011). In a pilot study with bexarotene (RXR activator), increased radioiodine uptake was observed in 8/11 patients, but no significant clinical benefit was demonstrated (Liu *et al.* 2006a).

A recent meta-analysis on retinoids in RAIR-DTC reported a pooled effect of 27.6% for increased radioiodine uptake and 17% for tumor response by RECIST criteria (Pak *et al.* 2018).

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HDAC inhibitors

Post-translational modification of histones bound to DNA such as acetylation is a well-known mechanism of tumorigenesis initiation and progression, especially for thyroid cancers (Russo *et al*. 2013). By altering the structural state of chromatin, histone acetylation by histone acetyl transferases favors transcriptional activation. On the other hand, histone deacetylation by histone deacetylase (HDACs) is associated with transcriptional repression. Several publications have demonstrated that the acetylation of histones of the *SLC5A5* promotor regulates its transcription and consequently NIS expression (Puppin *et al*. 2012, Zhang *et al*. 2014, Fu *et al*. 2019). Various HDAC inhibitors (HDACi), such as panobinostat (Pugliese *et al*. 2013, Wachter *et al*. 2018*a*,*c*, Fu *et al*. 2019), valproic acid (Shen *et al*. 2005, Frohlich *et al*. 2009, Haghpanah *et al*. 2014, Massimino *et al*. 2018), vorinostat (Puppin *et al*. 2005, 2012, Hou *et al*. 2010, Clinckspoor *et al*. 2011, Cheng *et al*. 2016, Wachter *et al*. 2018*a*), romidepsin (Kitazono *et al*. 2001, Furuya *et al*. 2004, Xu & Hershman 2006), sodium butyrate (Puppin *et al*. 2005), Trichostatin A (Zarnegar *et al*. 2002, Wachter *et al*. 2018*a*) or analogs targeting HDAC (Jang *et al*. 2015), have been shown in human thyroid cancer derived cell lines or patient-derived thyroid cancer tissue to reinduce NIS mRNA levels (Fig. 2) or even other thyroid differentiation genes such as Tg, TPO or TSH receptor. More interestingly, some studies have demonstrated in cellular or in *in vivo* models an increase in iodine uptake following HDACi treatment and/or proper targeting of the NIS to the plasma membrane where this transporter can be effective (Kitazono *et al*. 2001, Furuya *et al*. 2004, Hou *et al*. 2010, Puppin *et al*. 2012, Pugliese *et al*. 2013, Cheng *et al*. 2016, Massimino *et al*. 2018, Wachter *et al*. 2018*a*, Fu *et al*. 2019). However, the results of four clinical trials (Kelly *et al*. 2005, Amiri-Kordestani *et al*. 2013, Sherman *et al*. 2013, Nilubol *et al*. 2017) that tested HDACi in RAIR human thyroid cancers were in apparent contradiction with the promising results of preclinical studies. Nilubol *et al*. (2017) tested the effect of valproic acid in 13 patients with RAIR DTC without any improvement in radioiodine uptake in the ten patients evaluable. Two trials have tested romidepsin (Amiri-Kordestani *et al*. 2013, Sherman *et al*. 2013) in RAIR DTC. In these phase 1 and 2 trials, the 131I diagnostic WBS showed restoration of faint radioiodine uptake after various time periods of treatment in two out of six evaluable patients for the phase 1 study and 2 out of 16 patients for the phase 2. In the phase 2 trial (Sherman *et al*. 2013), the faint radioiodine uptake observed on the diagnostic WBS was confirmed on the therapeutic WBS but without long-term benefit from the treatment. In a phase 1 trial testing vorinostat, also known as Suberoylanilide Hydroxamic Acid (SAHA) (Kelly *et al*. 2005), in patients with advanced thyroid cancers, Kelly *et al*. reported an improvement in the radioiodine uptake evaluated by post-therapeutic WBS after SAHA administration for an unknown duration in one out of the three PTC patients included. These trials have classically been considered disappointing. However, several points should be highlighted before definitively buring HDACi as an effective redifferentiation strategy. First, from a clinical point of view, besides the small number of patients included that preclude any definite conclusion, the evaluation of a proper iodine uptake increase or restoration after HDACi treatment on a diagnostic WBS can be criticized as this imaging lacks sensitivity (Haugen *et al*. 2016). Second, preclinical studies show the complexity of triggering NIS and other thyroid-differentiation gene reinduction. In several of these studies the redifferentiation effect, that is, the increase in NIS mRNA and/or protein levels and/or increase in iodine uptake, was considerably variable among the human thyroid cancer-derived cell lines tested, suggesting variable effects according to the genetic background of the cell lines (Shen *et al*. 2005, Clinckspoor *et al*. 2011, Massimino *et al*. 2018, Fu *et al*. 2019). Illustrating the fine and complex regulation of the system, Shen *et al*. (2005) reported a paradoxical inefficacy to restore NIS mRNA levels of a higher dose of valproic acid (1.5 mM) in comparison with a lower dose (0.5 mM). Moreover, the timing of HDACi administration and the evaluation of the redifferentiation effect might also be critical, as *in vitro* the increase in radioiodine uptake may vary with the timing of the pharmacological inhibitor administration (Wachter *et al*. 2018*c*).

Several publications suggest that drug combination may be a powerful redifferentiation strategy. MAPK inhibition with a MEK inhibitor or a $BRAF^{V600E}$ inhibitor combined with HDACi, such as panobinostat or SAHA, has been shown to have a synergistic effect on NIS and other differentiation thyroid gene (TSH-R, TPO, Tg) reinduction in various human thyroid cancer cell lines, in comparison with either drug used alone (Hou *et al*. 2010, Cheng *et al*. 2016, Fu *et al*. 2019). This effect was even further enhanced by treatment with TSH, which not only enhanced the expression of thyroid genes but also promoted NIS targeting to the plasma membrane (Hou *et al*. 2010, Cheng *et al*. 2016). PI3K pathway inhibitors, such as mTOR or Akt inhibitors, in combination with HDACi also have the same synergistic effect (Hou *et al*. 2010).

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Finally, the triple combination of MEK inhibitor, Akt inhibitor and SAHA had a synergistic effect on radioiodine uptake, further enhanced by TSH, in human ATC derived cell lines (Hou *et al.* 2010). These pre-clinical data provide evidence which supports the proposal to use a drug combination strategy targeting the MAPK pathway and/or PI3K pathway with HDACi.

Finally, beyond HDACi, a poly ADP ribose polymerase-1 (PARP-1) pharmacological inhibitor has been demonstrated to increase radioiodine uptake in different thyroid cancer cell lines, through upregulation of histone modification activation marks, especially the acetylation of lysine 9 and 14 of histone 3 (H3K9K14Ac) (Lavarone *et al.* 2013).

PPARγ agonists

Peroxisome proliferator-activated receptor (PPAR)-γ belongs to the nuclear receptor family of transcription factors. PPAR-γ is the master regulator of adipogenesis and plays a role in tumorigenesis (Raman & Koenig 2014). Of note, the rearrangement of PPAR-γ/PAX8 occurs in 30–35% of FTC and in some follicular variants of PTC (Raman & Koenig 2014).

Different pre-clinical studies have demonstrated the re-differentiation effect of several thiazolidinediones, which bind to PPARγ and are known to be PPARγ agonists, such as rosiglitazone (Frohlich *et al.* 2005, 2009, troglitazone (Park *et al.* 2005) and pioglitazone (Frohlich *et al.* 2005), in addition to their anti-proliferative effects on DTC-derived cell lines (Fig. 2). Interestingly, Frohlich *et al.* (2005) demonstrated that, in FTC cell lines, thiazolidinediones increased radioiodine uptake and the amount of NIS in the membrane fraction, especially troglitazone which showed greater effects than other thiazolidinediones. However, these effects seem to be independent of PPARγ activation, as the addition of a PPARγ antagonist did not reverse their effects.

Several case reports (Elias & Lizotte 2006, Elola *et al.* 2011), pilot study and phase II clinical trials (Philips *et al.* 2004, Kebebew *et al.* 2006, 2009, Tepmongkol *et al.* 2008, Rosenbaum-Krumme *et al.* 2012) suggest a more or less efficient re-differentiation effect of the PPARγ agonist rosiglitazone. Kebebew *et al.* (2006, 2009) published the results of a phase II trial including 20 patients with RAIR DTC treated for 8 weeks with rosiglitazone of whom four showed a positive diagnostic WBS after treatment. Despite the administration of 50 to 213 mCi I131 in those four patients, none had complete or even partial response according to the RECIST 1.1 criteria at 3 months of follow-up. There was no relationship between the level of PPARγ mRNA and protein expression in patients who had radioiodine uptake compared with those who did not. These last results were in contradiction with the study of Tepmongkol *et al.* (2008). Out of 23 patients with RAIR DTC treated for 6 weeks with rosiglitazone, six patients demonstrated restoration of radioiodine uptake on a therapeutic WBS after rosiglitazone. Of these, five had strong PPARγ-positive staining in thyroid biopsies and one a weak staining. On the other hand, none of the seven patients with negative staining had positive therapeutic WBS after rosiglitazone therapy. In the study of Rosenbaum-Krumme *et al.* (2012), out of nine patients, four were retreated after 3 or 6 months of rosiglitazone and, of these, three had best partial response using RECIST 1.1 criteria after rosiglitazone therapy. A small pilot study in 2004 (Philips *et al.* 2004) had shown less encouraging results than the trials described, as out of five patients with RAIR DTC, only one experienced restoration of a faint uptake of radioiodine in lung metastases after 3 months of rosiglitazone.

Pre-clinical data for potential future targets

Demethylating agents

Hypermethylation of the promoter region of the *SCLC5A5* gene encoding for the NIS have been demonstrated in several publications, both in benign and malignant thyroid tumors (Venkataraman *et al.* 1999, Xing *et al.* 2003, Smith *et al.* 2007, Stephen *et al.* 2011, Galrao *et al.* 2013, 2014) (Fig. 2). In PTC, specific CpG-island regions of the promoter are hypermethylated through DNA methyltransferase 1 (DNMT1) up-regulation following NFκB activation by $BRAF^{V600E}$ (Choi *et al.* 2014). Moreover, an inverse correlation between NIS expression and the degree of methylation of some of this CpG island has been described (Galrao *et al.* 2014). However, preclinical studies analyzing the re-dedifferentiation effect of demethylating agents such as 5-azacytidine in human thyroid cancer cell lines had shown disappointing results without any significant effect on NIS mRNA reinduction and/or radioiodine uptake increase (Provenzano *et al.* 2007, Tuncel *et al.* 2007, Massimino *et al.* 2018), unless 5-azacytidine was associated with the sodium butyrate HDACi (Provenzano *et al.* 2007). Only one study showed an increase in radioiodine uptake *in vitro* after 5-azacytidine treatment, correlated with a reduced methylation level of *SCLC5A5* promoter (Galrao *et al.* 2014). To date, clinical trials evaluating demethylating agent effects on RAIR DTC redifferentiation are still lacking.

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NADPH oxidase 4 (NOX4)

The link between $BRAF^{V600E}$ mutation, reduction of NIS expression and dedifferentiation is well established. Even if a link between $BRAF^{V600E}$-induced TGFβ production and repression of NIS, Tg and TPO has been demonstrated (Kawaguchi *et al*. 1997, Nicolussi *et al*. 2003, Costamagna *et al*. 2004, Riesco-Eizaguirre *et al*. 2006), the whole underlying molecular mechanism remains largely unknown. Azouzi *et al*. (2017) shed light on the role of the NADPH oxidase NOX4, an enzyme specialized in reactive oxygen species production upregulated in thyroid cancers (Weyemi *et al*. 2010). Azouzi *et al*. demonstrated that NOX4 upregulation by the $BRAF^{V600E}$-activated TGFβ-Smad3 pathway correlated with NIS repression and dedifferentiation in thyroid cancer cell lines, patient tissue samples and in mouse $BRAF^{V600E}$-induced thyroid tumors. Remarkably NOX4 silencing by siRNA reversed $BRAF^{V600E}$-induced NIS repression at the mRNA and protein levels, making NOX4 a potential future therapeutic target (Fig. 2).

Consistent with this data, the antioxidant alpha-lipoic acid was able to increase radioiodine uptake in a human thyroid cancer derived cell line (Choi *et al*. 2012).

Bromodomain-containing protein 4 (BRD4)

BRD4 is a member of the BET (bromodomain and extra terminal) family which binds to acetylated histones promoting gene transcription and plays a role in carcinogenesis. BRD4 is up-regulated in thyroid cancer tissues and cell lines (Gao *et al*. 2016) and its pharmacological inhibition, besides having an antiproliferative effect, restored radioiodine uptake in thyroid cancer cell lines and in a mouse model of human papillary xenograft (Gao *et al*. 2016) (Fig 2).

Digitalis-like components

Autophagy, which facilitates the degradation of cytoplasmic components in response to stimuli such as DNA damage or hypoxia, has emerged as a key player in carcinogenesis. In thyroid cancers, autophagy has been shown to be associated with dedifferentiation and reduced clinical response to radioiodine (Plantinga *et al*. 2016). Based on this association, a systematic high-throughput screening has identified autophagy-activating digitalis-like compounds capable of restoring NIS expression and iodine uptake, at micromolecular concentrations, in PTC, FTC (Tesselaar *et al*. 2017) and ATC cell lines (Tesselaar *et al*. 2018) (Fig. 2). The increase in transcriptional activation of NIS following up-regulation of transcription factors such as FOS or ATF3 (activating transcription factor 3) (Tesselaar *et al*. 2017, 2018) after digitalis-like compound treatment may account for the redifferentiation effect. Future studies involving mouse models and addressing the issue of the narrow therapeutic index of digitalis-like compounds as well as the dosage required for thyroid cancer redifferentiation are mandatory before clinical implementation.

Modulation of NIS trafficking to the plasma membrane

The restoration of NIS expression in RAIR thyroid cancers can be insufficient as NIS targeting to the plasma membrane can be impaired (Fig. 1). Interestingly, overexpression of $BRAF^{V600E}$ in normal rat PCCL3 thyroid cells has the same effect as TSH withdrawal from the culture medium, that is, besides decreasing NIS protein levels, it also impairs NIS targeting to the plasma membrane (Riesco-Eizaguirre *et al*. 2006). Inhibition of the MAPK pathway by a MEK inhibitor partially restored NIS protein expression, albeit without recovering proper localization to the plasma membrane (Riesco-Eizaguirre *et al*. 2006).

Regarding the issue of the sub-cellular localization of NIS, several publications have shed light on the role of the proto-oncogene pituitary tumor transforming gene (PTTG)-binding factor (PBF) which may represent a novel therapeutic target for increasing radioiodine uptake (Smith *et al*. 2011). PBF, which specifically binds to PTTG, is over-expressed in thyroid cancers (Smith *et al*. 2011). After its phosphorylation on a tyrosine residue by the kinase Src, PBF represses iodide uptake *in vitro* and in murine models (Read *et al*. 2011) through two mechanisms: transcriptional inhibition of NIS expression (Boelaert *et al*. 2007) and the binding and redistribution of the NIS from the plasma membrane to the cytoplasm (Smith *et al*. 2009). Interestingly, a pharmacological Src inhibitor was able to stimulate radioiodine uptake in human thyroid cancer cell lines and in human primary thyroid cells (Smith *et al*. 2013). Future clinical therapeutic strategies may combine drugs targeting NIS protein reinduction associated with drugs modulating NIS sub-cellular localization, more specifically to the plasma membrane where the NIS is fully active.

Moreover, Thompson *et al*. (2019) recently demonstrated *in vitro* that NIS dimerization may be critical to its trafficking to the plasma membrane. Impaired dimerization of the NIS and its subsequent

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mis-localization in RAIR DTC remains a field for future investigation.

Lan *et al.* (2017) demonstrated that β-catenin activation subsequent to HIF1α overexpression relocated the NIS into intracellular location near the nucleus in FTC cells. As a consequence, radioiodine uptake decreased, but β-catenin knockdown restored the iodine uptake capacity and the NIS localization on the periphery of the cells. Experiments in xenograft mice tumors confirmed these data, as β-catenin silencing promotes the efficacy of radioiodine therapy in FTC cells overexpressing HIF-1α and β-catenin.

Finally, two novel NIS interactors namely ARF4 (ADP-ribosylation) and VCP (valosin-containing protein) have been involved in NIS trafficking to the plasma membrane. ARF4 enhanced NIS trafficking to the plasma membrane, while VCP governed NIS proteolysis. ARF4 overexpression and VCP inactivation resulted in increased iodine uptake in human thyroid cancer cells. Selective VCP inhibitors promoted RAI uptake in these same models as well as in primary thyrocytes isolated from a mouse model. Interestingly VCP and ARF4 are associated with poorer survival characteristics in RAI-treated patients (Fletcher *et al.* 2019).

Modulation of NIS post-transcriptional regulation

Few data are published on the role of miRNA, which are small interfering RNAs modulating the translation, on NIS and other thyroid differentiation genes expression.

Among the miRNA studied, the following ones appear to be good candidates to modulate NIS expression: miR-146b, miR-let-7f-5p, miR-21 and miR-106a (Shen *et al.* 2005, Li *et al.* 2015, Riesco-Eizaguirre *et al.* 2015, Damanakis *et al.* 2016, Haghpanah *et al.* 2016, Wachter *et al.* 2018*b*). Interestingly, in the TCGA miR-146b-5p, miR-146b-3p and miR-21 were inversely correlated with the thyroid differentiation score of the PTC studied (Cancer Genome Atlas Research 2014).

Gene therapy

Using various delivery techniques, radioiodine uptake was induced *in vitro* in different thyroid cancer cell lines and sometimes *in vivo* in xenograft models by NIS gene delivery and reviewed in 2009 by Sptizweg (Spitzweg 2009).

All studies (Smit *et al.* 2000, Haberkorn *et al.* 2003, Lee *et al.* 2003, Lin *et al.* 2004) demonstrated that NIS gene therapy was able to increase radioiodine uptake but almost always along with rapid iodide efflux which, as a consequence, was not sufficient to allow the therapeutic effects of radioiodine *in vivo* in animal models, except in one study (Smit *et al.* 2002).

Other studies suggested that gene therapy with both the transcription factors TTF1 and PAX8, known to control the expression of the NIS gene as well as Tg and TPO, was able to induce radioiodine uptake in thyroid cancer cell lines and could limit radioiodine efflux, while gene therapy with the transcription factor TTF1 alone was associated with rapid iodine efflux (Mu *et al.* 2012). The adenovirus mediated transfer of TTF1 in thyroid cancer cell lines that also stably express the NIS rescued this rapid radioiodine efflux issue (Furuya *et al.* 2004). Pax-8 gene transfer alone in thyroid cancer cell lines seems to be sufficient to promote radioiodine uptake and prolonged retention of radioiodine through up-regulation of NIS, TPO and Tg expression (Mu *et al.* 2012).

NIS gene transfer driven by telomerase promoters in a human thyroid cancer cell line markedly reduced cell viability after exposure to radioiodine (Riesco-Eizaguirre *et al.* 2011).

Finally, based on EGFR overexpression in ATC, EGFR-targeted synthetic polymers were used to target NIS expression in ATC cell lines as well as in DTC cell lines (Schmohl *et al.* 2017). Radioiodine uptake was significantly increased *in vitro* and *in vivo* in a mouse xenograft model, leading to significant reduction in tumor growth and prolonged survival in comparison with control animals.

Other potential strategies

A south Korean group recently published three papers describing the potential of the inverse agonist of estrogen-related receptor (ERRγ) on the redifferentiation of thyroid tumors (Singh *et al.* 2015, 2019, Kim *et al.* 2019). The inverse agonist of ERRγ increased radioiodine uptake in ATC cell lines by inducing the expression of iodine handling genes and by enhancing the membrane localization of the NIS (Singh *et al.* 2015, 2019, Kim *et al.* 2019). Surprisingly, pharmacological MEK inhibitors abolished the effects of the inverse agonist of ERRγ on radioiodine uptake of the cells (Singh *et al.* 2015, 2019). Moreover, one orally active inverse agonist of ERRγ has also been demonstrated *in vivo* in xenograft tumor mice models of ATC to increase

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radioiodine activity and reduction of tumor growth after radioiodine therapy (Singh *et al*. 2015, 2019).

TERT (telomerase reverse transcriptase) may be another potential candidate, as the mutation of its promoter are more prevalent in the less differentiated form of thyroid cancers, that is, PDTC and ATC (Landa *et al*. 2016), and are associated with a decrease in NIS mRNA levels in an analysis of the TCGA database (Tavares *et al*. 2018). Moreover, different studies suggest that TERT promoter mutation alone is associated with a loss of radioiodine avidity and even more so when associated with $BRAF^{V600E}$ mutation (Yang *et al*. 2017, Liu *et al*. 2019).

Lithium salts known to increase the trapping of iodide by the thyroid glands showed inconsistent and disappointing results in term of potentiation of radioiodine uptake in benign thyroid diseases and DTC in clinical series (Liu *et al*. 2006*b*).

Other miscellaneous studies have highlighted the potential of other agents as candidates for redifferentiation strategy (Marsee *et al*. 2004, Goncalves *et al*. 2013, 2018, Liu *et al*. 2017, Bauriaud-Mallet *et al*. 2019, Chai *et al*. 2019, Vella *et al*. 2019).

Perspectives and concluding remarks

The differentiation of follicular cells and more particularly the expression of the NIS and the iodide-metabolizing machinery is subject to very complex regulation. The data discussed in this review are promising for the redifferentiation of DTC and there is even hope for the redifferentiation of ATC. The redifferentiation of thyroid cancers offers the hope of obtaining prolonged remission or even cure of the disease, similar to what is observed for radioiodine avid metastatic thyroid cancers. However, there is neither long-term follow-up in any of the trials or case reports published nor data on repeated treatment and many issues remain to be addressed to optimize the redifferentiation approach.

The strategy to adopt and the objectives to achieve remain an open question. Three different scenarios are possible:

- The first would be a redifferentiation strategy implemented in the case of a RAIR DTC with a low metastatic tumoral volume (i.e. lesions under 1 cm) slowly progressing to achieve very prolonged stability of the disease or even remission.
- The second scenario would be a redifferentiation strategy implemented in the case of a RAIR DTC with a significant progressive metastatic tumoral volume (i.e. multiple lesions above 1 cm) to achieve stability of the disease or even a response to the strategy synergistically with the effect of antiproliferative systemic therapies.
- A third scenario of optimizing radioiodine uptake in thyroid cancer has emerged in the adjuvant situation after thyroid surgery and before the first radioiodine therapy for selected high risk DTC patients who present persistent/recurrent disease in 30 to 60 % of the cases.

In all scenarios, as already mentioned, the timing of redifferentiating drug administration before new radioiodine treatment is a key element for the success of radioiodine uptake restoration. The future is certainly to move toward strategies combining two or more drugs with a complementary mechanism of action, that is, drugs inhibiting the MAPK pathway output combined with drugs acting on the epigenetic regulation of the NIS and drugs that target the NIS to the plasma membrane for example. However, many other combinations could be considered. To address the side effect counterpart of these multiple combinations, the challenge is to find the minimal time period required to redifferentiate tumor cells and find the most efficient combination of drugs. In this context, novel data on the redifferentiation potential of the latest generation of well-tolerated and highly selective RET or NTRK inhibitors may emerge. To this end, recent tools such as ^{124}I PET/CT make a dosimetric approach possible and, when performed sequentially, could also be powerful in determining the optimal timing of drug administration before radioiodine therapy. Other clinical studies propose qualitative evaluations with 123-iodine SPECT/CT or diagnostic 131-iodine WBS and a fixed dose of 150 mCi of 131-iodine in patients with increased uptake after MEK or BRAF inhibitors with comparable efficiency. In the future, these different strategies need to be correctly evaluated with direct comparisons in trials dedicated to this in order to prove the superiority of the dosimetric approach from a clinical point of view.

The question of whether to discontinue one or more redifferentiating drugs after radioiodine treatment remains to be answered, except for the third scenario (in the adjuvant setting) in which obviously redifferentiating treatments can be stopped after the administration of radioiodine. This raises the issue of the efficacy *per se* of radioiodine uptake increase or restoration vs the antiproliferative effect of systemic drugs used. However, in the first scenario of a low metastatic volume (i.e. lesions under 1 cm, based on RECIST measurability criteria usually taken into account for long term systemic

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treatment introduction), with slow progression, the benefit over the risk ratio may be in favor of discontinuing the redifferentiating drugs. In this scenario, management would involve the administration of a shot of redifferentiating drugs for a minimum period followed by radioiodine therapy and discontinuation of the drugs. In the second scenario, systemic therapies may be continued in between radioiodine sessions. It is not described whether the continuation of systemic treatments could be associated with a loss of the redifferentiating effect and whether the temporary interruption of treatments followed by their recovery could be beneficial to this end.

Regarding the selection of the best candidates for redifferentiation strategy, several issues arise. Is there a need for imaging to evaluate the potential of iodine uptake and, if there is, what is the degree of this uptake to select patients for treatment with a therapeutic dose of radioiodine? What are the predictive factors of redifferentiation? A simple and reliable biochemical marker of redifferentiation is probably the increase in the plasmatic Tg level without morphological tumoral progression or even with a decrease in tumoral metastatic volume. Besides Tg level, is there a molecular profile able to predict responders to the redifferentiation strategy? Could plasma drug level monitoring be useful in improving redifferentiation strategy and in identifying responders? The identification of predictive factors of redifferentiation is a main field on the agenda for future research, especially when considering this strategy in adjuvant setting, where the benefit over the risk ratio should be carefully weighed up.

To conclude, at the time of redifferentiation strategy, the definition of RAIR thyroid cancers should be revisited. After the administration of redifferentiating drugs, three scenarios could be identified:

- Restored radioiodine responsive patients: an ideal situation where radioiodine uptake is increased or restored along with an objective radiological prolonged stabilization or a decrease in a progressive metastatic tumoral mass.
- True radioiodine resistance: a situation where there is a radioiodine uptake increase or restoration but with a radiological progression of the metastatic tumoral mass. In this case, the molecular mechanism underlying the resistance to radioiodine remains unclear.
- Persistent non avid patients: a situation where there is failure to restore radioiodine uptake. In this case, the molecular mechanism underlying the absence of a correct restoration of NIS activity remains to be elucidated.

In conclusion, redifferentiation strategy provides hope for the major issue of long-term adverse events of systemic therapies currently used for RAIR-DTC, thus creating new opportunities and a new choice for metastatic patients who should be back at the center of the decision-making process.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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