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Redifferentiation of Radioiodine-Refractory Thyroid Cancers

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Keywords:	NIS, radioiodine refractory thyroid cancers, redifferentiation, MAPK inhibition, NIS trafficking

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Manuscripts

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For Review Only

63 Abstract

64 The management of radioiodine refractory thyroid cancers (RAIR TC) is challenging for the
65 clinician. Tyrosine kinase inhibitors classically prescribed in this setting can fail due to
66 primary or acquired resistance or the necessity of drug withdrawal because of serious or
67 moderate but chronic and deleterious adverse effects. Thus, the concept of redifferentiation
68 strategy, which involves treating patients with one or more drugs capable of restoring
69 radioiodine sensitivity for RAIR TC, has emerged. The area of redifferentiation strategy leads
70 to the creation of new definitions of RAIR TC including persistent non radioiodine-avid
71 patients and “true” RAIR TC patients. The latter group presents a restored or increased
72 radioiodine uptake in metastatic lesions but with no radiological response on conventional
73 imaging i.e. progression of a metastatic disease thus proving that they are “truly” resistant to
74 the radiation delivered by radioiodine. Unlike these patients, metastatic TC patients with
75 restored radioiodine uptake offer the hope of prolonged remission or even cure of the disease
76 as for radioiodine-avid metastatic TC.

77 We review here the different redifferentiation strategies based on the underlying molecular
78 mechanism leading to the sodium iodide symporter (NIS) and radioiodine uptake reinduction
79 i.e. by modulating signaling pathways, NIS transcription, NIS trafficking to the plasma
80 membrane, NIS post-transcriptional regulation, by gene therapy and other potential strategies.
81 We discuss clinical trials and promising preclinical data of potential future targets.

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87 **Introduction**

88 Although the majority of patients with differentiated thyroid cancers (DTC) could be
89 successfully managed by surgery and radioiodine administration for which the indication is
90 decided according to published guidelines (Haugen et al., 2016, Luster et al., 2019, Ahuja et
91 al., 2019), the evolution of a minority of these DTC will be less favourable and will require
92 personalized management. Distant metastases encountered in less than 10% of DTC
93 (<https://seer.cancer.gov/statfacts/html/thyro.html>) can lead to serious complications and even
94 decrease the survival of patients (Wassermann et al., 2016). Radioiodine therapy is the
95 cornerstone of the treatment of distant metastases from DTC. This therapy is based on the
96 expression at the plasma membrane of normal and tumoral thyroid epithelial cells of a
97 symporter, the sodium iodide symporter (NIS), that transports two sodium ions and one iodide
98 ion into the cytosol. Iodide is then handled by an iodine-metabolizing machinery that
99 concentrates iodine into the thyroid cells which has probably a major impact on radioiodine
100 efficacy. One-third of metastatic DTC patients will be in remission after one or more
101 radioiodine sessions (Durante et al., 2006). The others are immediately or will later become
102 radioiodine refractory (RAIR) which is correlated with high tumor burden (multiple and
103 macrometastases) along with a less differentiated state of the tumor and worse prognosis
104 (Wassermann et al., 2016, Deandreis et al., 2017). Dedifferentiation is related to a decrease in
105 or even a loss of the NIS expression and/or targeting to the plasma membrane where NIS is
106 fully effective which results in the loss of iodine uptake in thyroid cells (Figure 1). Other
107 histological types are less or not at all sensitive to radioiodine such as poorly DTC (PDTC) or
108 anaplastic thyroid cancer (ATC) as these tumours have also lost the expression of thyroid
109 specific genes, particularly those encoding for the iodine-metabolizing machinery during the
110 dedifferentiation process. Therapeutic strategies for RAIR thyroid cancers include the

111 implementation of local therapy (surgery, external radiotherapy, interventional radiology
112 approach) whenever possible and in case of diffuse significant progression of distant
113 metastatic disease systemic therapy, mainly tyrosine kinase inhibitors (TKI). However, TKI
114 raise various issues such as primary and acquired resistance with tumor escape on the one
115 hand and on the other hand serious and/or moderate but chronic and deleterious adverse
116 effects, especially for antiangiogenic TKI. Thus, the concept of redifferentiation strategy has
117 emerged with a view to finding one or more drugs capable of restoring radioiodine sensitivity
118 for RAI-resistant thyroid cancers (Figure 1). One of the challenges of this redifferentiation strategy is
119 to find a treatment protocol involving the short-term use of systemic therapy so that even if
120 side effects occur, they only have to be tolerated temporarily and frequently vanish with the
121 discontinuation of re-differentiation treatment. Numerous compounds have been tested for
122 several years with inconsistent results. A renewed interest for this strategy came from the
123 publication of a pilot trial with a series of 24 patients showing promising results obtained with
124 a pharmacological MEK inhibitor prescribed for 4 weeks (Ho et al., 2013) which was in line
125 with the pre-clinical mouse model validating this strategy (Chakravarty et al., 2011).

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

134

135 1. Modulation of signaling pathways

136

137 **1.1. Modulation of the MAPK pathway: preclinical and clinical evidence**

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

145 Several *in vitro* studies have demonstrated an increase in NIS expression and/or radioiodine
146 uptake in human thyroid cancer-derived cell lines with various genetic backgrounds or
147 patient-derived tumor tissue of ATC and PDTC with different MAPK pathway inhibitors
148 namely BRAF^{V600E} inhibitors such as vemurafenib (Cheng et al., 2016, Zhang and Chen,
149 2018) or dabrafenib (Fu et al., 2019), multityrosine kinase inhibitor such as sorafenib (Ruan et
150 al., 2015, Wachter et al., 2018a), or cabozantinib (Ruan et al., 2015), MEK inhibitors such as
151 selumetinib (Fu et al., 2019, Wachter et al., 2018a, Wachter et al., 2018b), PD98059 (Hou et
152 al., 2010, Vadysirisack et al., 2007, Zhang and Chen, 2018) or refametinib (Hou et al., 2010).

153 The first clinical phase II study testing a pharmacological inhibitor of the MAPK pathway
154 reported the effect of the multityrosine kinase inhibitor sorafenib in 31 patients (Hoftijzer et
155 al., 2009). This study was based on *in vitro* data demonstrating that another multityrosine
156 kinase inhibitor namely sunitinib was able to induce NIS expression in PTC cell lines
157 harbouring RET/PTC1 rearrangement through inhibition of the MEK/ERK and SAPK/JNK
158 pathway (Fenton et al., 2010) and that sunitinib increased iodine uptake in normal rat thyroid
159 cells (Salem et al., 2008). Of the 20 patients evaluable for redifferentiation, after 26 weeks of
160 treatment with sorafenib, only one restored a faint uptake of radioiodine in an occipital

161 skeletal metastasis on the diagnostic whole body scan (WBS) performed, which was not
162 confirmed on the therapeutic WBS performed after the administration of 200 mCi (Hoftijzer
163 et al., 2009). A renewed interest for redifferentiation strategy with MAPK pathway
164 pharmacological inhibitors came from the publication by the James Fagin laboratory
165 (Memorial Sloan-Kettering Cancer Center, MSKCC) (Chakravarty et al., 2011). His team
166 developed an ingenious mouse model expressing the BRAF^{V600E} oncogene specifically in the
167 thyrocytes and with the ability to switch the expression of BRAF^{V600E} on and off with the
168 administration or withdrawal of doxycycline (Chakravarty et al., 2011). Switching on
169 BRAF^{V600E} expression induced thyroid cancers in mice that recapitulate most human
170 BRAF^{V600E}-tumor characteristics and was associated with a virtual abolition of thyroid-
171 specific gene expression along with radioiodine uptake. Switching off BRAF^{V600E} not only
172 restored thyroid follicular architecture but also thyroid-specific gene expression and
173 radioiodine incorporation. Pharmacological inhibitors of MEK i.e. selumetinib or BRAF^{V600E}
174 inhibitor i.e. dabrafenib for 2 weeks partially restored thyroid-specific gene expression and
175 radioiodine uptake. Following this preclinical *in vivo* model, Ho et al., from the MSKCC,
176 performed a clinical study in 24 patients with PTC or follicular thyroid cancer (FTC) or
177 poorly DTC confirming the ability of selumetinib to restore radioiodine in tumors previously
178 shown to be radioiodine resistant (Ho et al., 2013). In this study, patients were treated with
179 selumetinib for 4 weeks and then submitted to an iodine-124 PET-CT under thyrotropin
180 stimulation to estimate the activity of iodine-131 required to deliver an arbitrary dose of 2000
181 cGy and above to the metastatic lesions. If it appeared that at least one lesion could be treated
182 by an activity of less than 300 mCi the patient was then treated with a personalized
183 therapeutic dose of 131-iodine. Out of the 20 patients evaluable, selumetinib increased
184 radioiodine uptake in 12. Of these 12 patients, 8 reached the dosimetry threshold for
185 radioiodine therapy, including all 5 patients with NRAS mutation but only one out of 9

186 patients with BRAF^{V600E} mutation. Of the 8 patients treated with radioiodine, 7 had a
187 confirmed radiological partial response according to the RECIST 1.1 criteria, 6 months after
188 selumetinib withdrawal and radioiodine therapy. The authors attributed their good results in
189 large part to the dosimetric approach enabled by the iodine-124 PET-CT evaluation of the
190 patients. This publication has opened the field of successful redifferentiation strategy with
191 MAPK inhibitors (Table 1).

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

198 Despite these encouraging preliminary results on RAIR DTC patients, the results of the
199 ASTRA phase III study (NCT01843062) evaluating MEK inhibitors as an adjuvant therapy in
200 non-proved RAIR DTC were disappointing. This trial included patients with DTC at high risk
201 of recurrence after total thyroidectomy (i.e. pT> 4 cm, pT4, N1 with ≥ 5 lymph nodes or with
202 at least 1 lymph node ≥ 1 cm). Patients were randomized to receive placebo or selumetinib for
203 4 weeks prior radioiodine ablation to assess the effect of selumetinib on the complete
204 remission rate at 18 months. The addition of selumetinib to radioiodine did not improve the
205 complete remission rate (40% vs 38.5% in the placebo group) in this patient population, in
206 any subgroup of patients, even when genotype was taken into account. However, the placebo
207 group established a 38.5% complete remission rate with standard radioiodine alone in high-
208 risk patients, suggesting the need for improved therapeutic approaches. Subgroup analyses of
209 this trial suggest that treatment compliance and tailoring the targeted therapy approach to the

210 oncogenic driver mutation, especially the absence of a BRAF mutation, may be critical design
211 elements to consider for future trials (unpublished data from J. Fagin's team).

212 Owing to the relatively poor response of BRAF-mutated thyroid cancers in the study
213 published by Ho et al., others have evaluated the capacity of the selective BRAF^{V600E} inhibitor
214 [dabrafenib](#) to specifically redifferentiate BRAF^{V600E} (Rothenberg et al., 2015). Out of the 10
215 patients included with a RAIR BRAF-mutated PTC, defined as the absence of radioiodine
216 uptake on a first WBS (diagnostic or therapeutic dose), 6 weeks of dabrafenib restored
217 radioiodine uptake on a diagnostic WBS in 6 of them. Those 6 patients were then treated with
218 150 mCi iodine-131 with a therapeutic WBS confirming the data obtained with the diagnostic
219 scan. Two patients showed partial response and 4 stable disease on standard radiological
220 imaging obtained 3 months after radioiodine therapy and discontinuation of dabrafenib, while
221 out of the 4 patients without restoration of radioiodine uptake, one had progressive disease
222 and 3 stable disease. These results should be put into perspectives considering that at study
223 entry 5 patients out of 10 had progression per RECIST v1.1 within the 14 months prior to
224 enrolment. Ho and his team published another pilot trial (Dunn et al., 2019) evaluating
225 vemurafenib in 12 patients with a BRAF mutated-PTC or PDTC, with a methodology similar
226 to their first study (Ho et al., 2013). Out of the 10 patients evaluable, 4 reached the dosimetry
227 threshold on the iodine-124 PET scan and were retreated with a therapeutic dose of iodine
228 131. Of those 4 patients, radiological assessment revealed partial response (n = 2) and stable
229 disease (n = 2) 6 months after radioiodine therapy. Interestingly the serum Tg value among
230 iodine-124 responders was significantly higher than in non-responders. This suggests that
231 cancers with better differentiation at baseline (high serum Tg) have a better chance to respond
232 favourably to the redifferentiation strategy. Molecular tumor biopsy analysis performed
233 before and under vemurafenib revealed that to some extent the degree of iodine avidity

234 restoration is linked to the degree of MAPK pathway output inhibition and the induction of
235 thyroid-specific gene expression.

236 The retrospective analysis of patients with RAIR DTC (PTC, FTC or PDTC) treated with
237 MAPK inhibitors (BRAF^{V600E} and/or MEK inhibitors), for a mean of 14 months, also
238 confirmed the data described above (Jaber et al., 2018, Iravani et al., 2019). In the first study
239 (Jaber et al., 2018) 9 out of 13 patients were treated with a therapeutic dose of 150-250 mCi
240 of radioiodine based on meaningful uptake of radioiodine on a diagnostic WBS except for
241 one. All 9 patients had durable disease control. As previously reported, RAS-mutated tumors
242 were the best responders compared to BRAF-mutated ones. In the second study (Iravani et al.,
243 2019) out of 6 patients treated with MEK inhibitor (NRAS mutated) or BRAF and MEK
244 inhibitors (BRAF mutated) for 4 weeks, 4 were considered suitable for radioiodine therapy
245 based on the result of I124 PET/CT, with I131 post-therapeutic WBS confirming the
246 restoration of radioiodine uptake. In this study all BRAF-mutated patients responded to the
247 redifferentiation strategy while only one NRAS-mutated patient out of 3 did. Of these 4
248 patients 3 achieved a partial imaging response (while progressing or stable at study entry) and
249 one stable disease (while progressing at study entry) after a median follow-up of 16.6 months.
250 Additional case reports demonstrated the potential redifferentiating effect of pharmacological
251 MAPK inhibition in thyroid cancers (Huillard et al., 2017, Leboulleux et al., 2019). In one of
252 these case reports clinical hyperthyroidism developed, with free triiodothyronine and free
253 thyroxine levels increasing to 6.6 and 4.4 times their upper reference limit, as ultimate
254 evidence of efficient redifferentiation (Leboulleux et al. 2019). ~~In the study of Iravani et al. 4~~
255 ~~weeks of thyroid hormone withdrawal increased TSH to only 25.9 mUI/L in one patient~~
256 ~~submitted to a redifferentiation strategy with MEK and BRAF inhibitors suggesting the~~
257 ~~redifferentiation of functional thyroid cancer cells.~~

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

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

271 Future prospects to improve tumor redifferentiation, especially BRAF^{V600E}-mutated thyroid
272 cancers, through MAPK inhibition, might come from compounds able to profoundly inhibit
273 the MAPK pathway, such as CKI a MEK inhibitor that functions as a dominant-negative
274 inhibitor of RAF which reduces the feedback reactivation of ERK **signaling**. James Fagin's
275 laboratory published data, *in vitro*, in rat thyroid cells expressing BRAF^{V600E} and *in vivo* in a
276 mouse model with BRAF^{V600E}-induced thyroid cancer, demonstrating that a small increase in
277 ERK inhibition translates into a markedly increased expression of thyroid differentiation
278 genes and increased iodide accumulation in cancer (Nagarajah et al., 2016). A High-
279 throughput NIS enhancer screening platform also enabled the identification of a new tyrosine
280 kinase inhibitor which increased NIS promoter activity along with the expression of NIS and
281 other thyroid specific proteins (Tg, TPO, TSH-R, Pax 8 and TTF-1) in a BRAF^{V600E}-mutated
282 thyroid cancer cell line. Moreover this compound, which decreased the level of

283 phosphorylated ERK i.e. the activation of the MAPK pathway, increased ^{125}I uptake in the
284 same cell line and *in vivo* in xenograft mice models (Oh et al., 2018). The combination of
285 BRAF^{V600E} and MEK inhibition may be a promising strategy as the combination
286 synergistically increased radioiodine uptake in human BRAF-mutated thyroid cancer cell
287 lines possibly through inhibition of the rebound of ERK1/2 activation observed with only one
288 drug (Zhang and Chen, 2018). An ongoing clinical trial performed by the French RAIR
289 thyroid cancer TUTHYREF network is testing the association of MEK (Trametinib) alone
290 (for *RAS* mutated tumors) or in combination with BRAF^{V600E} (Dabrafenib) inhibitors (for
291 BRAF^{V600E} mutated tumors) followed by radioiodine therapy for the treatment of RAIR
292 metastatic DTC (NCT 03244956).

293 Another strategy for BRAF^{V600E}-mutated thyroid cancers would ~~consists in~~ be to combine
294 inhibitors of the human epidermal growth factor receptor (Her) belonging to the epidermal
295 growth factor receptor family with MAPK inhibitors. In thyroid cancer cells harbouring
296 BRAF^{V600E} mutation, inhibition of the MAPK pathway by pharmacological inhibitors were
297 transient due to the release, ~~by a RAF or MEK inhibitor,~~ of a transcriptional repressor from
298 the HER3 promoter and consequently induced HER3 gene overexpression (Montero-Conde et
299 al., 2013). An autocrine secretion by thyroid cancer cells of a ligand able to bind to and
300 activate ~~by dimerization~~ the tyrosine kinase receptors HER2/HER3 resulted in the reactivation
301 of the MAPK and PI3K pathway. The Her kinase inhibitor lapatinib prevented MAPK
302 rebound and overcame BRAF-mutated thyroid cancer cell resistance to MAPK inhibitors
303 (Montero-Conde et al., 2013). In line with these data, Cheng et al. (Cheng et al., 2017)
304 demonstrated *in vitro* in BRAF^{V600E}-mutated human thyroid cancer derived cell lines that the
305 combination of lapatinib with dabrafenib or selumetinib increased radioiodine uptake. A
306 clinical trial (NCT 02456701) performed at the MSKCC and which tests the ability of

307 vemurafenib combined with an anti HER3 monoclonal antibody to restore iodine
308 incorporation in BRAF mutant RAIR thyroid cancer patients is ongoing.

309 Iodide oxidation and tyrosine organification are not restored by MAPK blockade. As PI3K
310 inhibition seems to prolong radioiodine retention in thyroid cells (Lakshmanan et al., 2015),
311 the combination of MAPK and PI3K inhibitors may be an interesting strategy, but with the
312 disadvantage of potential synergistic side effects.

313 The potential of combining MAPK inhibitors with HDAC inhibitors is developed in the 2.2
314 section of this review.

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

320

321 **1.2. Modulation of other pathways: preclinical evidence**

322 **1.2.1. The PI3K pathway**

323

324 In a non-tumoral context, a central role for PI3K in the repression of NIS gene transcription
325 by IGF1 has been demonstrated in the FRTL-5 rat thyroid cell line (Garcia and Santisteban,
326 2002). Paradoxically, Quercetin, a compound with PI3K inhibitory properties, has been
327 shown to reduce NIS mRNA levels in FRTL-5 cells (Giuliani et al., 2008).

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

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

340 Interestingly pharmacological Akt inhibition increased radioiodine uptake despite a decrease
341 in NIS protein levels, in PCCL3 rat thyroid cells with doxycycline induced expression of
342 constitutively active MEK. Indeed, this Akt1/2 inhibitor acted on iodine uptake through a
343 decreased iodide efflux and a higher iodide affinity enhancing the NIS-mediated iodide
344 transport rate (Liu et al., 2012). The same research team further demonstrated that among
345 different pharmacological inhibitors targeting Akt, MEK, PI3K, Hsp90 or BRAF^{V600E} tested
346 in PCCL3 rat thyroid cells conditionally expressing RET/PTC3 or BRAF^{V600E}, the PI3K
347 inhibitor GDC-0941 outperformed other inhibitors in radioiodine increase (Lakshmanan et al.,
348 2015). Surprisingly the greatest effect was observed in BRAF^{V600E} expressing thyroid cells,
349 where MEK is the canonical downstream of BRAF^{V600E}. Again, a decrease in iodide efflux
350 was the main mechanism of action of this PI3K inhibitor which had limited effect on NIS
351 protein levels. Of note Lakshmanan et al. (Lakshmanan et al., 2015) demonstrated that
352 TGF β , present in the thyroid tumor microenvironment, reduces the extent of increase in
353 radioiodine uptake induced by the pharmacological inhibitors tested. They also showed that
354 Apigenin, a plant-derived flavonoid, associated with GDC-0941 provided the highest

355 radioiodine uptake level in both BRAF^{V600E} and RET/PTC3 expressing PCCL3 cells,
356 corroborating previous data obtained with an Akt pharmacological inhibitor in the same cells
357 and in a mouse thyroid tumor model (Lakshmanan et al., 2014).

358 Another PI3K inhibitor, rapamycin was found to induce NIS protein levels along with
359 radioiodine uptake in BRAF^{V600E} and RET/PTC1 PTC derived cell lines (but not in a FTC
360 derived cell line), possibly through a transcriptional effect dependent on the transcription
361 factor TTF1 (Plantinga et al., 2014). Rapamycin and its synthetic analog everolimus, led to
362 controversial results in a non-tumoral thyroid *in vitro* model (de Souza et al., 2010, Liu et al.,
363 2012).

364

365 1.3. Other pathways

366

367 1.3.1. The NOTCH signaling pathway

368 *Notch* genes encode receptors for a signaling pathway that controls cell fate by regulating cell
369 proliferation and differentiation, especially in oncogenic contexts. *Notch 1* and *Notch3* have
370 been described as tumor suppressor genes in PTC, FTC and ATC (Somnay et al., 2017, Xiao
371 et al., 2009, Yu et al., 2013). Moreover, *Notch1* and 3 expression are inversely correlated to
372 the degree of differentiation in thyroid cancers with ATC having the strongest decreased
373 expression of both *Notch* (Ferretti et al., 2008, Somnay et al., 2017). From a clinical point of
374 view, low *Notch3* expression levels correlated with overall survival independently from the
375 classical other prognostic factors (Somnay et al., 2017). *In vitro* Notch3 constitutive activation
376 in a FTC cell line increased the mRNA levels of thyroid specific genes i.e. *NIS*, *TSH-R*, *TTF-*
377 *1*, *TTF-2*. In a non-tumoral context, in the normal FRTL-5 rat thyroid cell line, overexpression
378 of Notch1 increased the expression of NIS and TPO (Ferretti et al., 2008). Pharmacological
379 Notch1 activating compounds (Figure 2) such as resveratrol (Yu et al., 2013) or Hesperetin

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

386 **1.3.2. PDGFR α blockade**

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

395 **2. Modulation of NIS transcription**

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

402 **2.1. Retinoic acid**

403

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

410 In thyroid carcinoma cell lines *in vitro* experiments showed that retinoids affect thyroid
411 specific functions (induction of type I 5'-deiodinase activity, NIS gene expression), cell-cell or
412 cell-matrix interaction, differentiation markers, growth, and tumorigenicity (Jeong et al.,
413 2006, Kurebayashi et al., 2000, Schmutzler and Kohrle, 2000, Schmutzler et al., 2002,
414 Schreck et al., 1994, Van Herle et al., 1990). Moreover, retinoids partly re-differentiate
415 follicular thyroid carcinoma cell lines (Schmutzler and Kohrle, 2000) (Figure 2).

416 The first clinical series of 10 RAIR-DTC patients treated with isotretinoin (13-cis-retinoid
417 acid, a first-generation retinoid) was published by Simon et al. Radioiodine uptake was
418 restored in 4 patients (Simon et al., 1996). Other pilot studies reported an increase in
419 radioiodine uptake in about 20 to 40% of patients (Courbon et al., 2006, Fernandez et al.,
420 2009, Gruning et al., 2003, Grunwald et al., 1994, Kim et al., 2009, Oh et al., 2011, Simon et
421 al., 2002). However, tumor response did not always correlate with increased radioiodine
422 uptake and other direct antiproliferative effects of isotretinoin might have been involved
423 (Simon et al., 2002). Prospective clinical trials failed to demonstrate a clinical utility of
424 isotretinoin. In an open-label phase II trial, isotretinoin did not significantly increase
425 radioiodine uptake in 16 RAIR-DTC (Short et al., 2004). In another published phase II trial,
426 increased radioiodine uptake was observed in 17% (n=9/53) of patients. However, this did not
427 translate into tumor response or clinical benefit (Handkiewicz-Junak et al., 2009).

428 Few clinical data exist with other retinoids. In a series of 11 patients treated with tretinoin
429 (all-trans-retinoic acid [RA]), also a first-generation retinoid, authors reported increased
430 radioiodine uptake in 4 patients and partial response in 5 patients (Zhang et al., 2007).
431 Another series of 13 patients treated with tretinoin for RAIR-DTC showed a weak radioiodine
432 uptake in 6 patients with no tumor response among those patients (Damle et al., 2011). In a
433 pilot study with bexarotene (RXR activator), increased radioiodine uptake was observed in
434 8/11 patients, but no significant clinical benefit was demonstrated (Liu et al., 2006a).
435 A recent meta-analysis on retinoids in RAIR-DTC reported a pooled effect of 27.6% for
436 increased radioiodine uptake and 17% for tumor response by RECIST criteria (Pak et al.,
437 2018).

438

439 **2.2. HDAC inhibitors**

440 Post-translational modification of histones bound to DNA such as acetylation is a well-known
441 mechanism of tumorigenesis initiation and progression, especially for thyroid cancers (Russo
442 et al., 2013). By altering the structural state of chromatin, histone acetylation by histone acetyl
443 transferases favors transcriptional activation. On the other hand, histone deacetylation by
444 histone deacetylase (HDACs) is associated with transcriptional repression. Several
445 publications have demonstrated that the acetylation of histones of the *SLC5A5* promotor
446 regulates its transcription and consequently NIS expression (Fu et al., 2019, Puppini et al.,
447 2012, Zhang et al., 2014). Various HDAC inhibitors (HDACi), such as panobinostat (Fu et
448 al., 2019, Pugliese et al., 2013, Wachter et al., 2018c, Wachter et al., 2018a), valproic acid
449 (Haghpanah et al., 2014, Massimino et al., 2018, Shen et al., 2005, Frohlich et al., 2009),
450 vorinostat (Cheng et al., 2016, Clinckspoor et al., 2011, Hou et al., 2010, Puppini et al., 2005,
451 Puppini et al., 2012, Wachter et al., 2018a), romidepsin (Furuya et al., 2004, Kitazono et al.,
452 2001, Xu and Hershman, 2006), sodium butyrate (Puppini et al., 2005), Trichostatin A

453 (Wachter et al., 2018a, Zarnegar et al., 2002) or analogs targeting HDAC (Jang et al., 2015),
454 have been shown in human thyroid cancer derived cell lines or patient-derived thyroid cancer
455 tissue to reinduce NIS mRNA levels (Figure 2) or even other thyroid differentiation genes
456 such as Tg, TPO or TSH receptor. More interestingly, some studies have demonstrated in
457 cellular or in *in vivo* models an increase in iodine uptake following HDACi treatment and/or
458 proper targeting of the NIS to the plasma membrane where this transporter can be effective
459 (Cheng et al., 2016, Fu et al., 2019, Furuya et al., 2004, Hou et al., 2010, Kitazono et al.,
460 2001, Massimino et al., 2018, Pugliese et al., 2013, Puppin et al., 2012, Wachter et al.,
461 2018a). However the results of four clinical trials (Amiri-Kordestani et al., 2013, Kelly et al.,
462 2005, Nilubol et al., 2017, Sherman et al., 2013) that tested HDACi in RAI R human thyroid
463 cancers were in apparent contradiction with the promising results of preclinical studies.
464 Nilubol et al. (Nilubol et al., 2017) tested the effect of valproic acid in 13 patients with RAI R
465 DTC without any improvement in radioiodine uptake in the 10 patients evaluable. Two trials
466 have tested romidepsin (Amiri-Kordestani et al., 2013, Sherman et al., 2013) in RAI R DTC.
467 In these phase 1 and 2 trials, the ¹³¹I diagnostic WBS showed restoration of faint radioiodine
468 uptake after various time periods of treatment in 2 out of 6 evaluable patients for the phase 1
469 study and 2 out of 16 patients for the phase 2. In the phase 2 trial (Sherman et al., 2013), the
470 faint radioiodine uptake observed on the diagnostic WBS was confirmed on the therapeutic
471 WBS but without long-term benefit from the treatment. In a phase 1 trial testing vorinostat
472 also known as Suberoylanilide Hydroxamic Acid (SAHA) (Kelly et al., 2005) in patients with
473 advanced thyroid cancers, Kelly et al. reported an improvement in the radioiodine uptake
474 evaluated by post-therapeutic WBS after SAHA administration for an unknown duration in
475 one out of the 3 PTC patients included. These trials have classically been considered
476 disappointing. However, several points should be highlighted before definitively buring
477 HDACi as an effective redifferentiation strategy. First, from a clinical point of view, besides

478 the small number of patients included that preclude any definite conclusion, the evaluation of
479 a proper iodine uptake increase or restoration after HDACi treatment on a diagnostic WBS
480 can be criticized as this imaging lacks sensitivity (Haugen et al., 2016). Second, preclinical
481 studies show the complexity of triggering NIS and other thyroid-differentiation gene
482 reinduction. In several of these studies the redifferentiation effect i.e. the increase in NIS
483 mRNA and/or protein levels and/or increase in iodine uptake was considerably variable
484 among the human thyroid cancer-derived cell lines tested, suggesting variable effects
485 according to the genetic background of the cell lines (Clinckspoor et al., 2011, Fu et al., 2019,
486 Massimino et al., 2018, Shen et al., 2005). Illustrating the fine and complex regulation of the
487 system, Shen et al. (Shen et al., 2005) reported a paradoxical inefficacy to restore NIS mRNA
488 levels of a higher dose of valproic acid (1.5 mM) in comparison with a lower dose (0.5 mM).
489 Moreover the timing of HDACi administration and the evaluation of the redifferentiation
490 effect might also be critical, as *in vitro* the increase in radioiodine uptake may vary with the
491 timing of the pharmacological inhibitor administration (Wachter et al., 2018c).

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

503 derived cell lines (Hou et al., 2010). These pre-clinical data provide evidence which supports
504 the proposal to use a drug combination strategy targeting the MAPK pathway and/or PI3K
505 pathway with HDACi.

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

510

511 **2.3. PPAR γ agonists**

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

516 Different pre-clinical studies have demonstrated the re-differentiation effect of several
517 thiazolidinediones, which bind to PPAR γ and are known to be PPAR γ agonists, such as
518 rosiglitazone (Frohlich et al., 2009, Frohlich et al., 2005, troglitazone {Frohlich, 2005 #265,
519 Park et al., 2005) and pioglitazone (Frohlich et al., 2005), in addition to their anti-proliferative
520 effects on DTC-derived cell lines (Figure 2). Interestingly Frohlich et al. (Frohlich et al., 2005)
521 demonstrated that in FTC cell lines thiazolidinediones increased radioiodine uptake and the
522 amount of NIS in the membrane fraction, especially troglitazone which showed greater effects
523 than other thiazolidinediones. However, these effects seem to be independent of PPAR γ
524 activation as the addition of a PPAR γ antagonist did not reverse their effects.

525 Several case reports (Elias and Lizotte, 2006, Elola et al., 2011), pilot study and phase II
526 clinical trials (Kebebew et al., 2009, Kebebew et al., 2006, Philips et al., 2004, Rosenbaum-
527 Krumme et al., 2012, Tepmongkol et al., 2008) suggest a more or less efficient re-
528 differentiation effect of the PPAR γ agonist rosiglitazone. Kebebew (Kebebew et al., 2009,
529 Kebebew et al., 2006) published the results of a phase II trial including 20 patients with RAIR
530 DTC treated for 8 weeks with rosiglitazone of whom 4 showed a positive diagnostic WBS
531 after treatment. Despite the administration of 50 to 213 mCi I131 in those 4 patients, none had
532 complete or even partial response according to the RECIST 1.1 criteria at 3 months of follow-
533 up. There was no relationship between the level of PPAR γ mRNA and protein expression in
534 patients who had radioiodine uptake compared with those who did not. These last results were
535 in contradiction with the study of Tepmongkol et al. (Tepmongkol et al., 2008). Out of 23
536 patients with RAIR DTC treated for 6 weeks with rosiglitazone, 6 patients demonstrated
537 restoration of radioiodine uptake on a therapeutic WBS after rosiglitazone. Of these, 5 had
538 strong PPAR γ -positive staining in thyroid biopsies and one a weak staining. On the other
539 hand, none of the seven patients with negative staining had positive therapeutic WBS after
540 rosiglitazone therapy. [In the study of](#), Rosenbaum-Krumme et al. (Rosenbaum-Krumme et al.,
541 2012) out of 9 patients, 4 were retreated after 3 or 6 months of rosiglitazone, and of these, 3
542 had at best partial response using RECIST 1.1 criteria after rosiglitazone therapy. A small
543 pilot study in 2004 (Philips et al., 2004) had shown less encouraging results than the trials
544 described above, as out of 5 patients with RAIR DTC only one experienced restoration of a
545 faint uptake of radioiodine in lung metastases after 3 months of rosiglitazone.

546

547 **2.4. Pre-clinical data for potential future targets**

548

549 **2.4.1. Demethylating agents**

550 Hypermethylation of the promoter region of the *SCLC5A5* gene encoding for the NIS have
551 been demonstrated in several publications, both in benign and malignant thyroid tumors
552 (Galrao et al., 2014, Galrao et al., 2013, Smith et al., 2007, Stephen et al., 2011,
553 Venkataraman et al., 1999, Xing et al., 2003) (Figure 2). In PTC, specific CpG-island regions
554 of the promoter are hypermethylated through DNA methyltransferase 1 (DNMT1) up-
555 regulation following NF κ B activation by BRAF^{V600E} (Choi et al., 2014). Moreover an inverse
556 correlation between NIS expression and the degree of methylation of some of this CpG island
557 has been described (Galrao et al., 2014). However, preclinical studies analyzing the re-
558 dedifferentiation effect of demethylating agents such as 5-azacytidine in human thyroid
559 cancer cell lines had shown disappointing results without any significant effect on NIS mRNA
560 reinduction and/or radioiodine uptake increase (Massimino et al., 2018, Provenzano et al.,
561 2007, Tuncel et al., 2007), unless 5-azacytidine was associated with the sodium butyrate
562 HDACi (Provenzano et al., 2007). Only one study showed an increase in radioiodine uptake
563 *in vitro* after 5-azacytidine treatment, correlated with a reduced methylation level of
564 *SCLC5A5* promoter (Galrao et al., 2014). To date clinical trials evaluating demethylating
565 agent effects on RAIR DTC redifferentiation are still lacking.

566

567 **2.4.2. NADPH oxidase 4 (NOX4)**

568

569 The link between BRAF^{V600E} mutation, reduction of NIS expression and dedifferentiation is
570 well established. Even if a link between BRAF^{V600E}-induced TGF β production and repression
571 of NIS, Tg and TPO has been demonstrated (Costamagna et al., 2004, Kawaguchi et al., 1997,
572 Nicolussi et al., 2003, Riesco-Eizaguirre et al., 2006), the whole underlying molecular
573 mechanism remains largely unknown. Azouzi et al. (Azouzi et al., 2017) shed light on the role
574 of the NADPH oxidase NOX4, an enzyme specialized in reactive oxygen species production

575 upregulated in thyroid cancers (Weyemi et al., 2010). Azouzi et al. demonstrated that NOX4
576 upregulation by the BRAF^{V600E}-activated TGFβ-Smad3 pathway correlated with NIS
577 repression and dedifferentiation in thyroid cancer cell lines, patient tissue samples and in
578 mouse BRAF^{V600E}-induced thyroid tumors. Remarkably NOX4 silencing by siRNA reversed
579 BRAF^{V600E}-induced NIS repression at the mRNA and protein levels making NOX4 a potential
580 future therapeutic target (Figure 2).

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

583

584 **2.4.3. Bromodomain-containing protein 4 (BRD4)**

585

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

592

593 **2.4.4. Digitalis-like components**

594 Autophagy, which facilitates the degradation of cytoplasmic components in response to
595 stimuli such as DNA damage or hypoxia, has emerged as a key player in carcinogenesis. In
596 thyroid cancers, autophagy has been shown to be associated with dedifferentiation and
597 reduced clinical response to radioiodine (Plantinga et al., 2016). Based on this association, a
598 systematic high-throughput screening has identified among autophagy-activating small
599 molecules 5 digitalis-like compounds capable of restoring NIS expression and iodine uptake,

600 at micromolecular concentrations, in PTC, FTC (Tesselaar et al., 2017) and ATC cell lines
601 (Tesselaar et al., 2018) (Figure 2). The increase in transcriptional activation of NIS following
602 up-regulation of transcription factors such as FOS or ATF3 (activating transcription factor 3)
603 (Tesselaar et al., 2018, Tesselaar et al., 2017) after digitalis-like compound treatment may
604 account for the redifferentiation effect. Future studies involving mouse models and addressing
605 the issue of the narrow therapeutic index of digitalis-like compounds as well as the dosage
606 required for thyroid cancer redifferentiation are mandatory before clinical implementation.

607

608 **3. Modulation of NIS trafficking to the plasma membrane**

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

616 Regarding the issue of the sub-cellular localization of NIS, several publications have shed
617 light on the role of the proto-oncogene pituitary tumor transforming gene (PTTG)-binding
618 factor (PBF) which may represent a novel therapeutic target for increasing radioiodine uptake
619 (Smith et al., 2011). PBF, which specifically binds to PTTG, is over-expressed in thyroid
620 cancers (Smith et al., 2011). After its phosphorylation on a tyrosine residue by the kinase Src,
621 PBF represses iodide uptake in vitro and in murine models (Read et al., 2011), through 2
622 mechanisms: transcriptional inhibition of NIS expression (Boelaert et al., 2007) and the
623 binding and redistribution of the NIS from the plasma membrane to the cytoplasm (Smith et
624 al., 2009). Interestingly, a pharmacological Src inhibitor was able to stimulate radioiodine

625 uptake in human thyroid cancer cell lines and in human primary thyroid cells (Smith et al.,
626 2013). Future clinical therapeutic strategies may combine drugs targeting NIS protein
627 reinduction associated with drugs modulating NIS sub-cellular localization, more specifically
628 to the plasma membrane where the NIS is fully active.

629 Moreover Thompson et al. (Thompson et al., 2019) recently demonstrated *in vitro* that NIS
630 dimerization may be critical to its trafficking to the plasma membrane. Impaired dimerization
631 of the NIS and its subsequent mis-localization in RAIR DTC remains a field for future
632 investigation.

633 Lan et al. (Lan et al., 2017) demonstrated that β -catenin activation subsequent to
634 HIF1 α overexpression relocated the NIS into intracellular location near the nucleus in FTC
635 cells. As a consequence, radioiodine uptake decreased, but β -catenin knockdown restored the
636 iodine uptake capacity and the NIS localization on the periphery of the cells. Experiments in
637 xenograft mice tumors confirmed these data as β -catenin silencing [promotes the efficacy of](#)
638 [radioiodine therapy](#) in FTC cells overexpressing HIF-1 α and β -catenin.

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

646

647 **4. Modulation of NIS post-transcriptional regulation**

648 Few data are published on the role of microRNAs (miR), which are small interfering RNAs
649 modulating the translation, on NIS and other thyroid differentiation genes expression.

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

656 **5. Gene therapy**

657 Using various delivery techniques radioiodine uptake was induced *in vitro* in different thyroid
658 cancer cell lines and sometimes *in vivo* in xenograft models by NIS gene delivery and
659 reviewed in 2009 by Spitzweg (Spitzweg, 2009). All studies (Haberkorn et al., 2003, Lee et
660 al., 2003, Lin et al., 2004, Smit et al., 2000) demonstrated that NIS gene therapy was able to
661 increase radioiodine uptake but almost always along with rapid iodide efflux which, as a
662 consequence, was not sufficient to allow the therapeutic effects of radioiodine *in vivo* in
663 animal models, except in one study (Smit et al., 2002).

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

671 uptake and prolonged retention of radioiodine through up-regulation of NIS, TPO and Tg
672 expression (Mu et al., 2012).

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

675 Finally, based on EGFR overexpression in ATC, EGFR-targeted synthetic polymers was used
676 to target NIS expression in ATC cell lines as well as in DTC cell lines (Schmohl et al., 2017).

677 Radioiodine uptake was significantly increased *in vitro* and *in vivo* in a mouse xenograft
678 model leading to significant reduction in tumor growth and prolonged survival in comparison
679 with control animals.

680 **6. Other potential strategies**

681 A south Korean group recently published 3 papers describing the potential of the inverse
682 agonist of estrogen-related receptor (ERR γ) on the redifferentiation of thyroid tumors (Kim et
683 al., 2019, Singh et al., 2015, Singh et al., 2019). The inverse agonist of ERR γ increased
684 radioiodine uptake in ATC cell lines by inducing the expression of iodine handling genes and
685 enhancing the membrane localization of the NIS (Kim et al., 2019, Singh et al., 2015, Singh et
686 al., 2019). Surprisingly pharmacological MEK inhibitors abolished the effects of the inverse
687 agonist of ERR γ on radioiodine uptake of the cells (Singh et al., 2015, Singh et al., 2019).
688 Moreover one orally active inverse agonist of ERR γ has also been demonstrated *in vivo* in
689 xenograft tumor mice models of ATC to increase radioiodine activity and reduction of tumor
690 growth after radioiodine therapy (Singh et al., 2015, Singh et al., 2019).

691 TERT (telomerase reverse transcriptase) may be another potential candidate as the mutation
692 of its promoter are more prevalent in the less differentiated form of thyroid cancers i.e. PDTC
693 and ATC (Landa et al., 2016) and are associated with a decrease in NIS mRNA levels in an
694 analysis of the TCGA database (Tavares et al., 2018). Moreover different studies suggest that

695 TERT promoter mutation alone is associated with a loss of radioiodine avidity and even more
696 so when associated with BRAF^{V600E} mutation (Liu et al., 2019, Yang et al., 2017).

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

700 Other miscellaneous studies have highlighted the potential of other agents as candidates for
701 redifferentiation strategy (Bauriaud-Mallet et al., 2019, Chai et al., 2019, Goncalves et al.,
702 2013, Goncalves et al., 2018, Liu et al., 2017, Marsee et al., 2004, Vella et al., 2019).

703 **Perspectives and concluding remarks**

704 The differentiation of follicular cells and more particularly the expression of the NIS and the
705 iodide-metabolizing machinery is subject to very complex regulation. The data discussed in
706 this review are promising for the redifferentiation of DTC and there is even hope for the
707 redifferentiation of ATC. The redifferentiation of thyroid cancers offers the hope of obtaining
708 prolonged remission or even cure of the disease, similar to what is observed for radioiodine
709 avid metastatic thyroid cancers. However, there is no long-term follow-up in any of the trials
710 or case reports published nor data on repeated treatment and many issues remain to be
711 addressed to optimize the redifferentiation approach.

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

- 714 - The first would be a redifferentiation strategy implemented in the case of a RAIR DTC
715 with a low metastatic tumoral volume (i.e. lesions under 1 cm) slowly progressing to
716 achieve very prolonged stability of the disease or even remission

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

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

741 correctly evaluated with direct comparisons in trials dedicated to this in order to prove the
742 superiority of the dosimetric approach from a clinical point of view.

743 The question of whether to discontinue one or more redifferentiating drugs after radioiodine
744 treatment remains to be answered, except for the third scenario (in the adjuvant setting) in
745 which obviously redifferentiating treatments can be stopped after the administration of
746 radioiodine. This raises the issue of the efficacy *per se* of radioiodine uptake increase or
747 restoration vs the antiproliferative effect of systemic drugs used. However, in the first scenario
748 of a low metastatic volume (i.e. lesions under 1 cm, based on RECIST measurability criteria
749 usually taken into account for long term systemic treatment introduction), with slow
750 progression, the benefit over the risk ratio may be in favor of discontinuing the
751 redifferentiating drugs. In this scenario management would involve the administration of a
752 shot of redifferentiating drugs for a minimum period followed by radioiodine therapy and
753 discontinuation of the drugs. In the second scenario, systemic therapies may be continued in
754 between radioiodine sessions. It is not described whether the continuation of systemic
755 treatments could be associated with a loss of the redifferentiating effect and whether the
756 temporary interruption of treatments followed by their recovery could be beneficial to this
757 end.

758 Regarding the selection of the best candidates for redifferentiation strategy several issues
759 arise. Is there a need for imaging to evaluate the potential of iodine uptake and, if there is,
760 what is the degree of this uptake to select patients for treatment with a therapeutic dose of
761 radioiodine? What are the predictive factors of redifferentiation? A simple and reliable
762 biochemical marker of redifferentiation is probably the increase in the plasmatic Tg level
763 without morphological tumoral progression or even with a decrease in tumoral metastatic
764 volume. Besides Tg level, is there a molecular profile able to predict responders to the
765 redifferentiation strategy? Could plasma drug level monitoring be useful in improving

766 redifferentiation strategy and in identifying responders? The identification of predictive
767 factors of redifferentiation is a main field on the agenda for future research, especially when
768 considering this strategy in adjuvant setting where the benefit over the risk ratio should be
769 carefully weighed up.

770 To conclude, at the time of redifferentiation strategy, the definition of RAIR thyroid cancers
771 should be revisited. After the administration of redifferentiating drugs, 3 scenarios could be
772 identified:

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

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

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1303 Figures/ Table legends

1304 **Figure 1 Differentiation and redifferentiation of thyroid cancer schematic.**

1305 The left panel illustrates a (re-)differentiated state where the Sodium iodide symporter (NIS)
1306 is normally expressed and localized at the plasma membrane, allowing radioactive iodine
1307 (RAI) to be uptaken by tumoral cells. The right panel illustrates a dedifferentiated state where
1308 the expression of the *SLC5A5* gene, encoding for the NIS, is reduced and/or not correctly
1309 targeted to the plasma membrane and consequently with no RAI uptake. The bottom panels
1310 illustrate the case of a patient with metastatic RAI refractory papillary thyroid cancer treated
1311 for 8 weeks with dabrafenib. The post-therapeutic WBS after dabrafenib demonstrates a clear
1312 restoration of RAI uptake in the metastatic sites (lung and neck lymphadenopathy).

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1315 **Figure 2 Molecular mechanism controlling NIS expression and current or future**
1316 **actionable targets**

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1318 **Table 1. Published trials or clinical cases of redifferentiating strategy with MAPK**
1319 **inhibitors**

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

References	TKI Duration of treatment before RAI	Patients (n)	Main results
2013 , NEJM, Ho et al.	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
2015, Clin Cancer Res, Rothenberg et al.	Dabrafenib (BRAFF^{V600E} i) 6 weeks	10 BRAFF ^{V600E}	- 60% (6/10) retreated with RAI - At 3 months: 2 PR and 4 SD
2017, JES, Huillard et al.	Vemurafenib, and then dabrafenib Several months, without withdrawal	1 BRAFF ^{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
2018, JCEM, Jaber et al.	Dabrafenib ± trametinib Vemurafenib (BRAFF^{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
2019, JCEM, Dunn et al.	Vemurafenib 4 weeks	12 BRAFF ^{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
2019, Thyroid, Iravani et al.	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
2019, Thyroid, Leboulleux et al.	Dabrafenib + trametinib 8 weeks	1 BRAFF ^{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

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

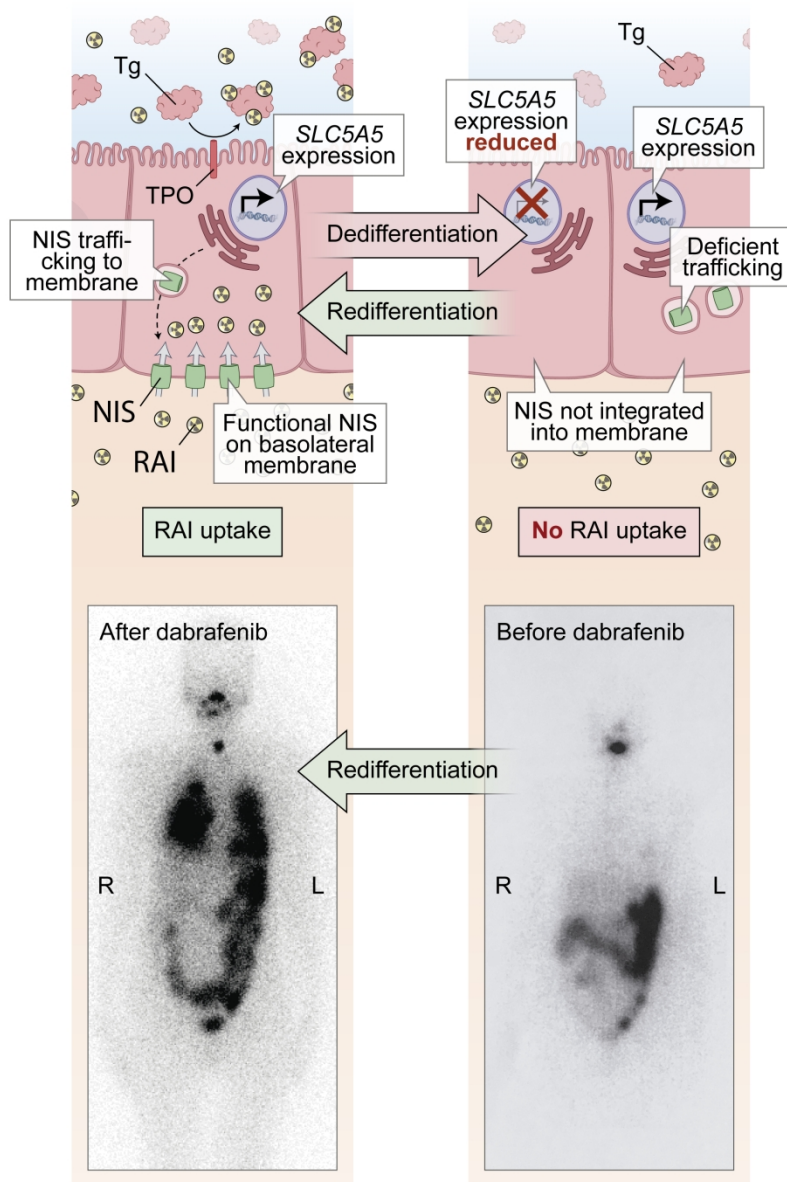


Figure 1 Differentiation and redifferentiation of thyroid cancer schematic.

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 a personal 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).

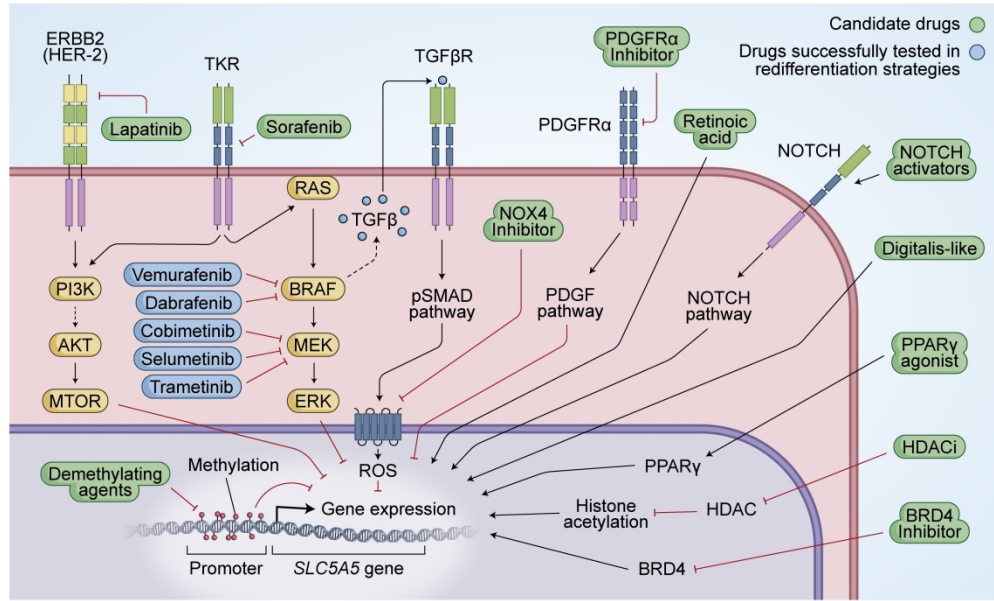


Figure 2 Molecular mechanism controlling NIS expression and current or future actionable targets

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