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Camille Buffet, Johanna Wassermann, Fabio Hecht, Laurence Leenhardt, Corinne Dupuy, Lionel Groussin, Charlotte Lussey-Lepoutre

► **To cite this version:**

Camille Buffet, Johanna Wassermann, Fabio Hecht, Laurence Leenhardt, Corinne Dupuy, et al.. Redifferentiation of radioiodine-refractory thyroid cancers. *Endocrine-Related Cancer*, 2020, 27 (5), pp.R113-R132. 10.1530/ERC-19-0491 . hal-03030427

HAL Id: hal-03030427

<https://cnrs.hal.science/hal-03030427v1>

Submitted on 25 Oct 2023

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**ENDOCRINE-RELATED
CANCER**



Redifferentiation of Radioiodine-Refractory Thyroid Cancers

Journal:	<i>Endocrine-Related Cancer</i>
Manuscript ID	ERC-19-0491.R2
Manuscript Type:	Invited Review
Date Submitted by the Author:	n/a
Complete List of Authors:	Buffet, Camille; Sorbonne Universite, Faculté de médecine; Hopital Universitaire Pitie Salpetriere, Thyroid and Endocrine Tumor Unit Wassermann, Johanna; Sorbonne Universite, ile de France; University Hospital Pitié Salpêtrière, Department of oncology Hecht, Fabio; Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho Leenhardt, Laurence; Sorbonne Universite, faculte de medecine; Hopital Universitaire Pitie Salpetriere, Thyroid and Endocrine Tumor Unit Dupuy, Corinne; Gustave Roussy, UMR 8200 CNRS Groussin, Lionel; Hopital Cochin, Endocrinologie Lussey-Lepoutre, Charlotte; Sorbonne Universite, faculte de medecine; University Hospital Pitié Salpêtrière, Department of nuclear medicine; PARCC, INSERM
Keywords:	NIS, radioiodine refractory thyroid cancers, redifferentiation, MAPK inhibition, NIS trafficking

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Manuscripts

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2 Camille Buffet, Johanna Wassermann, Fabio Hecht, Laurence Leenhardt, Corinne Dupuy,
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4

5 Camille Buffet, Sorbonne Université, Unité Thyroïde-Tumeur endocrine, Groupe de
6 Recherche Clinique n°16, Groupe de Recherche Clinique Tumeurs Thyroïdiennes, AP-HP,
7 Hôpital Pitié-Salpêtrière, 75013 Paris, France

8 Johanna Wassermann, Sorbonne Université, service d'oncologie, Groupe de Recherche
9 Clinique n°16, Groupe de Recherche Clinique Tumeurs Thyroïdiennes, AP-HP, Hôpital Pitié-
10 Salpêtrière, 75013 Paris, France

11 Fabio Hecht Instituto de Biofisica Carlos Chagas Filho, Universidade Federal do Rio de
12 Janeiro, UFRJ, Brazil

13 Laurence Leenhardt, Sorbonne Université, Unité Thyroïde-Tumeur endocrine, Groupe de
14 Recherche Clinique n°16, Groupe de Recherche Clinique Tumeurs Thyroïdiennes, AP-HP,
15 Hôpital Pitié-Salpêtrière, 75013 Paris, France

16 Corinne Dupuy, UMR 8200 CNRS, Villejuif, France; Université Paris-Saclay et Gustave
17 Roussy, Villejuif

18 Lionel Groussin, INSERM Unité 1016, CNRS, UMR 8104, Institut Cochin, Paris, France ;
19 Université Paris Descartes, Sorbonne Paris Cité, Paris, France ; Department of Endocrinology,
20 APHP, Cochin Hospital, Paris, France

21

22

23 Charlotte Lussey-Lepoutre, Sorbonne Université, service de médecine nucléaire, Groupe de
24 Recherche Clinique n°16, Groupe de Recherche Clinique Tumeurs Thyroïdiennes, AP-HP,
25 Hôpital Pitié-Salpêtrière, 75013 Paris, France and PARCC, INSERM, Equipe Labellisée par
26 la Ligue contre le Cancer, F-75015 Paris, France

27

28 **Co-Corresponding author's postal and email address**

29 Charlotte Lussey-Lepoutre

30 Hôpital Pitié-Salpêtrière

31 Service de médecine nucléaire

32 Unité de radiothérapie interne vectorisée

33 47-83 bvd de l'Hôpital

34 75013 Paris

35 email address : charlotte.lepoutre@aphp.fr

36 Camille Buffet

37 Hôpital Pitié-Salpêtrière

38 Unité Thyroïde Tumeurs Endocrines

39 47-83 bvd de l'Hôpital

40 75013 Paris

41 email address : camille.buffet@aphp.fr

42 **Short title** Redifferentiation of thyroid cancers

43 **Keywords:** NIS, radioiodine refractory thyroid cancers, redifferentiation, MAPK inhibition,
44 NIS trafficking, epigenetic regulation.

45 Word count of the full article, excluding abstract, references and figure legends: 8684

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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 RAIR 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 RAIR
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 RAIR 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 burying
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.

787 **Declaration of interest:** The authors declare that they have no conflict of interest for this
788 review

789 **Funding:** Not funding was received for this work

790 **References**

- 791 AHUJA, S., AVRAM, A. M., DILLEHAY, G., GREENSPAN, B. S., GULEC, S. & VAN NOSTRAND, D. 2019.
792 The Martinique Principles. *J Nucl Med*, 60, 1334-1335.
- 793 AMIRI-KORDESTANI, L., LUCHENKO, V., PEER, C. J., GHAFOURIAN, K., REYNOLDS, J., DRAPER, D., FRYE,
794 R., WOO, S., VENZON, D., WRIGHT, J., et al. 2013. Phase I trial of a new schedule of
795 romidepsin in patients with advanced cancers. *Clin Cancer Res*, 19, 4499-507.
- 796 AZOUZI, N., CAILLOUX, J., CAZARIN, J. M., KNAUF, J. A., CRACCHIOLO, J., AL GHUZLAN, A., HARTL, D.,
797 POLAK, M., CARRE, A., EL MZIBRI, M., et al. 2017. NADPH Oxidase NOX4 Is a Critical Mediator
798 of BRAF(V600E)-Induced Downregulation of the Sodium/Iodide Symporter in Papillary
799 Thyroid Carcinomas. *Antioxid Redox Signal*, 26, 864-877.
- 800 BAURIAUD-MALLET, M., VIJA-RACARU, L., BRILLOUET, S., MALLINGER, A., DE MEDINA, P., RIVES, A.,
801 PAYRE, B., POIROT, M., COURBON, F. & SILVENTE-POIROT, S. 2019. The cholesterol-derived
802 metabolite dendrogenin A functionally reprograms breast adenocarcinoma and
803 undifferentiated thyroid cancer cells. *J Steroid Biochem Mol Biol*, 192, 105390.
- 804 BOELAERT, K., SMITH, V. E., STRATFORD, A. L., KOGAI, T., TANNAHILL, L. A., WATKINSON, J. C., EGGO,
805 M. C., FRANKLYN, J. A. & MCCABE, C. J. 2007. PTTG and PBF repress the human sodium iodide
806 symporter. *Oncogene*, 26, 4344-56.
- 807 BROWN, S. R., HALL, A., BUCKLEY, H. L., FLANAGAN, L., GONZALEZ DE CASTRO, D., FARNELL, K.,
808 MOSS, L., GREGORY, R., NEWBOLD, K., DU, Y., et al. 2019. Investigating the potential clinical
809 benefit of Selumetinib in resensitising advanced iodine refractory differentiated thyroid
810 cancer to radioiodine therapy (SEL-I-METRY): protocol for a multicentre UK single arm phase
811 II trial. *BMC Cancer*, 19, 582.
- 812 CANCER GENOME ATLAS RESEARCH, N. 2014. Integrated genomic characterization of papillary
813 thyroid carcinoma. *Cell*, 159, 676-90.
- 814 CHAI, W., YE, F., ZENG, L., LI, Y. & YANG, L. 2019. HMGB1-mediated autophagy regulates
815 sodium/iodide symporter protein degradation in thyroid cancer cells. *J Exp Clin Cancer Res*,
816 38, 325.
- 817 CHAKRAVARTY, D., SANTOS, E., RYDER, M., KNAUF, J. A., LIAO, X. H., WEST, B. L., BOLLAG, G.,
818 KOLESNICK, R., THIN, T. H., ROSEN, N., et al. 2011. Small-molecule MAPK inhibitors restore
819 radioiodine incorporation in mouse thyroid cancers with conditional BRAF activation. *J Clin
820 Invest*, 121, 4700-11.
- 821 CHENG, L., JIN, Y., LIU, M., RUAN, M. & CHEN, L. 2017. HER inhibitor promotes BRAF/MEK inhibitor-
822 induced redifferentiation in papillary thyroid cancer harboring BRAFV600E. *Oncotarget*, 8,
823 19843-19854.
- 824 CHENG, W., LIU, R., ZHU, G., WANG, H. & XING, M. 2016. Robust Thyroid Gene Expression and
825 Radioiodine Uptake Induced by Simultaneous Suppression of BRAF V600E and Histone
826 Deacetylase in Thyroid Cancer Cells. *J Clin Endocrinol Metab*, 101, 962-71.
- 827 CHOI, H. J., KIM, T. Y., RUIZ-LLORENTE, S., JEON, M. J., HAN, J. M., KIM, W. G., SHONG, Y. K. & KIM, W.
828 B. 2012. Alpha-lipoic acid induces sodium iodide symporter expression in TPC-1 thyroid
829 cancer cell line. *Nucl Med Biol*, 39, 1275-80.
- 830 CHOI, Y. W., KIM, H. J., KIM, Y. H., PARK, S. H., CHWAE, Y. J., LEE, J., SOH, E. Y., KIM, J. H. & PARK, T. J.
831 2014. B-RafV600E inhibits sodium iodide symporter expression via regulation of DNA
832 methyltransferase 1. *Exp Mol Med*, 46, e120.
- 833 CLINCKSPOOR, I., VERLINDEN, L., OVERBERGH, L., KORCH, C., BOUILLON, R., MATHIEU, C., VERSTUYF,
834 A. & DECALLONNE, B. 2011. 1,25-dihydroxyvitamin D3 and a superagonistic analog in
835 combination with paclitaxel or suberoylanilide hydroxamic acid have potent antiproliferative
836 effects on anaplastic thyroid cancer. *J Steroid Biochem Mol Biol*, 124, 1-9.

- 837 COSTAMAGNA, E., GARCIA, B. & SANTISTEBAN, P. 2004. The functional interaction between the
838 paired domain transcription factor Pax8 and Smad3 is involved in transforming growth
839 factor-beta repression of the sodium/iodide symporter gene. *J Biol Chem*, 279, 3439-46.
- 840 COURBON, F., ZERDOUD, S., BASTIE, D., ARCHAMBAUD, F., HOFF, M., ECHE, N., BERRY, I. & CARON, P.
841 2006. Defective efficacy of retinoic acid treatment in patients with metastatic thyroid
842 carcinoma. *Thyroid*, 16, 1025-31.
- 843 DAMLE, N., PATNECHA, M., KUMAR, P., MAHARJAN, S. & BAL, C. 2011. Retinoic acid therapy in
844 patients with radioiodine negative differentiated thyroid cancer and clinical or biochemical
845 evidence of disease: An initial experience. *Indian J Nucl Med*, 26, 144-8.
- 846 DE SOUZA, E. C., PADRON, A. S., BRAGA, W. M., DE ANDRADE, B. M., VAISMAN, M., NASCIUTTI, L. E.,
847 FERREIRA, A. C. & DE CARVALHO, D. P. 2010. MTOR downregulates iodide uptake in
848 thyrocytes. *J Endocrinol*, 206, 113-20.
- 849 DEANDREIS, D., RUBINO, C., TALA, H., LEBoulLEUX, S., TERROIR, M., BAUDIN, E., LARSON, S., FAGIN,
850 J. A., SCHLUMBERGER, M. & TUTTLE, R. M. 2017. Comparison of Empiric Versus Whole-
851 Body/-Blood Clearance Dosimetry-Based Approach to Radioactive Iodine Treatment in
852 Patients with Metastases from Differentiated Thyroid Cancer. *J Nucl Med*, 58, 717-722.
- 853 DUNN, L. A., SHERMAN, E. J., BAXI, S. S., TCHEKMEDYIAN, V., GREWAL, R. K., LARSON, S. M.,
854 PENTLOW, K. S., HAQUE, S., TUTTLE, R. M., SABRA, M. M., et al. 2019. Vemurafenib
855 Redifferentiation of BRAF Mutant, RAI-Refractory Thyroid Cancers. *J Clin Endocrinol Metab*,
856 104, 1417-1428.
- 857 DURANTE, C., HADDY, N., BAUDIN, E., LEBoulLEUX, S., HARTL, D., TRAVAGLI, J. P., CAILLOU, B.,
858 RICARD, M., LUMBROSO, J. D., DE VATHAIRE, F., et al. 2006. Long-term outcome of 444
859 patients with distant metastases from papillary and follicular thyroid carcinoma: benefits and
860 limits of radioiodine therapy. *J Clin Endocrinol Metab*, 91, 2892-9.
- 861 ELIAS, A. N. & LIZOTTE, P. 2006. Enhanced radioiodine uptake in a patient with poorly differentiated
862 papillary thyroid cancer after treatment with rosiglitazone. *Clin Nucl Med*, 31, 517-9.
- 863 ELMOKH, O., TAELMAN, V., RADOJEWSKI, P., ROELLI, M. A., STOSS, A., DUMONT, R. A., DETTMER, M.
864 S., PHILLIPS, W. A., WALTER, M. A. & CHARLES, R. P. 2019. MEK Inhibition Induces
865 Therapeutic Iodine Uptake in a Murine Model of Anaplastic Thyroid Cancer. *J Nucl Med*, 60,
866 917-923.
- 867 ELOLA, M., YOLDI, A., EMPARANZA, J. I., MATTEUCCI, T., BILBAO, I. & GOENA, M. 2011.
868 [Redifferentiation therapy with rosiglitazone in a case of differentiated thyroid cancer with
869 pulmonary metastases and absence of radioiodine uptake]. *Rev Esp Med Nucl*, 30, 241-3.
- 870 FENTON, M. S., MARION, K. M., SALEM, A. K., HOGEN, R., NAEIM, F. & HERSHMAN, J. M. 2010.
871 Sunitinib inhibits MEK/ERK and SAPK/JNK pathways and increases sodium/iodide symporter
872 expression in papillary thyroid cancer. *Thyroid*, 20, 965-74.
- 873 FERNANDEZ, C. A., PUIG-DOMINGO, M., LOMENA, F., ESTORCH, M., CAMACHO MARTI, V., BITTINI, A.
874 L., MARAZUELA, M., SANTAMARIA, J., CASTRO, J., MARTINEZ DE ICAYA, P., et al. 2009.
875 Effectiveness of retinoic acid treatment for redifferentiation of thyroid cancer in relation to
876 recovery of radioiodine uptake. *J Endocrinol Invest*, 32, 228-33.
- 877 FERRETTI, E., TOSI, E., PO, A., SCIPIONI, A., MORISI, R., ESPINOLA, M. S., RUSSO, D., DURANTE, C.,
878 SCHLUMBERGER, M., SCREPANTI, I., et al. 2008. Notch signaling is involved in expression of
879 thyrocyte differentiation markers and is down-regulated in thyroid tumors. *J Clin Endocrinol*
880 *Metab*, 93, 4080-7.
- 881 FLETCHER, A., READ, M. L., THORNTON, C. E. M., LARNER, D. P., POOLE, V. L., BROOKES, K., NIETO, H.
882 R., ALSHAHRANI, M., THOMPSON, R. J., LAVERY, G. G., et al. 2019. Targeting novel sodium
883 iodide symporter interactors ADP-ribosylation factor 4 (ARF4) and valosin-containing protein
884 (VCP) enhances radioiodine uptake. *Cancer Res*.
- 885 FROHLICH, E., BROSSART, P. & WAHL, R. 2009. Induction of iodide uptake in transformed thyrocytes:
886 a compound screening in cell lines. *Eur J Nucl Med Mol Imaging*, 36, 780-90.

- 887 FROHLICH, E., MACHICAO, F. & WAHL, R. 2005. Action of thiazolidinediones on differentiation,
888 proliferation and apoptosis of normal and transformed thyrocytes in culture. *Endocr Relat*
889 *Cancer*, 12, 291-303.
- 890 FU, H., CHENG, L., JIN, Y., CHENG, L., LIU, M. & CHEN, L. 2019. MAPK Inhibitors Enhance HDAC
891 Inhibitor-Induced Redifferentiation in Papillary Thyroid Cancer Cells Harboring BRAF (V600E):
892 An In Vitro Study. *Mol Ther Oncolytics*, 12, 235-245.
- 893 FURUYA, F., SHIMURA, H., SUZUKI, H., TAKI, K., OHTA, K., HARAGUCHI, K., ONAYA, T., ENDO, T. &
894 KOBAYASHI, T. 2004. Histone deacetylase inhibitors restore radioiodide uptake and retention
895 in poorly differentiated and anaplastic thyroid cancer cells by expression of the
896 sodium/iodide symporter thyroperoxidase and thyroglobulin. *Endocrinology*, 145, 2865-75.
- 897 GALRAO, A. L., CAMARGO, R. Y., FRIGUGLIETTI, C. U., MORAES, L., CERUTTI, J. M., SERRANO-
898 NASCIMENTO, C., SUZUKI, M. F., MEDEIROS-NETO, G. & RUBIO, I. G. 2014. Hypermethylation
899 of a New Distal Sodium/Iodide Symporter (NIS) enhancer (NDE) is associated with reduced
900 NIS expression in thyroid tumors. *J Clin Endocrinol Metab*, 99, E944-52.
- 901 GALRAO, A. L., SODRE, A. K., CAMARGO, R. Y., FRIGUGLIETTI, C. U., KULCSAR, M. A., LIMA, E. U.,
902 MEDEIROS-NETO, G. & RUBIO, I. G. 2013. Methylation levels of sodium-iodide symporter
903 (NIS) promoter in benign and malignant thyroid tumors with reduced NIS expression.
904 *Endocrine*, 43, 225-9.
- 905 GAO, X., WU, X., ZHANG, X., HUA, W., ZHANG, Y., MAIMAITI, Y., GAO, Z. & ZHANG, Y. 2016. Inhibition
906 of BRD4 suppresses tumor growth and enhances iodine uptake in thyroid cancer. *Biochem*
907 *Biophys Res Commun*, 469, 679-85.
- 908 GARCIA, B. & SANTISTEBAN, P. 2002. PI3K is involved in the IGF-I inhibition of TSH-induced
909 sodium/iodide symporter gene expression. *Mol Endocrinol*, 16, 342-52.
- 910 GIULIANI, C., NOGUCHI, Y., HARI, N., NAPOLITANO, G., TATONE, D., BUCCI, I., PIANTELLI, M.,
911 MONACO, F. & KOHN, L. D. 2008. The flavonoid quercetin regulates growth and gene
912 expression in rat FRTL-5 thyroid cells. *Endocrinology*, 149, 84-92.
- 913 GONCALVES, C. F., SANTOS, M. C., GINABREDA, M. G., FORTUNATO, R. S., CARVALHO, D. P. & FREITAS
914 FERREIRA, A. C. 2013. Flavonoid rutin increases thyroid iodide uptake in rats. *PLoS One*, 8,
915 e73908.
- 916 GONCALVES, C. F. L., DE FREITAS, M. L., FORTUNATO, R. S., MIRANDA-ALVES, L., CARVALHO, D. P. &
917 FERREIRA, A. C. F. 2018. Rutin Scavenges Reactive Oxygen Species, Inactivates 5'-Adenosine
918 Monophosphate-Activated Protein Kinase, and Increases Sodium-Iodide Symporter
919 Expression in Thyroid PCCL3 Cells. *Thyroid*, 28, 265-275.
- 920 GRUNING, T., TIEPOLT, C., ZOPHEL, K., BREDOW, J., KROPP, J. & FRANKE, W. G. 2003. Retinoic acid for
921 redifferentiation of thyroid cancer--does it hold its promise? *Eur J Endocrinol*, 148, 395-402.
- 922 GRUNWALD, F., SCHOMBURG, A., MENZEL, C., STEINECKER, S., SPATH, G., BOCKISCH, A., FIMMERS,
923 R., HOTZE, A. L. & BIERSACK, H. J. 1994. [Changes in the blood picture after radioiodine
924 therapy of thyroid cancer]. *Med Klin (Munich)*, 89, 522-8.
- 925 HABERKORN, V., OZIOL, L. & GOUDONNET, H. 2003. 9-cis-Retinoic acid regulation of four UGT
926 isoforms in hepatocytes from rats with various thyroid states. *Pharm Res*, 20, 1568-73.
- 927 HAGHPANAH, V., MALEHMIR, M., LARIJANI, B., AHMADIAN, S., ALIMOGHADDAM, K., HESHMAT, R.,
928 GHAVAMZADEH, A., ADABI, K. & GHAFFARI, S. H. 2014. The Beneficial Effects of Valproic Acid
929 in Thyroid Cancer Are Mediated through Promoting Redifferentiation and Reducing Stemness
930 Level: An In Vitro Study. *J Thyroid Res*, 2014, 218763.
- 931 HANDKIEWICZ-JUNAK, D., ROSKOSZ, J., HASSE-LAZAR, K., SZPAK-ULCZOK, S., PUCH, Z., KUKULSKA, A.,
932 OLCZYK, T., PIELA, A., PALICZKA-CIESLIK, E. & JARZAB, B. 2009. 13-cis-retinoic acid re-
933 differentiation therapy and recombinant human thyrotropin-aided radioiodine treatment of
934 non-Functional metastatic thyroid cancer: a single-center, 53-patient phase 2 study. *Thyroid*
935 *Res*, 2, 8.
- 936 HAUGEN, B. R., ALEXANDER, E. K., BIBLE, K. C., DOHERTY, G. M., MANDEL, S. J., NIKIFOROV, Y. E.,
937 PACINI, F., RANDOLPH, G. W., SAWKA, A. M., SCHLUMBERGER, M., et al. 2016. 2015
938 American Thyroid Association Management Guidelines for Adult Patients with Thyroid

- 939 Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines
940 Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid*, 26, 1-133.
- 941 HO, A. L., GREWAL, R. K., LEBOEUF, R., SHERMAN, E. J., PFISTER, D. G., DEANDREIS, D., PENTLOW, K.
942 S., ZANZONICO, P. B., HAQUE, S., GAVANE, S., et al. 2013. Selumetinib-enhanced radioiodine
943 uptake in advanced thyroid cancer. *N Engl J Med*, 368, 623-32.
- 944 HOFTIJZER, H., HEEMSTRA, K. A., MORREAU, H., STOKKEL, M. P., CORSSMIT, E. P., GELDERBLUM, H.,
945 WEIJERS, K., PEREIRA, A. M., HUIJBERTS, M., KAPITEIJN, E., et al. 2009. Beneficial effects of
946 sorafenib on tumor progression, but not on radioiodine uptake, in patients with
947 differentiated thyroid carcinoma. *Eur J Endocrinol*, 161, 923-31.
- 948 HOU, P., BOJDANI, E. & XING, M. 2010. Induction of thyroid gene expression and radioiodine uptake
949 in thyroid cancer cells by targeting major signaling pathways. *J Clin Endocrinol Metab*, 95,
950 820-8.
- 951 HUIILLARD, O., TENENBAUM, F., CLERC, J., GOLDWASSER, F. & GROUSSIN, L. 2015. Redifferentiation
952 of Iodine-Refractory BRAF V600E-Mutant Metastatic Papillary Thyroid Cancer with
953 Dabrafenib-Letter. *Clin Cancer Res*, 21, 5639.
- 954 HUIILLARD, O., TENENBAUM, F., CLERC, J., GOLDWASSER, F. & GROUSSIN, L. 2017. Restoring
955 Radioiodine Uptake in BRAF V600E-Mutated Papillary Thyroid Cancer. *J Endocr Soc*, 1, 285-
956 287.
- 957 IRAVANI, A., SOLOMON, B., PATTISON, D. A., JACKSON, P., RAVI KUMAR, A. S., KONG, G., HOFMAN,
958 M. S., AKHURST, T. & HICKS, R. J. 2019. Mitogen-activated protein kinase pathway inhibition
959 for re-differentiation of radioiodine-refractory differentiated thyroid cancer: an evolving
960 protocol. *Thyroid*.
- 961 JABER, T., WAGUESPACK, S. G., CABANILLAS, M. E., ELBANAN, M., VU, T., DADU, R., SHERMAN, S. I.,
962 AMIT, M., SANTOS, E. B., ZAFEREO, M., et al. 2018. Targeted Therapy in Advanced Thyroid
963 Cancer to Resensitize Tumors to Radioactive Iodine. *J Clin Endocrinol Metab*, 103, 3698-3705.
- 964 JANG, S., YU, X. M., ODORICO, S., CLARK, M., JASKULA-SZTUL, R., SCHIENEBECK, C. M., KUPCHO, K. R.,
965 HARRISON, A. D., WINSTON-MCPHERSON, G. N., TANG, W., et al. 2015. Novel analogs
966 targeting histone deacetylase suppress aggressive thyroid cancer cell growth and induce re-
967 differentiation. *Cancer Gene Ther*, 22, 410-6.
- 968 JEONG, H., KIM, Y. R., KIM, K. N., CHOE, J. G., CHUNG, J. K. & KIM, M. K. 2006. Effect of all-trans
969 retinoic acid on sodium/iodide symporter expression, radioiodine uptake and gene
970 expression profiles in a human anaplastic thyroid carcinoma cell line. *Nucl Med Biol*, 33, 875-
971 82.
- 972 KAWAGUCHI, A., IKEDA, M., ENDO, T., KOGAI, T., MIYAZAKI, A. & ONAYA, T. 1997. Transforming
973 growth factor-beta1 suppresses thyrotropin-induced Na⁺/I⁻ symporter messenger RNA and
974 protein levels in FRTL-5 rat thyroid cells. *Thyroid*, 7, 789-94.
- 975 KEBEBEW, E., LINDSAY, S., CLARK, O. H., WOEBER, K. A., HAWKINS, R. & GREENSPAN, F. S. 2009.
976 Results of rosiglitazone therapy in patients with thyroglobulin-positive and radioiodine-
977 negative advanced differentiated thyroid cancer. *Thyroid*, 19, 953-6.
- 978 KEBEBEW, E., PENG, M., REIFF, E., TRESELER, P., WOEBER, K. A., CLARK, O. H., GREENSPAN, F. S.,
979 LINDSAY, S., DUH, Q. Y. & MORITA, E. 2006. A phase II trial of rosiglitazone in patients with
980 thyroglobulin-positive and radioiodine-negative differentiated thyroid cancer. *Surgery*, 140,
981 960-6; discussion 966-7.
- 982 KELLY, W. K., O'CONNOR, O. A., KRUG, L. M., CHIAO, J. H., HEANEY, M., CURLEY, T., MACGREGORE-
983 CORTELLI, B., TONG, W., SECRIST, J. P., SCHWARTZ, L., et al. 2005. Phase I study of an oral
984 histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced
985 cancer. *J Clin Oncol*, 23, 3923-31.
- 986 KIM, J., SONG, J., JI, H. D., YOO, E. K., LEE, J. E., LEE, S. B., OH, J. M., LEE, S., HWANG, J. S., YOON, H., et
987 al. 2019. Discovery of Potent, Selective, and Orally Bioavailable Estrogen-Related Receptor-
988 gamma Inverse Agonists To Restore the Sodium Iodide Symporter Function in Anaplastic
989 Thyroid Cancer. *J Med Chem*, 62, 1837-1858.

- 990 KIM, T. H., YOO, Y. H., KANG, D. Y., SUH, H., PARK, M. K., PARK, K. J. & KIM, S. H. 2009. Efficacy on
991 anaplastic thyroid carcinoma of valproic acid alone or in combination with doxorubicin, a
992 synthetic chenodeoxycholic acid derivative, or lactacystin. *Int J Oncol*, 34, 1353-62.
- 993 KITAZONO, M., ROBEY, R., ZHAN, Z., SARLIS, N. J., SKARULIS, M. C., AIKOU, T., BATES, S. & FOJO, T.
994 2001. Low concentrations of the histone deacetylase inhibitor, depsipeptide (FR901228),
995 increase expression of the Na(+)/I(-) symporter and iodine accumulation in poorly
996 differentiated thyroid carcinoma cells. *J Clin Endocrinol Metab*, 86, 3430-5.
- 997 KOGAI, T., SAJID-CROCKETT, S., NEWMARCH, L. S., LIU, Y. Y. & BRENT, G. A. 2008. Phosphoinositide-3-
998 kinase inhibition induces sodium/iodide symporter expression in rat thyroid cells and human
999 papillary thyroid cancer cells. *J Endocrinol*, 199, 243-52.
- 1000 KUREBAYASHI, J., TANAKA, K., OTSUKI, T., MORIYA, T., KUNISUE, H., UNO, M. & SONOO, H. 2000. All-
1001 trans-retinoic acid modulates expression levels of thyroglobulin and cytokines in a new
1002 human poorly differentiated papillary thyroid carcinoma cell line, KTC-1. *J Clin Endocrinol*
1003 *Metab*, 85, 2889-96.
- 1004 LAKSHMANAN, A., DOSEFF, A. I., RINGEL, M. D., SAJI, M., ROUSSET, B., ZHANG, X. & JHIANG, S. M.
1005 2014. Apigenin in combination with Akt inhibition significantly enhances thyrotropin-
1006 stimulated radioiodide accumulation in thyroid cells. *Thyroid*, 24, 878-87.
- 1007 LAKSHMANAN, A., SCARBERRY, D., GREEN, J. A., ZHANG, X., SELMI-RUBY, S. & JHIANG, S. M. 2015.
1008 Modulation of thyroidal radioiodide uptake by oncological pipeline inhibitors and Apigenin.
1009 *Oncotarget*, 6, 31792-804.
- 1010 LAN, L., BASOURAKOS, S., CUI, D., ZUO, X., DENG, W., HUO, L., CHEN, L., ZHANG, G., DENG, L., SHI, B.,
1011 et al. 2017. Inhibiting beta-catenin expression promotes efficiency of radioiodine treatment
1012 in aggressive follicular thyroid cancer cells probably through mediating NIS localization. *Oncol*
1013 *Rep*, 37, 426-434.
- 1014 LANDA, I., IBRAHIMPASIC, T., BOUCAI, L., SINHA, R., KNAUF, J. A., SHAH, R. H., DOGAN, S., RICARTE-
1015 FILHO, J. C., KRISHNAMOORTHY, G. P., XU, B., et al. 2016. Genomic and transcriptomic
1016 hallmarks of poorly differentiated and anaplastic thyroid cancers. *J Clin Invest*, 126, 1052-66.
- 1017 LANDA, I., POZDEYEV, N., KORCH, C., MARLOW, L. A., SMALLRIDGE, R. C., COPLAND, J. A.,
1018 HENDERSON, Y. C., LAI, S. Y., CLAYMAN, G. L., ONODA, N., et al. 2019. Comprehensive
1019 Genetic Characterization of Human Thyroid Cancer Cell Lines: A Validated Panel for
1020 Preclinical Studies. *Clin Cancer Res*, 25, 3141-3151.
- 1021 LAVARONE, E., PUPPIN, C., PASSON, N., FILETTI, S., RUSSO, D. & DAMANTE, G. 2013. The PARP
1022 inhibitor PJ34 modifies proliferation, NIS expression and epigenetic marks in thyroid cancer
1023 cell lines. *Mol Cell Endocrinol*, 365, 1-10.
- 1024 LEBoulLEUX, S., DUPUY, C., LACROIX, L., ATTARD, M., GRIMALDI, S., CORRE, R., RICARD, M., NASR, S.,
1025 BERDELOU, A., HADOUX, J., et al. 2019. Redifferentiation of a BRAF(K601E)-Mutated Poorly
1026 Differentiated Thyroid Cancer Patient with Dabrafenib and Trametinib Treatment. *Thyroid*,
1027 29, 735-742.
- 1028 LEE, W. W., LEE, B., KIM, S. J., JIN, J., MOON, D. H. & LEE, H. 2003. Kinetics of iodide uptake and efflux
1029 in various human thyroid cancer cells by expressing sodium iodide symporter gene via a
1030 recombinant adenovirus. *Oncol Rep*, 10, 845-9.
- 1031 LIN, J. T., WU, M. S., WANG, W. S., YEN, C. C., CHIOU, T. J., LIU, J. H., YANG, M. H., CHAO, T. C., CHOU,
1032 S. C. & CHEN, P. M. 2003. All-trans retinoid acid increases Notch1 transcript expression in
1033 acute promyelocytic leukemia. *Adv Ther*, 20, 337-43.
- 1034 LIN, X., FISCHER, A. H., RYU, K. Y., CHO, J. Y., SFERRA, T. J., KLOOS, R. T., MAZZAFERRI, E. L. & JHIANG,
1035 S. M. 2004. Application of the Cre/loxP system to enhance thyroid-targeted expression of
1036 sodium/iodide symporter. *J Clin Endocrinol Metab*, 89, 2344-50.
- 1037 LIU, J., LIU, R., SHEN, X., ZHU, G., LI, B. & XING, M. 2019. The Genetic Duet of BRAF V600E and TERT
1038 Promoter Mutations Robustly Predicts the Loss of Radioiodine Avidity in Recurrent Papillary
1039 Thyroid Cancer. *J Nucl Med*.

- 1040 LIU, L., LI, D., CHEN, Z., YANG, J., MA, Y., CAI, H., SHAN, C., LV, Z. & ZHANG, X. 2017. Wild-Type P53
1041 Induces Sodium/Iodide Symporter Expression Allowing Radioiodide Therapy in Anaplastic
1042 Thyroid Cancer. *Cell Physiol Biochem*, 43, 905-914.
- 1043 LIU, Y. Y., STOKKEL, M. P., PEREIRA, A. M., CORSSMIT, E. P., MORREAU, H. A., ROMIJN, J. A. & SMIT, J.
1044 W. 2006a. Bexarotene increases uptake of radioiodide in metastases of differentiated thyroid
1045 carcinoma. *Eur J Endocrinol*, 154, 525-31.
- 1046 LIU, Y. Y., VAN DER PLUIJM, G., KARPERIEN, M., STOKKEL, M. P., PEREIRA, A. M., MORREAU, J., KIEVIT,
1047 J., ROMIJN, J. A. & SMIT, J. W. 2006b. Lithium as adjuvant to radioiodine therapy in
1048 differentiated thyroid carcinoma: clinical and in vitro studies. *Clin Endocrinol (Oxf)*, 64, 617-
1049 24.
- 1050 LIU, Y. Y., ZHANG, X., RINGEL, M. D. & JHIANG, S. M. 2012. Modulation of sodium iodide symporter
1051 expression and function by LY294002, Akti-1/2 and Rapamycin in thyroid cells. *Endocr Relat*
1052 *Cancer*, 19, 291-304.
- 1053 LOPEZ-CAMPISTROUS, A., ADEWUYI, E. E., BENESCH, M. G. K., KO, Y. M., LAI, R., THIESEN, A.,
1054 DEWALD, J., WANG, P., CHU, K., GHOSH, S., et al. 2016. PDGFRalpha Regulates Follicular Cell
1055 Differentiation Driving Treatment Resistance and Disease Recurrence in Papillary Thyroid
1056 Cancer. *EBioMedicine*, 12, 86-97.
- 1057 LUSTER, M., AKTOLUN, C., AMENDOEIRA, I., BARCZYNSKI, M., BIBLE, K. C., DUNTAS, L. H., ELISEI, R.,
1058 HANDKIEWICZ-JUNAK, D., HOFFMANN, M., JARZAB, B., et al. 2019. European Perspective on
1059 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid
1060 Nodules and Differentiated Thyroid Cancer: Proceedings of an Interactive International
1061 Symposium. *Thyroid*, 29, 7-26.
- 1062 MARSEE, D. K., VENKATESWARAN, A., TAO, H., VADYSIRISACK, D., ZHANG, Z., VANDRE, D. D. &
1063 JHIANG, S. M. 2004. Inhibition of heat shock protein 90, a novel RET/PTC1-associated
1064 protein, increases radioiodide accumulation in thyroid cells. *J Biol Chem*, 279, 43990-7.
- 1065 MASSIMINO, M., TIRRO, E., STELLA, S., FRASCA, F., VELLA, V., SCIACCA, L., PENNISI, M. S., VITALE, S.
1066 R., PUMA, A., ROMANO, C., et al. 2018. Effect of Combined Epigenetic Treatments and
1067 Ectopic NIS Expression on Undifferentiated Thyroid Cancer Cells. *Anticancer Res*, 38, 6653-
1068 6662.
- 1069 MONTERO-CONDE, C., RUIZ-LLORENTE, S., DOMINGUEZ, J. M., KNAUF, J. A., VIALE, A., SHERMAN, E.
1070 J., RYDER, M., GHOSSEIN, R. A., ROSEN, N. & FAGIN, J. A. 2013. Relief of feedback inhibition
1071 of HER3 transcription by RAF and MEK inhibitors attenuates their antitumor effects in BRAF-
1072 mutant thyroid carcinomas. *Cancer Discov*, 3, 520-33.
- 1073 MU, D., HUANG, R., LI, S., MA, X., LOU, C. & KUANG, A. 2012. Combining transfer of TTF-1 and Pax-8
1074 gene: a potential strategy to promote radioiodine therapy of thyroid carcinoma. *Cancer Gene*
1075 *Ther*, 19, 402-11.
- 1076 NAGARAJAH, J., LE, M., KNAUF, J. A., FERRANDINO, G., MONTERO-CONDE, C., PILLARSETTY, N.,
1077 BOLAENDER, A., IRWIN, C., KRISHNAMOORTHY, G. P., SAQCENA, M., et al. 2016. Sustained
1078 ERK inhibition maximizes responses of BrafV600E thyroid cancers to radioiodine. *J Clin Invest*,
1079 126, 4119-4124.
- 1080 NICOLUSSI, A., D'INZEO, S., SANTULLI, M., COLLETTA, G. & COPPA, A. 2003. TGF-beta control of rat
1081 thyroid follicular cells differentiation. *Mol Cell Endocrinol*, 207, 1-11.
- 1082 NILUBOL, N., MERKEL, R., YANG, L., PATEL, D., REYNOLDS, J. C., SADOWSKI, S. M., NEYCHEV, V. &
1083 KEBEBEW, E. 2017. A phase II trial of valproic acid in patients with advanced, radioiodine-
1084 resistant thyroid cancers of follicular cell origin. *Clin Endocrinol (Oxf)*, 86, 128-133.
- 1085 OH, J. M., KALIMUTHU, S., GANGADARAN, P., BAEK, S. H., ZHU, L., LEE, H. W., RAJENDRAN, R. L.,
1086 HONG, C. M., JEONG, S. Y., LEE, S. W., et al. 2018. Reverting iodine avidity of radioactive-
1087 iodine refractory thyroid cancer with a new tyrosine kinase inhibitor (K905-0266) excavated
1088 by high-throughput NIS (sodium iodide symporter) enhancer screening platform using dual
1089 reporter gene system. *Oncotarget*, 9, 7075-7087.

- 1090 OH, S. W., MOON, S. H., PARK, D. J., CHO, B. Y., JUNG, K. C., LEE, D. S. & CHUNG, J. K. 2011. Combined
1091 therapy with ¹³¹I and retinoic acid in Korean patients with radioiodine-refractory papillary
1092 thyroid cancer. *Eur J Nucl Med Mol Imaging*, 38, 1798-805.
- 1093 PAK, K., SHIN, S., KIM, S. J., KIM, I. J., CHANG, S., KOO, P., KWAK, J. & KIM, J. H. 2018. Response of
1094 Retinoic Acid in Patients with Radioactive Iodine-Refractory Thyroid Cancer: A Meta-Analysis.
1095 *Oncol Res Treat*, 41, 100-104.
- 1096 PARK, J. W., ZARNEGAR, R., KANAUCHI, H., WONG, M. G., HYUN, W. C., GINZINGER, D. G., LOBO, M.,
1097 COTTER, P., DUH, Q. Y. & CLARK, O. H. 2005. Troglitazone, the peroxisome proliferator-
1098 activated receptor-gamma agonist, induces antiproliferation and redifferentiation in human
1099 thyroid cancer cell lines. *Thyroid*, 15, 222-31.
- 1100 PATEL, P. N., YU, X. M., JASKULA-SZTUL, R. & CHEN, H. 2014. Hesperetin activates the Notch1
1101 signaling cascade, causes apoptosis, and induces cellular differentiation in anaplastic thyroid
1102 cancer. *Ann Surg Oncol*, 21 Suppl 4, S497-504.
- 1103 PHILIPS, J. C., PETITE, C., WILLI, J. P., BUCHEGGER, F. & MEIER, C. A. 2004. Effect of peroxisome
1104 proliferator-activated receptor gamma agonist, rosiglitazone, on dedifferentiated thyroid
1105 cancers. *Nucl Med Commun*, 25, 1183-6.
- 1106 PLANTINGA, T. S., HEINHUIS, B., GERRITS, D., NETEA, M. G., JOOSTEN, L. A., HERMUS, A. R., OYEN, W.
1107 J., SCHWEPPE, R. E., HAUGEN, B. R., BOERMAN, O. C., et al. 2014. mTOR Inhibition promotes
1108 TTF1-dependent redifferentiation and restores iodine uptake in thyroid carcinoma cell lines. *J*
1109 *Clin Endocrinol Metab*, 99, E1368-75.
- 1110 PLANTINGA, T. S., TESSELAAR, M. H., MORREAU, H., CORSSMIT, E. P., WILLEMSSEN, B. K., KUSTERS, B.,
1111 VAN ENGEN-VAN GRUNSVEN, A. C., SMIT, J. W. & NETEA-MAIER, R. T. 2016. Autophagy
1112 activity is associated with membranous sodium iodide symporter expression and clinical
1113 response to radioiodine therapy in non-medullary thyroid cancer. *Autophagy*, 12, 1195-205.
- 1114 PROVENZANO, M. J., FITZGERALD, M. P., KRAGER, K. & DOMANN, F. E. 2007. Increased iodine uptake
1115 in thyroid carcinoma after treatment with sodium butyrate and decitabine (5-Aza-dC).
1116 *Otolaryngol Head Neck Surg*, 137, 722-8.
- 1117 PUGLIESE, M., FORTUNATI, N., GERMANO, A., ASIOLI, S., MARANO, F., PALESTINI, N., FRAIRIA, R.,
1118 BOCCUZZI, G. & CATALANO, M. G. 2013. Histone deacetylase inhibition affects sodium iodide
1119 symporter expression and induces ¹³¹I cytotoxicity in anaplastic thyroid cancer cells.
1120 *Thyroid*, 23, 838-46.
- 1121 PUPPIN, C., D'AURIZIO, F., D'ELIA, A. V., CESARATTO, L., TELL, G., RUSSO, D., FILETTI, S., FERRETTI, E.,
1122 TOSI, E., MATTEI, T., et al. 2005. Effects of histone acetylation on sodium iodide symporter
1123 promoter and expression of thyroid-specific transcription factors. *Endocrinology*, 146, 3967-
1124 74.
- 1125 PUPPIN, C., PASSON, N., HERSHMAN, J., FILETTI, S., BULOTTA, S., CELANO, M., RUSSO, D. &
1126 DAMANTE, G. 2012. Cooperative effects of SAHA and VPA on NIS gene expression and
1127 proliferation of thyroid cancer cells. *Journal of Molecular Endocrinology*, 48, 217-227.
- 1128 RAMAN, P. & KOENIG, R. J. 2014. Pax-8-PPAR-gamma fusion protein in thyroid carcinoma. *Nat Rev*
1129 *Endocrinol*, 10, 616-23.
- 1130 READ, M. L., LEWY, G. D., FONG, J. C., SHARMA, N., SEED, R. I., SMITH, V. E., GENTILIN, E., WARFIELD,
1131 A., EGGO, M. C., KNAUF, J. A., et al. 2011. Proto-oncogene PBF/PTTG1IP regulates thyroid cell
1132 growth and represses radioiodide treatment. *Cancer Res*, 71, 6153-64.
- 1133 RIESCO-EIZAGUIRRE, G., DE LA VIEJA, A., RODRIGUEZ, I., MIRANDA, S., MARTIN-DUQUE, P., VASSAUX,
1134 G. & SANTISTEBAN, P. 2011. Telomerase-driven expression of the sodium iodide symporter
1135 (NIS) for in vivo radioiodide treatment of cancer: a new broad-spectrum NIS-mediated
1136 antitumor approach. *J Clin Endocrinol Metab*, 96, E1435-43.
- 1137 RIESCO-EIZAGUIRRE, G., GUTIERREZ-MARTINEZ, P., GARCIA-CABEZAS, M. A., NISTAL, M. &
1138 SANTISTEBAN, P. 2006. The oncogene BRAF V600E is associated with a high risk of recurrence
1139 and less differentiated papillary thyroid carcinoma due to the impairment of Na⁺/I⁻ targeting
1140 to the membrane. *Endocr Relat Cancer*, 13, 257-69.

- 1141 ROSENBAUM-KRUMME, S. J., FREUDENBERG, L. S., JENTZEN, W., BOCKISCH, A. & NAGARAJAH, J.
1142 2012. Effects of rosiglitazone on radioiodine negative and progressive differentiated thyroid
1143 carcinoma as assessed by (1)(2)(4)I PET/CT imaging. *Clin Nucl Med*, 37, e47-52.
- 1144 ROTHENBERG, S. M., DANIELS, G. H. & WIRTH, L. J. 2015. Redifferentiation of Iodine-Refractory BRAF
1145 V600E-Mutant Metastatic Papillary Thyroid Cancer with Dabrafenib-Response. *Clin Cancer*
1146 *Res*, 21, 5640-1.
- 1147 RUAN, M., LIU, M., DONG, Q. & CHEN, L. 2015. Iodide- and glucose-handling gene expression
1148 regulated by sorafenib or cabozantinib in papillary thyroid cancer. *J Clin Endocrinol Metab*,
1149 100, 1771-9.
- 1150 RUSSO, D., DURANTE, C., BULOTTA, S., PUPPIN, C., PUXEDDU, E., FILETTI, S. & DAMANTE, G. 2013.
1151 Targeting histone deacetylase in thyroid cancer. *Expert Opin Ther Targets*, 17, 179-93.
- 1152 SALEM, A. K., FENTON, M. S., MARION, K. M. & HERSHMAN, J. M. 2008. Effect of sunitinib on growth
1153 and function of FRTL-5 thyroid cells. *Thyroid*, 18, 631-5.
- 1154 SCHMOHL, K. A., DOLP, P., SCHUG, C., KNOOP, K., KLUTZ, K., SCHWENK, N., BARTENSTEIN, P.,
1155 NELSON, P. J., OGRIS, M., WAGNER, E., et al. 2017. Reintroducing the Sodium-Iodide
1156 Symporter to Anaplastic Thyroid Carcinoma. *Thyroid*, 27, 1534-1543.
- 1157 SCHMUTZLER, C. & KOHRLE, J. 2000. Retinoic acid redifferentiation therapy for thyroid cancer.
1158 *Thyroid*, 10, 393-406.
- 1159 SCHMUTZLER, C., SCHMITT, T. L., GLASER, F., LOOS, U. & KOHRLE, J. 2002. The promoter of the
1160 human sodium/iodide-symporter gene responds to retinoic acid. *Mol Cell Endocrinol*, 189,
1161 145-55.
- 1162 SCHRECK, R., SCHNIEDERS, F., SCHMUTZLER, C. & KOHRLE, J. 1994. Retinoids stimulate type I
1163 iodothyronine 5'-deiodinase activity in human follicular thyroid carcinoma cell lines. *J Clin*
1164 *Endocrinol Metab*, 79, 791-8.
- 1165 SCHWEPPE, R. E., KLOPPER, J. P., KORCH, C., PUGAZHENTHI, U., BENEZRA, M., KNAUF, J. A., FAGIN, J.
1166 A., MARLOW, L. A., COPLAND, J. A., SMALLRIDGE, R. C., et al. 2008. Deoxyribonucleic acid
1167 profiling analysis of 40 human thyroid cancer cell lines reveals cross-contamination resulting
1168 in cell line redundancy and misidentification. *J Clin Endocrinol Metab*, 93, 4331-41.
- 1169 SHEN, W. T., WONG, T. S., CHUNG, W. Y., WONG, M. G., KEBEBEW, E., DUH, Q. Y. & CLARK, O. H.
1170 2005. Valproic acid inhibits growth, induces apoptosis, and modulates apoptosis-regulatory
1171 and differentiation gene expression in human thyroid cancer cells. *Surgery*, 138, 979-84;
1172 discussion 984-5.
- 1173 SHERMAN, E. J., SU, Y. B., LYALL, A., SCHODER, H., FURY, M. G., GHOSSEIN, R. A., HAQUE, S., LISA, D.,
1174 SHAHA, A. R., TUTTLE, R. M., et al. 2013. Evaluation of romidepsin for clinical activity and
1175 radioactive iodine reuptake in radioactive iodine-refractory thyroid carcinoma. *Thyroid*, 23,
1176 593-9.
- 1177 SHORT, S. C., SUOVUORI, A., COOK, G., VIVIAN, G. & HARMER, C. 2004. A phase II study using
1178 retinoids as redifferentiation agents to increase iodine uptake in metastatic thyroid cancer.
1179 *Clin Oncol (R Coll Radiol)*, 16, 569-74.
- 1180 SIMON, D., KOHRLE, J., SCHMUTZLER, C., MAINZ, K., REINERS, C. & ROHER, H. D. 1996.
1181 Redifferentiation therapy of differentiated thyroid carcinoma with retinoic acid: basics and
1182 first clinical results. *Exp Clin Endocrinol Diabetes*, 104 Suppl 4, 13-5.
- 1183 SIMON, D., KORBER, C., KRAUSCH, M., SEGERING, J., GROTH, P., GORGES, R., GRUNWALD, F.,
1184 MULLER-GARTNER, H. W., SCHMUTZLER, C., KOHRLE, J., et al. 2002. Clinical impact of
1185 retinoids in redifferentiation therapy of advanced thyroid cancer: final results of a pilot
1186 study. *Eur J Nucl Med Mol Imaging*, 29, 775-82.
- 1187 SINGH, T. D., JEONG, S. Y., LEE, S. W., HA, J. H., LEE, I. K., KIM, S. H., KIM, J., CHO, S. J., AHN, B. C., LEE,
1188 J., et al. 2015. Inverse Agonist of Estrogen-Related Receptor gamma Enhances Sodium Iodide
1189 Symporter Function Through Mitogen-Activated Protein Kinase Signaling in Anaplastic
1190 Thyroid Cancer Cells. *J Nucl Med*, 56, 1690-6.
- 1191 SINGH, T. D., SONG, J., KIM, J., CHIN, J., JI, H. D., LEE, J. E., LEE, S. B., YOON, H., YU, J. H., KIM, S. K., et
1192 al. 2019. A Novel Orally Active Inverse Agonist of Estrogen-related Receptor Gamma

- 1193 (ERRgamma), DN200434, A Booster of NIS in Anaplastic Thyroid Cancer. *Clin Cancer Res*, 25,
1194 5069-5081.
- 1195 SMIT, J. W., SCHRODER-VAN DER ELST, J. P., KARPERIEN, M., QUE, I., STOKKEL, M., VAN DER HEIDE, D.
1196 & ROMIJN, J. A. 2002. Iodide kinetics and experimental (¹³¹I) therapy in a xenotransplanted
1197 human sodium-iodide symporter-transfected human follicular thyroid carcinoma cell line. *J*
1198 *Clin Endocrinol Metab*, 87, 1247-53.
- 1199 SMIT, J. W., SHRODER-VAN DER ELST, J. P., KARPERIEN, M., QUE, I., VAN DER PLUIJM, G., GOSLINGS,
1200 B., ROMIJN, J. A. & VAN DER HEIDE, D. 2000. Reestablishment of in vitro and in vivo iodide
1201 uptake by transfection of the human sodium iodide symporter (hNIS) in a hNIS defective
1202 human thyroid carcinoma cell line. *Thyroid*, 10, 939-43.
- 1203 SMITH, J. A., FAN, C. Y., ZOU, C., BODENNER, D. & KOKOSKA, M. S. 2007. Methylation status of genes
1204 in papillary thyroid carcinoma. *Arch Otolaryngol Head Neck Surg*, 133, 1006-11.
- 1205 SMITH, V. E., FRANKLYN, J. A. & MCCABE, C. J. 2011. Expression and function of the novel proto-
1206 oncogene PBF in thyroid cancer: a new target for augmenting radioiodine uptake. *J*
1207 *Endocrinol*, 210, 157-63.
- 1208 SMITH, V. E., READ, M. L., TURNELL, A. S., WATKINS, R. J., WATKINSON, J. C., LEWY, G. D., FONG, J. C.,
1209 JAMES, S. R., EGGO, M. C., BOELAERT, K., et al. 2009. A novel mechanism of sodium iodide
1210 symporter repression in differentiated thyroid cancer. *J Cell Sci*, 122, 3393-402.
- 1211 SMITH, V. E., SHARMA, N., WATKINS, R. J., READ, M. L., RYAN, G. A., KWAN, P. P., MARTIN, A.,
1212 WATKINSON, J. C., BOELAERT, K., FRANKLYN, J. A., et al. 2013. Manipulation of PBF/PTTG1IP
1213 phosphorylation status; a potential new therapeutic strategy for improving radioiodine
1214 uptake in thyroid and other tumors. *J Clin Endocrinol Metab*, 98, 2876-86.
- 1215 SOMNAY, Y. R., YU, X. M., LLOYD, R. V., LEVERSON, G., ABURJANIA, Z., JANG, S., JASKULA-SZTUL, R. &
1216 CHEN, H. 2017. Notch3 expression correlates with thyroid cancer differentiation, induces
1217 apoptosis, and predicts disease prognosis. *Cancer*, 123, 769-782.
- 1218 SONG, J., QIU, W., DENG, X., QIU, Z., FAN, Y. & YANG, Z. 2018. A somatic mutation of RasGRP3
1219 decreases Na(+)/I(-) symporter expression in metastases of radioactive iodine-refractory
1220 thyroid cancer by stimulating the Akt signaling pathway. *Am J Cancer Res*, 8, 1847-1855.
- 1221 SPITZWEG, C. 2009. Gene therapy in thyroid cancer. *Horm Metab Res*, 41, 500-9.
- 1222 STEPHEN, J. K., CHITALE, D., NARRA, V., CHEN, K. M., SAWHNEY, R. & WORSHAM, M. J. 2011. DNA
1223 methylation in thyroid tumorigenesis. *Cancers (Basel)*, 3, 1732-43.
- 1224 TAVARES, C., COELHO, M. J., ELOY, C., MELO, M., DA ROCHA, A. G., PESTANA, A., BATISTA, R.,
1225 FERREIRA, L. B., RIOS, E., SELMI-RUBY, S., et al. 2018. NIS expression in thyroid tumors,
1226 relation with prognosis clinicopathological and molecular features. *Endocr Connect*, 7, 78-90.
- 1227 TEPMONGKOL, S., KEELAWAT, S., HONSAWEK, S. & RUANGVEJVORACHAI, P. 2008. Rosiglitazone
1228 effect on radioiodine uptake in thyroid carcinoma patients with high thyroglobulin but
1229 negative total body scan: a correlation with the expression of peroxisome proliferator-
1230 activated receptor-gamma. *Thyroid*, 18, 697-704.
- 1231 TESSELAAR, M. H., CREZEE, T., SCHUURMANS, I., GERRITS, D., NAGARAJAH, J., BOERMAN, O. C., VAN
1232 ENGEN-VAN GRUNSVEN, I., SMIT, J. W. A., NETEA-MAIER, R. T. & PLANTINGA, T. S. 2018.
1233 Digitalislike Compounds Restore hNIS Expression and Iodide Uptake Capacity in Anaplastic
1234 Thyroid Cancer. *J Nucl Med*, 59, 780-786.
- 1235 TESSELAAR, M. H., CREZEE, T., SWARTS, H. G., GERRITS, D., BOERMAN, O. C., KOENDERINK, J. B.,
1236 STUNNENBERG, H. G., NETEA, M. G., SMIT, J. W., NETEA-MAIER, R. T., et al. 2017. Digitalis-
1237 like Compounds Facilitate Non-Medullary Thyroid Cancer Redifferentiation through
1238 Intracellular Ca²⁺, FOS, and Autophagy-Dependent Pathways. *Mol Cancer Ther*, 16, 169-181.
- 1239 THOMPSON, R. J., FLETCHER, A., BROOKES, K., NIETO, H., ALSHAHRANI, M. M., MUELLER, J. W., FINE,
1240 N. H. F., HODSON, D. J., BOELAERT, K., READ, M. L., et al. 2019. Dimerization of the
1241 Sodium/Iodide Symporter. *Thyroid*.
- 1242 TUNCEL, M., AYDIN, D., YAMAN, E., TAZEBAY, U. H., GUC, D., DOGAN, A. L., TASBASAN, B. & UGUR, O.
1243 2007. The comparative effects of gene modulators on thyroid-specific genes and radioiodine
1244 uptake. *Cancer Biother Radiopharm*, 22, 281-8.

- 1245 VADYSIRISACK, D. D., VENKATESWARAN, A., ZHANG, Z. & JHIANG, S. M. 2007. MEK signaling
1246 modulates sodium iodide symporter at multiple levels and in a paradoxical manner. *Endocr*
1247 *Relat Cancer*, 14, 421-32.
- 1248 VAN HERLE, A. J., AGATEP, M. L., PADUA, D. N., 3RD, TOTANES, T. L., CANLAPAN, D. V., VAN HERLE, H.
1249 M. & JUILLARD, G. J. 1990. Effects of 13 cis-retinoic acid on growth and differentiation of
1250 human follicular carcinoma cells (UCLA R0 82 W-1) in vitro. *J Clin Endocrinol Metab*, 71, 755-
1251 63.
- 1252 VELLA, V., NICOLOSI, M. L., CANTAFIO, P., MASSIMINO, M., LAPPANO, R., VIGNERI, P., CIUNI, R.,
1253 GANGEMI, P., MORRIONE, A., MALAGUARNERA, R., et al. 2019. DDR1 regulates thyroid
1254 cancer cell differentiation via IGF-2/IR-A autocrine signaling loop. *Endocr Relat Cancer*, 26,
1255 197-214.
- 1256 VENKATARAMAN, G. M., YATIN, M., MARCINEK, R. & AIN, K. B. 1999. Restoration of iodide uptake in
1257 dedifferentiated thyroid carcinoma: relationship to human Na⁺/I⁻-symporter gene
1258 methylation status. *J Clin Endocrinol Metab*, 84, 2449-57.
- 1259 WACHTER, S., DAMANAKIS, A. I., ELXNAT, M., ROTH, S., WUNDERLICH, A., VERBURG, F. A.,
1260 FELLINGER, S. A., BARTSCH, D. K. & DI FAZIO, P. 2018a. Epigenetic Modifications in Thyroid
1261 Cancer Cells Restore NIS and Radio-Iodine Uptake and Promote Cell Death. *J Clin Med*, 7.
- 1262 WACHTER, S., WUNDERLICH, A., GREENE, B. H., ROTH, S., ELXNAT, M., FELLINGER, S. A., VERBURG, F.
1263 A., LUSTER, M., BARTSCH, D. K. & DI FAZIO, P. 2018b. Selumetinib Activity in Thyroid Cancer
1264 Cells: Modulation of Sodium Iodide Symporter and Associated miRNAs. *Int J Mol Sci*, 19.
- 1265 WACHTER, S., WUNDERLICH, A., ROTH, S., MINTZIRAS, I., MAURER, E., HOFFMANN, S., VERBURG, F.
1266 A., FELLINGER, S. A., HOLZER, K., BARTSCH, D. K., et al. 2018c. Individualised Multimodal
1267 Treatment Strategies for Anaplastic and Poorly Differentiated Thyroid Cancer. *J Clin Med*, 7.
- 1268 WASSERMANN, J., BERNIER, M. O., SPANO, J. P., LEPOUTRE-LUSSEY, C., BUFFET, C., SIMON, J. M.,
1269 MENEGAUX, F., TISSIER, F., LEBAN, M. & LEENHARDT, L. 2016. Outcomes and Prognostic
1270 Factors in Radioiodine Refractory Differentiated Thyroid Carcinomas. *Oncologist*, 21, 50-8.
- 1271 WEYEMI, U., CAILLOU, B., TALBOT, M., AMEZIANE-EL-HASSANI, R., LACROIX, L., LAGENT-CHEVALLIER,
1272 O., AL GHUZLAN, A., ROOS, D., BIDART, J. M., VIRION, A., et al. 2010. Intracellular expression
1273 of reactive oxygen species-generating NADPH oxidase NOX4 in normal and cancer thyroid
1274 tissues. *Endocr Relat Cancer*, 17, 27-37.
- 1275 XIAO, X., NING, L. & CHEN, H. 2009. Notch1 mediates growth suppression of papillary and follicular
1276 thyroid cancer cells by histone deacetylase inhibitors. *Mol Cancer Ther*, 8, 350-6.
- 1277 XING, M., USADEL, H., COHEN, Y., TOKUMARU, Y., GUO, Z., WESTRA, W. B., TONG, B. C., TALLINI, G.,
1278 UDELSMAN, R., CALIFANO, J. A., et al. 2003. Methylation of the thyroid-stimulating hormone
1279 receptor gene in epithelial thyroid tumors: a marker of malignancy and a cause of gene
1280 silencing. *Cancer Res*, 63, 2316-21.
- 1281 XU, J. & HERSHMAN, J. M. 2006. Histone deacetylase inhibitor depsipeptide represses nicotinamide
1282 N-methyltransferase and hepatocyte nuclear factor-1beta gene expression in human
1283 papillary thyroid cancer cells. *Thyroid*, 16, 151-60.
- 1284 YANG, X., LI, J., LI, X., LIANG, Z., GAO, W., LIANG, J., CHENG, S. & LIN, Y. 2017. TERT Promoter
1285 Mutation Predicts Radioiodine-Refractory Character in Distant Metastatic Differentiated
1286 Thyroid Cancer. *J Nucl Med*, 58, 258-265.
- 1287 YU, X. M., JASKULA-SZTUL, R., AHMED, K., HARRISON, A. D., KUNNIMALAIYAAN, M. & CHEN, H. 2013.
1288 Resveratrol induces differentiation markers expression in anaplastic thyroid carcinoma via
1289 activation of Notch1 signaling and suppresses cell growth. *Mol Cancer Ther*, 12, 1276-87.
- 1290 ZARNEGAR, R., BRUNAUD, L., KANAUCHI, H., WONG, M., FUNG, M., GINZINGER, D., DUH, Q. Y. &
1291 CLARK, O. H. 2002. Increasing the effectiveness of radioactive iodine therapy in the
1292 treatment of thyroid cancer using Trichostatin A, a histone deacetylase inhibitor. *Surgery*,
1293 132, 984-90; discussion 990.
- 1294 ZHANG, H. & CHEN, D. 2018. Synergistic inhibition of MEK/ERK and BRAF V600E with PD98059 and
1295 PLX4032 induces sodium/iodide symporter (NIS) expression and radioiodine uptake in BRAF
1296 mutated papillary thyroid cancer cells. *Thyroid Res*, 11, 13.

1297 ZHANG, Y., JIA, S., LIU, Y., LI, B., WANG, Z., LU, H. & ZHU, C. 2007. A clinical study of all-trans-retinoid-
1298 induced differentiation therapy of advanced thyroid cancer. *Nucl Med Commun*, 28, 251-5.
1299 ZHANG, Z., LIU, D., MURUGAN, A. K., LIU, Z. & XING, M. 2014. Histone deacetylation of NIS promoter
1300 underlies BRAF V600E-promoted NIS silencing in thyroid cancer. *Endocr Relat Cancer*, 21,
1301 161-73.

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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.

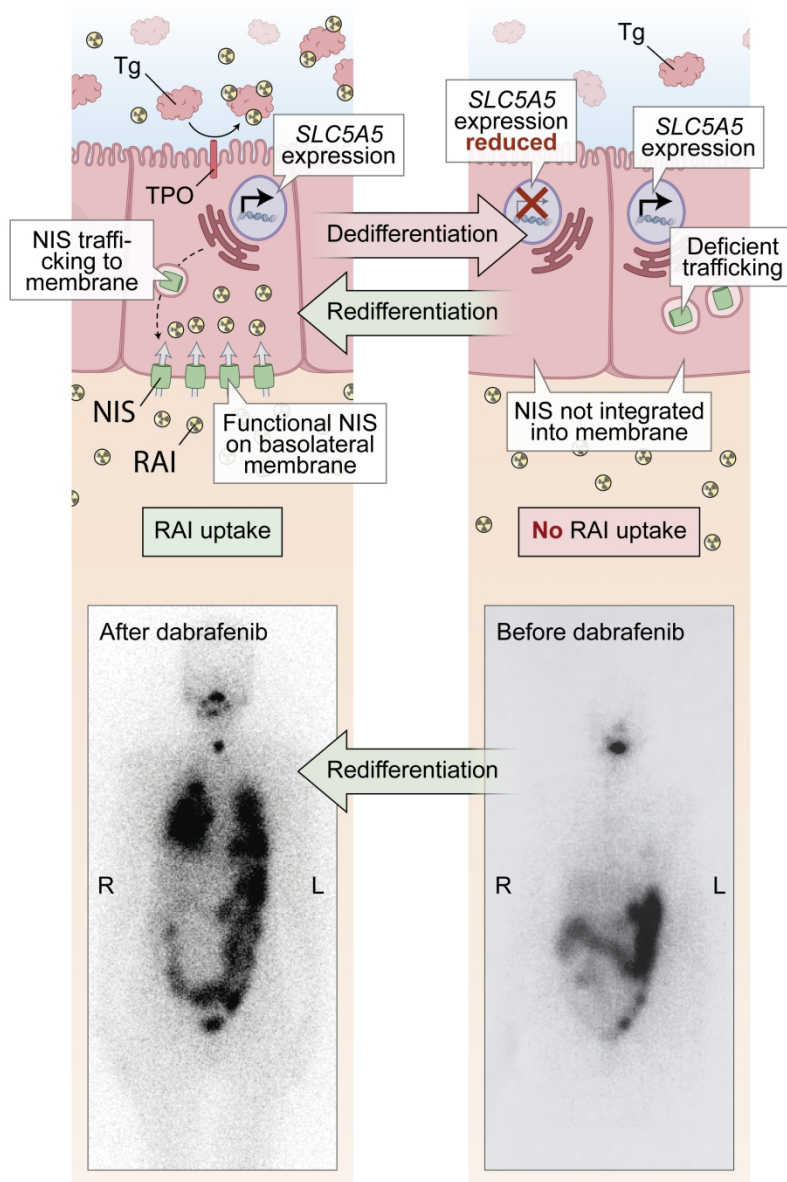


Figure 1 Differentiation and redifferentiation of thyroid cancer schematic.

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).

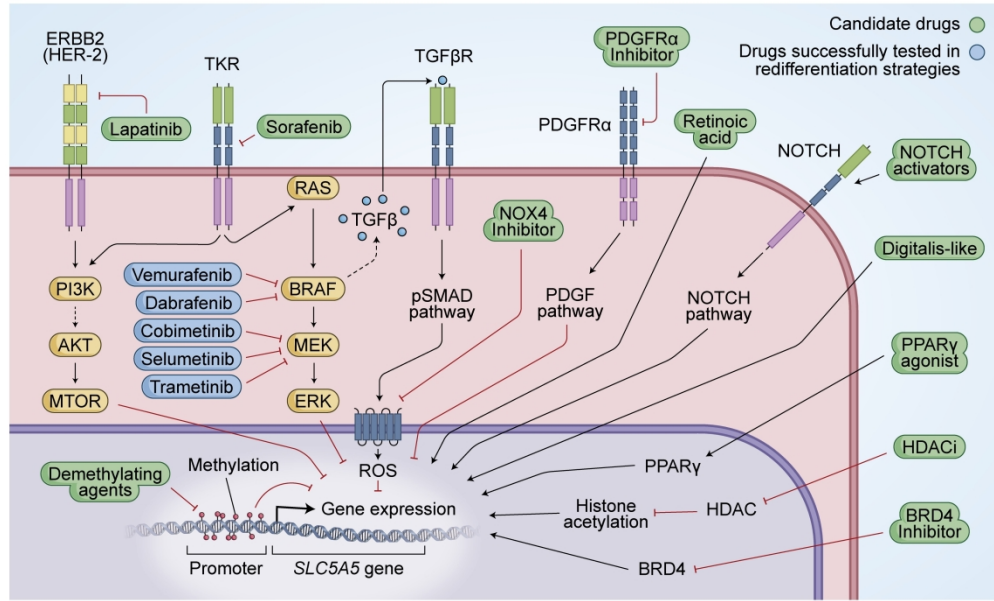


Figure 2 Molecular mechanism controlling NIS expression and current or future actionable targets

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