Redifferentiation-facilitated radioiodine therapy in thyroid cancer

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Introduction

Since the 40s, treatment of distant metastases from thyroid cancer with radioiodine uptake based on the administration of a high activity of ^{131}I was the first available targeted therapy for metastatic disease that provided long-term benefits in a significant percentage of patients (Hertz 1946, Siedlin 1946, Coliez 1951). Also, short-term toxicity rate was lower than that observed with other cancer treatment modalities and on a long-term basis an increased risk of secondary leukemia and cancers was observed only following the administration of high cumulative activities of ^{131}I (>22GBq) (Rubino 2003, Verburg 2020).

However, large retrospective studies have shown that long-term benefits are observed in one third of metastatic patients and that the other two thirds will become refractory to radioiodine treatment. The refractoriness to radioactive iodine treatment is mainly due to a loss of differentiation of the thyroid cancer cells with an impairment of iodine metabolism. The life expectancy in the latter group can be as low as <10% at 10 years (Durante 2006, Schlumberger 2014) and these patients will need other treatment modalities.

The improved knowledge on biology of thyroid cancer has led to therapeutic trials for redifferentiating refractory thyroid cancer that will then permit their treatment with radioactive iodine. This exciting field of research led to several prospective phase 2 trials, and was recently the purpose of reviews and editorials (Buffet 2020, Jhiang 2020, Wirth 2019).

Abnormalities of iodine metabolism in differentiated thyroid cancer tissue

The two major steps of iodine metabolism—uptake and organification—are altered in thyroid cancer tissues. Indeed, impaired uptake of iodide is one of the most peculiar abnormalities present in thyroid neoplastic tissue. Organification defects result in a rapid discharge of radioiodine from thyroid cells, resulting in a short effective half-life of iodine in the cells, and in a low rate of thyroid hormone synthesis (Schlumberger 2007). These abnormalities are related to abnormal expression of thyroid functional genes (Lazar 1999,

Bidart 2000, Lacroix 2001). In hypofunctioning (cold) benign adenomas, there is a decrease in Natrium/Iodide symporter (NIS) mRNA levels but the expression of the other functional genes is similar to that observed in the normal thyroid tissue. In papillary and follicular thyroid carcinomas, more extensive alterations are detected : NIS and thyroid peroxidase (TPO) mRNA expressions are strongly reduced, but thyroglobulin (Tg) and SLC26A4 (which encodes pendrin) gene expressions are only moderately decreased. Dual oxidase (Duox) gene expression is either normal, decreased or increased and thyroid stimulating hormone receptor (TSH-R) gene expression is present in most differentiated thyroid cancers.

Immunohistochemistry confirmed that NIS protein expression is profoundly decreased in differentiated thyroid cancer tissues, and in positive samples, NIS protein is detected in only a few malignant cells that appear to be polarized (Caillou 1998). Other studies suggested that the NIS protein is expressed and is present in the intra-cellular compartments in some thyroid cancer tissues but is not transported to the cell membrane, and this may explain why it is not biologically active (Dohan 2001). TPO biological activity is reduced (Fragu 1977) and TPO immunostaining is weak or absent in most carcinomas when using the monoclonal antibody MoAb47, because of both a decreased expression and the presence of a large proportion of spliced variants that do not react with this antibody and that are enzymatically inactive (Di Cristofaro 2006).

In these studies, serum TSH was in the normal range at surgery, consequently the amount of NIS mRNA obtained represented the unstimulated levels. The expression of NIS and of most thyroid functional genes is increased following TSH stimulation. In accordance with clinical data, this suggests that prolonged and intense TSH stimulation should be performed before any administration of ^{131}I in thyroid cancer patients, in order to optimize the ability of thyroid cancer tissue to take-up and retain ^{131}I.

Furthermore, several alterations have been described in the post-translational modifications and targeting of the NIS protein to the plasma membrane and in its degradation that can have impact on the capability of thyroid cells to concentrate iodine. Dimerisation of NIS molecules might be critical for its trafficking to the plasma membrane (Thomson 2019). Pituitary tumor transforming gene 1 (PTTG1) binding factor overexpression in thyroid cancers results in decreased NIS levels (Read 2011). ADP-ribosylation factor 4 (ARF4)

enhances NIS vesicular trafficking from the Golgi to the plasma membrane and valosin-containing protein (VCP), a principal component of endoplasmic reticulum associated degradation governs NIS proteolysis. NIS cell surface trafficking can be compromised in thyroid cancer over-expressing VCP that can be reverted by a VCP inhibitor (ebastine or clotrimazole) (Fletcher 2020).

Radiation and dosimetry concepts

The outcome of ^{131}I therapy is related to the absorbed dose of radiation delivered to thyroid cancer cells and to the sensitivity of these cells to radiations. The absorbed dose depends on the initial radioactive concentration of ^{131}I in the tissue, namely the ratio between total uptake and the mass of thyroid tissue and on its effective half-life in the tissue, i.e. the time after which the radioactivity in the tissue has decreased by a factor of 2. The effective half-life is related to its physical half-life (8.02 days) and to its biological half-life, which is related to its elimination from the tissue. The volume in which uptake occurs can be estimated with the current use of three dimensional imaging, using ^{131}I and single photon emission computed tomography/computed tomography (SPECT/CT). The poor spatial resolution of this imaging technique is a limiting factor for accurate measurement of small volumes. Moreover, the absolute quantification of ^{131}I concentration in small lesions is challenging because of the complex detection and image reconstruction processes. As recommended by the Medical Internal Radiation Dose committee (Dewaraja et al 2013), the radioactive uptake and then the absorbed dose might not be calculated without correction for volume below to 2-3 times the spatial resolution of the detection device, i.e 3 cm in diameter for ^{131}I SPECT/CT imaging with high energy collimator. Therefore, it is difficult to estimate the absorbed doses to lesions in patients with multiple small metastases or with metastases that are not visualized with anatomical imaging techniques, which is frequently the case for lung metastases with diffuse radioiodine uptake. Advanced image reconstructions, high energy photon interaction compensation and partial-volume correction would help to improve dosimetry calculation, but these techniques are not always available on commercial software. Positron emission tomography/computed tomography (PET/CT) imaging provides a higher spatial resolution and semi-quantitative measurement of radioactivity. Despite complex radiation decay, ^{124}I can be used for pre-therapeutic dosimetry assessment and had shown positive results to predict ^{131}I absorbed dose and lesion response (Wierts et al 2016).

Most of the absorbed dose is delivered by beta particles that do not penetrate deep into tissue (maximum of 2.4 mm in depth) and gamma radiation contributes only by 10 % of the total absorbed dose. As virtually no beta particles escape from large tumor deposits with ^{131}I uptake, large absorbed doses may be delivered without damaging surrounding tissues. However, in case of multiple small diffuse lung metastases with ^{131}I uptake, the absorbed dose delivered to the normal lung tissue might be significant and in some patients with high lung uptake, high ^{131}I activities led to radiation induced pneumonitis (Rall 1957).

Even though the administered activity is high, the absorbed dose delivered to neoplastic foci might be suboptimal for successful therapy due to a low radioiodine concentration and a short effective half-life in the lesion. In fact, iodine uptake is about 1%/g of the administered activity in normal thyroid tissues, whereas it ranges from 0.1% to 0.001%, or is even lower in neoplastic tissues. Furthermore, the effective half-life of iodine ranges from 6-8 days in normal thyroid tissues and only 2-5 days (and sometimes much less) in neoplastic tissues (Schlumberger, 2007).

Radioiodine uptake and biological half-life differ from one patient to another and in a given patient among metastases but also in a given focus, and can also vary with time, resulting in wide differences in the absorbed doses among various tumor foci among patients but also in a given patient (Sgouros 2004, Wierts et al 2016). Dose distribution is also heterogeneous at the cellular level in neoplastic foci, due to the spotty distribution of radioiodine (which is well visualized on autoradiographs and is in accordance with the heterogeneous NIS staining) (Caillou, 1998) and to the short path of beta particles emitted by ^{131}I. This heterogeneity that is not reflected in the absorbed dose estimate provided by scintigraphic methods because of large voxel size and poor spatial resolution, may explain some pitfalls of radioiodine treatment.

A positive correlation has been shown between the radiation dose delivered to neoplastic thyroid foci and outcome of ^{131}I therapy in patients with distant metastases and the mean radiation dose recommended to treat metastases is 100 Gy (Maxon 1983, Maxon 1990).

Many attempts have been made to improve the efficacy of ^{131}I therapy. Prolonged withdrawal of thyroid hormone treatment provides higher metastatic uptake than rhTSH injections (Potzi, 2006; Plyku 2017) and indeed iodine contamination should be avoided. Two

dosimetric methods might optimize the activity to be administered for treatment (Lassmann et al 2010). One is pre-therapeutic lesion dosimetry from repeated three dimensional imaging with ^{131}I SPECT/CT or ^{124}I PET/CT after a tracer administration. It enables reconstruction of the time–activity curve within the lesion and will determine the activity to be administered to deliver an optimal radiation dose to the lesion. However, the dose estimated with a low activity may differ from the dose effectively delivered during a treatment with a high ^{131}I activity due to lower uptake and shorter retention in the lesion induced by high absorbed doses. The other method is whole body/blood clearance dosimetry to determine the maximal activity that can be administered so that absorbed dose to blood does not exceed 200 cGy and the whole-body retention does not exceed 2.96 GBq (80 mCi) at 48 h after administration in the presence of iodine-avid diffuse lung metastases, respectively in order to prevent toxic effects on the bone marrow and lungs (Benua 1962). In fact, as estimated from external beam radiation therapy, mean absorbed dose to the lung should be lower than 25-27 Gy to prevent lung damage. These values should be taken with caution because radiation emission in molecular radiotherapy is different (dose rate, source distribution and change over time). Advanced methods based on a three dimensional dosimetry calculation using Monte Carlo simulation (Dewaraja et al 2012) can take into account uptake heterogeneity and improve the accuracy of the absorbed dose estimate (Sgouros et al 2006).

In a study comparing the outcome of patients with distant metastases with ^{131}I uptake, overall survival was similar in the different groups of patients classified according to prognostic factors (age and size of the metastases) and treated either with a standard activity of 3.7 GBq every 6-12 months or with higher activities determined with whole body/blood clearance (Deandreis 2017). This study has raised concerns, but unfortunately scientific data demonstrating that either method for dosimetry is beneficial for these metastatic patients are still controversial (Klubo-Gwiezdzinska, 2011, Wierts 2016). However, these methods might be useful for determining the optimal time of treatment and the ^{131}I activity to be administered in redifferentiation trials (see below). Between ^{131}I treatment courses, thyroid hormone treatment is given at doses that decrease serum TSH to low or undetectable levels in the absence of contraindication.

Following ^{131}I treatment, complete tumor responses were observed in young patients with small metastases from a well differentiated DTC and with high initial ^{131}I uptake (Durante 2006), but unfortunately not all tumor foci with ^{131}I uptake do respond to ^{131}I treatment, and this is the case in older patients with large metastases from a poorly differentiated thyroid cancer or in the presence of high fluorodesoxyglucose (FDG) uptake on PET/CT (Robbins 2006). Almost all complete responses were observed after the administration of a cumulative activity of 22 GBq or less; higher cumulative activities may be administered but it is then unlikely to obtain a complete response (Durante 2006). Sufficient uptake of ^{131}I for effective treatment of metastases occurs only in two-thirds of the patients, and complete responses are achieved in only one third of patients (Durante 2006). Therefore, about two-thirds of patients with extended disease will be considered refractory to ^{131}I therapy at some points of their life, with a reduced 10-year life expectancy of ~10%, and then will require other treatment modalities (Schlumberger, 2014).

Genomic alterations and relationships with functional defects

Genomic alterations have been characterized in differentiated thyroid cancers (Fagin 2016). In the TCGA on 500 papillary thyroid cancer tissues, the density of mutation is low, with one driver alteration found in 96.5% of tumors. These driver alterations are in most tumors mutually exclusive (Thyroid Cancer Genome Atlas (TCGA) [https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga/studied-cancers/thyroid). In papillary thyroid cancers, the *BRAF V600E* point mutation is the most frequent alteration being found in 45-60 %, followed by *RAS* and then *TERT* promoter point mutations; gene fusions (*TRK 1/3, RET, ALK, BRAF*) are found in 15% of tumor samples. Aberrant activation of the MAPK pathway present in 85% of papillary thyroid cancers has a crucial role in the impairment of the iodide uptake and metabolism. In advanced follicular cancers, *RAS* mutations are the most frequent (25%), followed by *TERT* promoter*, TP53, EIF1AX, PTEN, RB1, GNAS* point mutations and *PAX8-PPARG, ALK, NTRK 1/3, RET* fusions are also found (Yoo 2016, Pozdeyev 2018). In Hürthle cell carcinoma, the spectrum of mutations is different and includes chromosomal losses, mitochondrial DNA mutations, and changes in the expression profile of the PI3K-AKT-mTOR and Wnt/β-catenin pathways (Ganly 2018). In poorly differentiated thyroid cancers, the density of mutations is higher, *BRAF* and *RAS* point

mutations are the most frequent alterations, and *EIF1AX, PIK3CA, PTEN, TP53* and *TERT* promoter mutations are also found (Landa 2016).

Histological and genomic characteristics have a prognostic impact and may guide treatment. PTC classified as *BRAF*-like tumors are more dedifferentiated than *RAS*-like PTC; *RAS* mutations are more frequently found in tumors with a follicular pattern, including FTC, follicular adenomas and follicular variants of PTC (Fagin 2016). Metastases with RAI uptake are enriched in RAS mutated tumors (Sabra 2013). PTC patients with a *BRAF V600E* mutation have a poor clinical outcome, especially if a *TERT* promoter mutation is also present (Xing 2013, Xing 2015), and the expression of thyroid functional genes, including NIS, TSH-R, TPO, Tg and SLC26A4 (which encodes pendrin) is decreased or absent in BRAFV600E mutated PTC (Durante 2007; Romei 2008). In patients, BRAFV600E mutation is associated with the loss of radioiodine avidity and with radioiodine treatment failure and is more frequently found in recurrent radioiodine-refractory than in the primary papillary thyroid cancers (Sabra 2013). Also, as already reported about 30% of all patients with metastases do not demonstrate any radioiodine uptake (Durante 2006), and this percentage increases to 70% when a *BRAF V600E* is present and to 97% when both *BRAF V600E* and *TERT* promoter mutations are present, whereas it is only 20% in the absence of these two mutations (Liu 2020). These data indeed suggest that genomic studies should be performed on all thyroid cancer tissues, either extended or limited to the neck and whenever mutations are found they should be taken into account for optimizing the protocol of treatment.

An induced expression of BRAF V600E in thyroid cells impaired the expression of almost all these genes, which was restored by ceasing its expression or by suppressing the MAPK pathway with a BRAF or a MEK inhibitor (Knauf 2005, Chakravarty 2011). Similarly, in thyroid cells expressing RET–PTC1 or a RAS mutated gene, NIS expression was increased following treatment with a MEK inhibitor (Knauf 2003). These reversible effects suggest that the inhibition of NIS expression and of other thyroid functional genes are related to epigenetic changes.

Loss of differentiation features correlates with the degree of MAPK activation, which is higher in tumors with *BRAF V600E* mutation than in those with receptor tyrosine kinase (*RET*, *TRK* or *ALK*) or *RAS* mutations. In fact, in rat thyroid cells expressing BRAFV600E and, in vivo in a mouse model with BRAFV600E-induced thyroid cancer, a small increase in ERK

inhibition translates into a markedly increased expression of thyroid differentiation genes and increased iodide accumulation in cancer cells (Nagarajah et al. 2016; Oh et al. 2018). In case of *BRAF* mutation, the BRAF protein is activated as a monomer and do not respond to the negative feedback of the ERK activation, whereas in RAS mutated tumors the BRAF protein still acts as a dimer sensitive to the negative feedback of ERK activation (Poulikakos 2011). Redifferentiation process might therefore differ depending on the driver-mutation. In human *BRAF*-mutated thyroid cancer cell lines, the combination of BRAFV600E and MEK inhibition synergistically increased radioiodine uptake possibly through an inhibition of the rebound of ERK1/2 activation observed with only one drug (Lito 2012, Zhang & Chen 2018). Hence, inhibition of the MEK-1 and -2 downstream of RAF with drugs such as selumetinib or trametinib could redifferentiate RAI refractory thyroid cancer. Indeed, the addition of a BRAF inhibitor such as vemurafenib or dabrafenib in case of *BRAF* mutation will reinforce the inhibition of the MAPK pathway and will avoid its reactivation due to the disappearance of a negative feedback.

One mechanism for the inhibition of the expression of NIS and of other thyroid functional genes is that MAPkinase pathway activation stimulates Transforming growth factor (TGF) beta that in turns stimulates the SMAD pathway that will stimulate the expression of the NADPH oxidase NOX4. NOX4 will then produce ROS that will induce epigenetic changes such as histone acetylation or methylation at gene promoter regions that might reduce their expression (Riesco-Eizaguirre 2009, Azouzi 2016). The acetylation of histones of the promoter of the SLC5A5 gene encoding for NIS regulates its transcription and consequently NIS expression. Hypermethylation of the promoter region of the SCLC5A5 gene has been demonstrated in both benign and malignant thyroid tumors. All these steps offer potential use of specific inhibitors that might reinduce RAI uptake and organification in thyroid tumor cells.

Activation of the PI3K–AKT pathway was also shown to down-regulate iodide uptake and metabolism in thyroid cells both in vitro and in vivo. In vitro, inhibition of the PI3K–AKT pathway induced NIS expression and iodide uptake in human thyroid cancer cells and also the expression of TSHR, TPO and Tg (Hou 2010). The involvement of both MAPK and PI3K–AKT pathways in the silencing of thyroid iodide-handling genes is consistent with the

accumulation of genetic alterations in both pathways as thyroid tumors progress, with a parallel increasing loss of radioiodine avidity.

Manipulating NIS expression: gene reactivation and gene therapy.

In the past, lithium salts have been used for increasing the effective half-life of ^{131}I in neoplastic thyroid cells, but their clinical efficacy is still not proven (Luo 2018).

Retinoids act on the nuclear receptors, retinoic acid receptor (RAR) and retinoid X receptor (RXR). These complexes bind to the responsive elements in gene promoter sites and activate the transcription of their target genes. Prospective clinical trials failed to demonstrate a clinical utility of isotretinoin (the 13-cis-rétinoic acid). In a phase II trial, isotretinoin did not significantly increase radioiodine uptake in 16 RAIR-DTC (Short et al. 2004). In another phase II trial on 53 patients, increased radioiodine uptake was observed in 9 (17%), but this did not translate into clinical benefit (Handkiewicz-Junak. 2009).

Histone deacetylase inhibitors (Sherman 2013) did not induce clinical benefits in patients with radioiodine refractory thyroid cancer and clinical trials with methylation inhibitors are still lacking. However, their combination with drugs that inhibit the MAPkinase pathway may produce a synergistic effect on NIS expression.

Inhibition of the BRAF kinase with sorafenib, a weak BRAF inhibitor did not reinduce significant ^{131}I uptake in 20 patients evaluable for redifferentiation (Hoftijzer 2009).

Gene therapy

Transfection of NIS cDNA into malignant non-thyroid cells enabled the transfected cells to take-up radioiodine in culture or after subcutaneous injections to animals (Shimura 1997, Mandel 1999, Boland 2000, Spiztweg 2002). This approach allowed *in vivo* radioimaging of human tumors xenografted in mice. Moreover, *in vitro* experiments indicated that NIS-infected tumor cells can be selectively killed by inducing ^{131}I uptake therein. However, NIS-infected tumor cells do not organify iodide efficiently, and this results in a short half-life in the cells and in insufficient radiation doses for treatment efficacy. This is the reason why

combinations with other drugs such as anti-angiogenic drugs have been tested (Magnon 2007), but with limited increased efficacy.

Current treatment of advanced refractory thyroid cancer

When there is no radioiodine uptake in the metastases or when metastases progress despite the presence of uptake, the disease is considered refractory and radioiodine administration is abandoned (Schlumberger 2014). Other treatment modalities might be indicated in patients with large tumor burden, with symptoms or risk of local complication or with documented rapid tumor progression. In these patients, focal treatment modalities, including thermal ablation, surgery and external beam radiation therapy might be used and the systemic treatment is currently based on tyrosine kinase inhibitors (TKI). Two phase 3 trials with anti-angiogenic TKI, one with sorafenib and the other with lenvatinib showed significant improvement of median Progression Free Survival (PFS) over placebo and this led to their approval by FDA and EMA (Brose 2014, Schlumberger 2015). Other anti-angiogenic TKIs have been effective in phase 2 trials, such as cabozantinib (Cabanillas 2017), pazopanib (Bible 2010) and axitinib (Locati 2014) and might be used as second line treatment. Unfortunately, the long-term use of these TKIs is associated with toxic effects that may decrease the quality of life of the patients and their compliance to treatment and that may be fatal in some (Lamartina 2016). More recently, TKIs selectively directed against an abnormally active oncoprotein have been available, but their use is restricted to tumors with a point mutation such as *RAS* or *BRAF* or a fusion such as *RET, TRK* or *ALK*. The use of specific inhibitors directed against fusions has produced profound tumor responses in a large proportion of treated patients (Drilon 2018, Wirth 2020). In patients with a *BRAF V600E* mutation, a BRAF inhibitor (dabrafenib) alone or in association with a MEK inhibitor (trametinib) induced a tumor response in up to 54% of patients when administered on a long-term basis (Shah 2017). The toxicity profile of these selective TKIs is often more favorable than that observed with anti-angiogenic TKIs. Therefore, the presence of selectively druggable abnormalities should be screened in thyroid cancer tissue in all patients with extended disease, and whenever present, a specific inhibitor should be considered as first line treatment.

Because of their toxicity and the absence of demonstrated benefits on overall survival, the use of multikinase inhibitors is often limited to patients with radioiodine-refractory DTC who have large tumor burden and clinically meaningful disease progression. Patients with radioiodine-refractory DTC who are asymptomatic and have a low burden and a slowly progressive disease are often followed with active surveillance alone. Although the asymptomatic period of slow tumor growth might last for a long period of time, their disease-specific survival is not favorable, and a treatment approach that would be more effective than active surveillance is clearly needed.

Redifferentiation: current experience

Based on experimental data, inhibition of the MAPkinase pathway might be used on a short term basis (4-6 weeks) to induce a redifferentiation of thyroid tumor cells. Then, in case of reappearance of a significant tumor uptake following rhTSH stimulation, a ^{131}I treatment is administered (Ho 2013). Prospective trials are ongoing to evaluate the benefits of this strategy in DTC patients with advanced RAI-refractory (RAI-R) disease and to ascertain the absence of significant toxicity.

In a clinical study in 24 patients with advanced RAI-R DTC, patients were treated with selumetinib (a MEK inhibitor) for 4 weeks and then submitted to an ^{124}I PET-CT following rhTSH stimulation to estimate the activity of ^{131}I required to deliver an arbitrary dose of 2000 cGy and above to the metastatic lesions (Ho et al. 2013). If it appeared that at least one lesion could be treated with an activity of less than 300 mCi, the patient was then treated with ^{131}I. Of the 20 evaluable patients, selumetinib increased radioiodine uptake in 12. Of these 12 patients, eight reached the dosimetry threshold for radioiodine therapy, and were treated with radioiodine. Seven of these 8 patients had a confirmed RECIST 1.1 partial response, 6 months after radioiodine therapy but the duration of response was not reported. The eight patients who reached the dosimetry threshold included all five patients with NRAS mutation but only one of the nine patients with BRAFV600E mutation. This led to hypothesize that in *BRAFV600E*-mutant tumors, pre-radioiodine treatment by a more robust MAPK inhibition might be needed to effectively induce redifferentiation. To confirm these preliminary data, a multicenter UK single arm phase II trial (SEL-I-METRY, ISRCTN17468602) is ongoing with a similar design (treatment with selumetinib for 4 weeks) and patients showing

a significant iodine uptake following rhTSH stimulation are treated with a fixed activity of 150 mCi of ^{131}I; the potential role of lesional dosimetry will be assessed but will not be taken into account for treatment decision (Brown et al. 2019).

In another pilot trial, vemurafenib (a BRAF inhibitor) was evaluated in 12 patients with a BRAF mutated advanced RAI-R DTC, with a methodology similar to the previous study (Dunn et al. 2019). Four of the 10 evaluable patients reached the dosimetry threshold on the ^{124}I PET/CT and were treated with ^{131}I: 6 months after radioiodine therapy, two achieved a partial response and two patients had a stable disease. Of the four I-131–treated patients, two required subsequent thyroid cancer treatment at 9 and 18 months, and the other two patients have not required further therapy at 22 and 33 months, respectively suggesting prolonged benefits. Molecular tumor biopsy analysis performed before and under vemurafenib treatment in 3 patients revealed a decrease in the MAPK pathway transcriptional output and an induction of thyroid-specific gene expression and suggested that these two modifications are related.

The BRAFV600E inhibitor dabrafenib has been evaluated in 10 patients with a BRAFV600E advanced RAI-R thyroid cancer (Rothenberg et al. 2015). These patients had no radioiodine uptake on a baseline WBS, and 5 of them had a documented progression during the 14 months prior to enrolment. Radioiodine uptake was detectable after 6 weeks of dabrafenib treatment on a diagnostic WBS in six patients who were treated with a standard activity of 150 mCi ^{131}I. At 3 months after radioiodine therapy, two patients showed a partial response and four a stable disease.

Overall, these feasibility studies suggests that the use of a selective BRAF inhibitor either dabrafenib or vemurafenib for redifferentiation in *BRAFV600E*-mutant radioiodine-refractory DTC is a strategy that may offer some clinical benefits.

The retrospective analysis of patients with RAI-R DTC treated with MAPK inhibitors (BRAFV600E and/or MEK inhibitors) confirmed these data (Jaber et al. 2018, Iravani et al. 2019). In a study on 13 patients treated with a BRAF or a MEK inhibitor, 9 were treated with a therapeutic activity of 150–250 mCi of ^{131}I based on meaningful uptake of radioiodine on a diagnostic WBS, except for one. All nine patients had durable disease control, and RAS-mutated tumors were the best responders compared to BRAF-mutated ones (Jaber et al.

2018). In another study, six patients were treated with a MEK inhibitor (NRAS mutated) or with a combination of a BRAF inhibitor and a MEK inhibitor (BRAF mutated) for 4 weeks, and four were considered suitable for radioiodine therapy based on the results of ^{124}I PET/CT (Iravani et al. 2019). All three BRAF-mutated patients responded to the redifferentiation strategy, while only one of the three NRAS mutated patients did. Of these four patients, three achieved a partial tumor response and one had a stable disease with a median follow-up of 16.6 months.

Additional case reports demonstrated the potential redifferentiating effect of pharmacological MAPK inhibition in thyroid cancer patients. In one patient with a *BRAFV600E* mutated papillary thyroid cancer, both vemurafenib and dabrafenib sequentially induced tumor uptake and RAI treatments induced a partial response (Huillard et al. 2017). In another case report, clinical thyrotoxicosis developed in a patient with a BRAFK601E mutated papillary carcinoma treated with dabrafenib and trametinib (Leboulleux et al. 2019). In both cases, this redifferentiation effect was transitory and RAI uptake disappeared after a short period of discontinuation of the redifferentiation drugs as well as thyrotoxicosis in the second case. A rise in serum thyroglobulin levels might indicate the success of redifferentiation rather than disease progression (Huillard et al. (2017), Leboulleux et al. (2019), Dunn et al. (2019)).

This redifferentiation concept might apply to refractory thyroid cancers with other mutation using a specific inhibitor, as shown by the redifferentiation observed in a patient with TRK mutation following treatment with larotrectinib, a NTRK inhibitor (Groussin 2020).

These preliminary data clearly demonstrate that the redifferentiating drugs induce a re-expression of NIS that is responsible for the increased iodine uptake but also of all the other thyroid functional proteins that are needed for the organification of radioiodine and its retention inside thyroid tumor cells.

In these studies, it is difficult to distinguish between tumor response resulting from a cytotoxic effect of the pharmacological inhibitor and the effect of ^{131}I therapy after the restoration of radioiodine uptake or a combination of both effects. However, a treatment given for 4 to 6 weeks is unlikely to produce long-term tumor responses. This short term

treatment will induce a low toxicity, if any that will then be much lower than toxicities observed during long term TKI treatments.

Future prospects to improve tumor redifferentiation, especially of BRAFV600E-mutated thyroid cancers, through MAPK inhibition, might come from a profound inhibition of the MAPK pathway. An ongoing multicentric clinical trial performed by the French RAI-R thyroid cancer TUTHYREF network is testing for the treatment of metastatic RAI-R DTC a MEK inhibitor (Trametinib) alone (for RAS mutated tumors) or in combination with a BRAFV600E inhibitor (Dabrafenib) (for BRAFV600E mutated tumors) followed by a standard therapeutic activity of 150 mCi of ^{131}I after rhTSH stimulation in case of radioiodine uptake (NCT 03244956). The interest of lesional dosimetry will be evaluated. This trial includes selected patients with relatively small metastatic burden and slow progression rate, that are the best candidates for response to ^{131}I treatment. Whether results could be applied to metastatic patients with other characteristics, such as larger metastases or higher progression rates will need further studies.

To date, it remains unknown why some DTC patients do not have restoration or enhanced RAI uptake after BRAF/MEK inhibitor therapy. The comparison of genomic characteristics of responsive and non-responsive patients might be informative as tumor driver mutation alone may not fully reflect thetumor genetic background and the influence from tumor microenvironment. Furthermore, a standard protocol is applied, but probably some optimization might be warranted in the length of treatment or in the protocol used for TSH stimulation. This optimization might be guided during treatment by the trend in serum Tg level and the decrease of FDG uptake on PET/CT, and by lesion dosimetry assessed following rhTSH stimulation with a diagnostic activity of radioiodine. The risk of radiation induced toxicity, such as lung irradiation due to high RAI uptake in diffuse lung metastases should also be minimized and the use of whole body/blood clearance dosimetry to improve tolerance might also be relevant.

Redifferentiation: potential advances

Several strategies based on the current knowledge of the biology of refractory thyroid cancer might improve the efficacy and tolerance of redifferentiation.

For *BRAFV600E*-mutated thyroid cancers, a strategy would be to combine inhibitors of the human EGF receptor (Her) with MAPK inhibitors. In thyroid cancer cells harboring BRAFV600E mutation, inhibition of the MAPK pathway by a RAF or a MEK inhibitor might be transient due to the release of a transcriptional repressor from the HER3 promoter and consequently induced HER3 gene overexpression (Montero-Conde et al. 2013). An autocrine secretion by thyroid cancer cells of a ligand able to bind to and activate by dimerization the tyrosine kinase receptors HER2/HER3 resulted in the reactivation of the MAPK and PI3K pathway. The Her kinase inhibitor lapatinib prevented MAPK rebound and overcame BRAF-mutated thyroid cancer cell resistance to MAPK inhibitors (Montero-Conde et al. 2013). In BRAFV600E-mutated human thyroid cancer derived cell lines, the combination of lapatinib with dabrafenib or selumetinib increased radioiodine uptake (Cheng et al. (2017). A clinical trial (NCT 02456701) which tests the ability of vemurafenib combined with an anti HER3 monoclonal antibody to restore iodine incorporation in BRAF mutant RAI-R thyroid cancer patients is ongoing.

As PI3K inhibition seems to prolong radioiodine retention in thyroid cells (Lakshmanan et al. 2015), the combination of MAPK and PI3K inhibitors may be an interesting strategy, but with the disadvantage of potential synergistic side effects.

As previously discussed NOX4 is activated in BRAF V600E mutated tumors and by producing ROS might be responsible for a decreased expression of thyroid functional genes, including NIS. These inhibitory effects might be achieved through epigenetic changes such as acetylation of histones of the SLC5A5 promoter gene encoding for the NIS or its hypermethylation of its promoter region that might reduce its expression. Therefore, drugs inhibiting NOX4, acetylation of histones (HDAC inhibitors) or hypermethylation of the NIS promoter might be used in these patients and probably in association with inhibitors of the MAPkinase pathway.

Additionally, since the efficacy of RAI therapy depends not only on sufficient absorbed doses to metastatic lesions but also on the radiosensitivity of the lesions, and MEK inhibitor has been reported to radiosensitize tumors derived from several tissue types to external beam radiation therapy. Thyroid cancer cell lines carrying the *BRAFV600E* mutation were associated with resistance to ionizing radiation, and the BRAF

inhibitor, vemurafenib, selectively radiosensitized BRAFV600E tumor cells by inhibiting DNA double-strand break repair. Vemurafenib in combination with radiation therapy resulted in marked and sustained regression of thyroid tumor xenografts carrying BRAFV600E mutation. Unpublished data also showed that the MEK inhibitor trematinib increased RAI radiosensitivity in RAI-R human thyroid cancer cell lines expressing exogenous NIS (Jhiang2020). Thus, BRAF/MEK inhibitors likely not only enhance RAI delivery but also increase RAI radiosensitivity. To date, differential RAI radiosensitivity among lesions within individual patients or between patients has not been investigated.

Redifferentiation: applications for adjuvant treatment

When benefits of redifferentiation will be demonstrated in advanced RAI-R DTC, this therapeutic strategy might also be used for post-operative RAI administration in patients with a localized thyroid cancer. It is often recommended to perform an aggressive treatment that includes the post-operative administration of RAI in patients with either an aggressive histological form or a *BRAFV600E* mutation. However, RAI may be ineffective in these patients due to a low or absent RAI uptake in tumor tissue. The ASTRA phase III study (NCT01843062) in DTC patients at high risk of recurrence after total thyroidectomy (i.e. pT >4 cm, pT4, N1 with ≥5 lymph nodes or with at least 1 lymph node ≥1 cm) was disappointing. Patients were randomized to receive placebo or selumetinib for 4 weeks prior post-operative radioiodine ablation. The complete remission rate at 18 months was not improved by the addition of selumetinib to radioiodine (40% vs 38.5% in the placebo group) in this patient population, in any subgroup of patients, even when genotype was taken into account (Ho, 2018). However, patients with a low probability of radioiodine uptake, such as patients with a BRAF mutated tumor in whom RAI administration is associated with a high probability of being ineffective because of the lack of radioiodine uptake might benefit from a redifferentiation strategy before post-operative RAI administration.

Conclusion

Redifferentiation appears to be an alternative treatment for RAI-R thyroid cancers, but clinical data are still preliminary. It is feasible, based on genomic studies of the thyroid cancer tissue and has already provided significant clinical benefits in few patients. However, it needs to be optimized, its toxicity should be assessed and its role in treating these patients

has to be delineated in future trials. Furthermore, this method might be also applied to patients with aggressive disease because of the presence of a BRAFV600E mutation, even confined to the neck, when no uptake of radioiodine is expected rendering RAI treatment inefficient. Finally, whether this strategy might afford benefits in patients with an anaplastic thyroid cancer carrying a targetable mutation has to be tested in specific trials.

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