Clinical impact of retinoids in redifferentiation therapy of advanced thyroid cancer: final results of a pilot study

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Abstract. Differentiated thyroid cancer is a malignant tumour that has a fairly good prognosis, with patients surviving for many years. Multimodal therapy with surgery, radioiodine therapy and TSH suppressive medication is of proven efficacy. However, loss of differentiation is observed in up to one-third of patients with differentiated thyroid cancer, paralleled by an increase in tumour grading and loss of thyroid-specific functions (thyrotropin receptor, iodine accumulation). Such tumours may no longer be amenable to standard treatment protocols, including TSH suppression and radioiodide therapy. Retinoic acids have been shown to exert re-differentiating effects on thyrocytes in various experimental studies and case reports, and it was on this basis that this pilot study was initiated. Patients with advanced thyroid cancer and without the therapeutic options of operation or radioiodide therapy were treated with 13-*cis*-retinoic acid at a dosage of 1.5 mg/kg body weight daily over 5 weeks. Parameters for assessment of the therapeutic effect were serum thyroglobulin (TG) levels, radioiodine uptake, and tumour size prior to and after retinoid treatment. Fifty patients were evaluated for response, classified as reduction in tumour size and TG levels, stable disease or disease progression. Thirteen patients showed a clear increase in radioiodine uptake, and eight a mild increase. TG levels were unchanged or decreased in 20 patients. Tumour size was assessable in 37 patients; tumour regression was observed in six, and there was no change in 22. In total, a response was seen in 19 patients (38%). Response to retinoid therapy did not always correlate with increased radioiodine uptake, so other direct antiproliferative effects have to be assumed. The encouraging results of the study and the low rate of side-effects with good tolerability of retinoids warrant further studies with altered inclusion criteria and employment of other redifferentiating drugs or combinations of agents.

Keywords: Thyroid cancer – Redifferentiation – Retinoid treatment

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Introduction

Therapy of differentiated thyroid cancer (DTC) primarily takes three forms: surgical removal of the tumour-bearing thyroid gland and of extraglandular tumour spread in lymph nodes or at distant sites; radioiodine therapy, which is especially effective for ablation of thyroid remnants and distant metastases; and thyrotropin (TSH) suppressive thyroxine therapy. In the course of tumour progression, distinct morphological and functional characteristics of DTCs disappear. This occurs in about one-third of thyroid carcinomas, which show a change in histological grading and altered iodide uptake [1]. Clinically, more aggressive growth and metastatic spread are observed.

Retinoic acids (RAs) are biologically active metabolites of vitamin A that play an important role in the morphogenesis, differentiation and proliferation of many cell types [2, 3, 4]. RA signals are transduced by specific re-

ceptors, the retinoic acid receptors RARs and RXRs, which belong to the superfamily of nuclear receptors [5, 6, 7]. These act as ligand-binding transcription factors modulating the activity of RA-responsive genes.

Retinoids have been administered for anti-cancer and tumour-preventive treatment in various clinical trials, and promising results have been achieved in the therapy of acute promyelocytic leukaemia (APL), head and neck cancers and lung and skin tumours [8, 9, 10, 11, 12, 13, 14, 15, 16]. The paradigm of redifferentiation via retinoids is APL, complete remission of disease being achieved in a high percentage of patients [8]. Due to the unique genetic abnormality involved in APL, the effects of retinoids in the treatment of APL cannot be transferred to other tumour entities. In the lung, vitamin A deficiency has been found to be a potential risk factor for cancer development [26].

Experimental data provide evidence that differentiated functions of thyrocytes and of iodide metabolism can be re-induced by RAs [2, 17, 18, 19]. In follicular thyroid tumour cells, retinoids have proved able to inhibit tumour growth and induce iodine uptake. A redifferentiating effect on follicular thyroid cancer cells could be shown by induction of type I iodothyronine-5'-deiodinase (5'-DI) and alkaline phosphatase as well as by stimulation of intercellular adhesion molecule-1 (ICAM-1) in thyroid carcinoma cell lines [17, 18, 19, 20, 21, 22]. Furthermore, treatment of follicular thyroid tumour cells with RAs leads to loss of tumourigenicity in athymic nude mice. The redifferentiating effects of RAs are confined to at least partly differentiated thyroid cancers and are not seen in anaplastic thyroid cancer [23].

Loss of differentiation is a common event in DTC and entails the loss of thyroid-specific functions, e.g. there may be reduced or missing expression of thyrotropin receptor or reduced ability to accumulate iodine. Consequently these tumours may no longer be amenable to standard treatment protocols, including TSH suppression and radioiodine therapy. The promising experimental results achieved with RAs prompted us to perform clinical studies of the effectiveness of 13-*cis*-retinoic acid in patients with advanced thyroid cancer. Here, the results of clinical application will be discussed in the context of our experience with 50 patients.

Materials and methods

The study was designed as a prospective multicentre trial without controls. University hospitals in Düsseldorf, Essen, Würzburg, Rostock and Bonn participated in the study. The pilot study was approved by the local ethical committees. All patients provided informed consent to participation in the study.

Inclusion criteria for retinoid treatment were:

1. Advanced poorly DTC of papillary, follicular or oxyphilic origin
2. Inoperability due to extensive local and irresectable invasive disease or distant metastatic spread
3. Absent or insufficient radioiodine uptake

In addition, patients had to have undergone previous radioiodine treatment with primary failure of radioiodine uptake or decrease or loss of initially sufficient radioiodine uptake. Exclusion criteria were pregnancy, anaplastic carcinoma and liver insufficiency. Drop-outs were defined as those patients in whom therapy was interrupted owing to non-compliance or intolerable side-effects.

The treatment was performed with 13-*cis*-retinoic acid (Roaccutan) at a dosage of 1.5 mg/kg body weight daily over 5 weeks. Dose reduction to 1.0 mg/kg body weight daily was allowed in the event of severe side-effects. L-Thyroxine (T_4) supplementation was discontinued for the duration of retinoid treatment and replaced by triiodothyronine (T_3) until 2 weeks prior to post-therapeutic radioiodide scan.

Clinical response to retinoid therapy was defined by serum thyroglobulin (TG) levels, radioiodine uptake and tumour burden (size or number of metastases). Response was measured immediately after retinoid therapy and 6 months later. If longer follow-up was available, these data were included in the evaluation. The cut-off for follow-up was the year 1998. Prior to the retinoid treatment and after 5 weeks of therapy, serum TG was measured under stimulated conditions (TSH >30 ng/ml), a post-therapy radioiodine whole-body scan with radioactivity between 3 and 10 GBq iodine-131 was performed, and tumour size was assessed by computed tomography (CT) or magnetic resonance imaging (MRI). Diagnostic imaging for the assessment of tumour size was performed in only some of the patients, in order to avoid iodine contamination and because of the difficulty in quantifying the tumour mass (e.g. owing to diffuse pulmonary metastases).

Thyroglobulin levels were measured in various laboratories with varying TG assays. Therefore TG levels were classified as increased or decreased only. TG levels with less than 10% variation between pre- and post-therapeutic values were classified as unchanged.

The post-therapy scan was performed according to the guidelines of the German Society of Nuclear Medicine. A standardized dosimetric approach for the evaluation of iodine accumulation in thyroid cancer tissue was not available at the beginning of the study. However, six patients underwent dosimetry later in the course of the study according to a protocol that had been developed for studies with recombinant human TSH (rhTSH).

For the post-therapy scan, a high-energy collimator was used, and 300,000 counts were acquired for the head and neck region. The whole-body scan was performed with a gamma camera speed of 12 cm/min. In most cases, a former post-therapy scan obtained less than 6 months previously was available. Therefore, comparison of the scans was possible for classification of response. A marked increase in uptake was defined on the basis of comparison with background and physiological structures such as the liver. For classification of response, the tumour burden or size of local recurrence was measured with ultrasound, CT or MRI and the sites of localisation were known.

Side-effects were documented according to the common toxicity criteria of the EORTC. Weekly controls were performed of symptoms like dryness of skin, lips, and mucosa, pruritus, nose bleeding, hair loss, arthritis and headache, and there was also weekly assessment of red and white blood cell count, cholesterol, triglycerides and liver enzymes (glutamic-oxaloacetic transaminase, alkaline phosphatase, bilirubin).

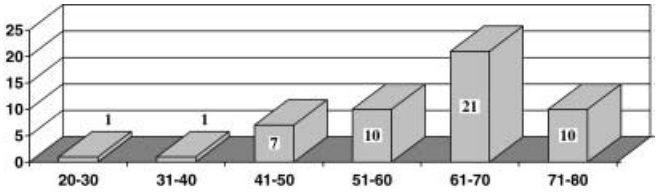

Fig. 1. Age distribution of the 50 thyroid carcinoma patients

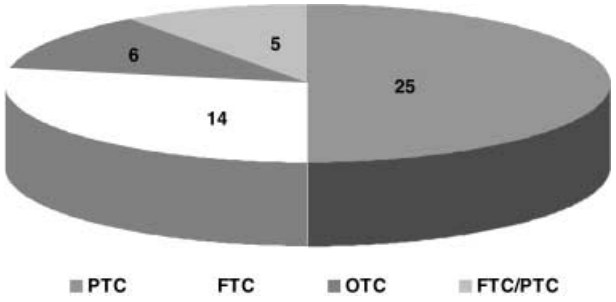

Fig. 2. Distribution of histological types of thyroid cancer in the 50 patients treated with retinoids who were included in the further evaluation. PTC, Papillary thyroid cancer; FTC, follicular thyroid cancer; OTC, mixed oxyphilic-follicular thyroid tumours; FTC/PTC, mixed follicular-papillary thyroid tumours

Table 1. Side-effects of retinoid therapy (grade I and II CTC/EORTC)

Side-effects	No.	%
Dry skin	19	38%
Dry lips	18	36%
Dry mucosa	4	8%
Nausea	1	2%
Nose bleeding	1	2%
Hair loss	2	4%
Change in blood count	2	4%
Vision	2	4%
Coughing	1	2%
None	21	42%
Total	29	58%

Table 2. Correlation of serum TG level and outcome

Serum TG level	No.	Response	Stable	Disease progression
Increase	30	0	1 (3%)	29 (97%)
No change	8	1 (13%)	6 (75%)	1 (13%)
Decrease	12	9 (75%)	2 (17%)	1 (8%)

Results

By 1998, 75 patients had been enrolled in this study. Due to violations of the study protocol, only 50 of these patients with comparable and completely documented data sets were included in the further evaluation. Serum TG as a parameter of retained differentiated thyroid function was measured in all patients. A radioiodine scan was performed prior to and after retinoid treatment in all patients as well. Assessment of tumour size by CT scan or MRI was possible in 37 patients (74%). In the remaining 13 patients, diffuse pulmonary metastases precluded measurement of tumour size.

Thirty-one patients were female and 19 male; the mean age was 62 years (range 30–79 years), and the median age, 61 years (Fig. 1). Histology demonstrated papillary cancer in 25 patients, follicular cancer in 14, oxyphilic and mixed oxyphilic-follicular tumours in six and mixed follicular-papillary tumours in five (Fig. 2). There was variable primary tumour stage (pT2–4) and lymph node involvement. All but two patients had distant metastases, either in lung or bone or both. All patients had undergone previous radioiodine therapy on one to eight occasions with variable cumulative radioactivity (3.7–59.4 GBq). All patients had also undergone one or multiple operations (range 1–8), and some had received additional external radiation therapy. The mean follow-up was 16 months, with a median of 18 months (range 5–46 months). Long-term follow-up beyond 1998 was possible in 13 of the enrolled patients, with an additional mean follow-up of 20 months (6–30 months). During the observation period of the study no patient died; during further follow-up one tumour-related death was documented.

Side-effects occurred in 29 (58%) patients, representing grade 1 and 2 toxicity only. Therapy was well tolerated except in one case in which it had to be disrupted because of a significant increase in liver enzymes. In none of the other patients were significant changes in blood count, liver enzymes, cholesterol or triglycerides registered. Side-effects included dryness of skin, lips, mucosa and conjunctiva (n=29; 58%) and other rare side-effects (hair loss, arthritis, nose bleeding) (Table 1).

Serum TG levels increased in 30 patients (60%), were unchanged in 8 (16%) and decreased in 12 (24%) (Table 2). Radioiodine uptake increased in 21 patients (42%), with a marked increase in 13 (26%), was unchanged in 29 patients (58%) and decreased in none of the patients (Table 3). In six patients, post-therapeutic dosimetry was possible by assessment of uptake, effective half-time and volumetric definition of the tumour mass. The radiation doses determined in the thyroid tumour mass were 6, 9, 12, 15, 50 and 53 Gy. Tumour size was assessable in 37 patients (74%), as explained above; an increase in tumour size was found in nine patients (18%), no change in 22 (44%) and a decrease in six (12%) (Table 4).

With regard to clinical outcome, patients were assigned to three categories: responders, with increased radioiodine uptake and decrease in TG level or tumour

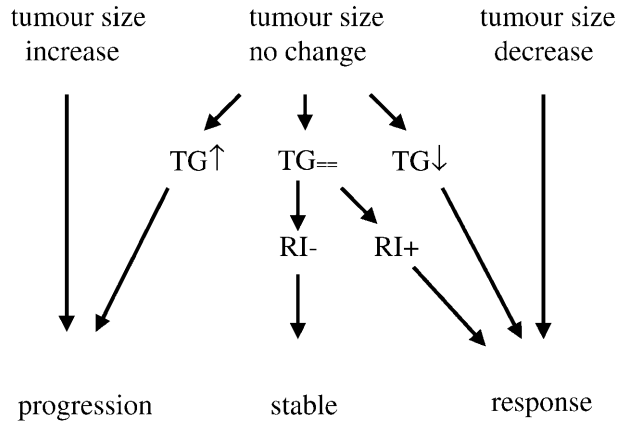

Fig. 3. Decision tree for assignment to outcome category. TG, Serum thyroglobulin; RI, radioiodine; ↑, increase; ↓, decrease; =, no change

Table 3. Correlation of radioiodine uptake and outcome

Radioiodine uptake	No.	Response	Stable	Disease progression
Marked increase	13	6	2	5
Mild increase	8	1	1	6
No change	29	3	6	20
Total	50	10	9	31

Table 4. Response of radioiodine uptake and tumour size after RA treatment, and assignment to clinical outcome category

Parameter	Increase	No change	Decrease
Iodine uptake	21 (42%)	29 (58%)	0
Tumour size	9 (18%)	22 (44%)	6 (12%)
	Response	Stable	Disease progression
Outcome	10 (20%)	9 (18%)	31 (62%)

size; stable disease, with no or insignificant changes in any of the parameters; and progressive disease, with failure of radioiodine uptake and increase in tumour size or TG levels. Tumour size was found to have the greatest influence on classification, followed by TG level and iodine uptake (Fig. 3). Patients were assigned to one of the three outcome categories by three independent examiners. Based on the aforementioned parameters, there were ten responders (20%) and nine patients (18%) with stable disease, accounting for 19 (38%) of the patients (Table 4). In this group radioiodine uptake increased in seven patients. Progressive disease was seen in 31 patients (62%). This group included 11 patients with disease progression despite increased radioiodine uptake (Table 3).

Discussion

Clinical setting

Differentiated thyroid cancer is a malignant tumour that has a fairly good prognosis, with patients surviving for many years. Multimodal therapy with surgery, radioiodine therapy and TSH suppressive therapy is of proven efficacy. However, loss of differentiation is observed in up to one-third of patients with DTC [1]. This is accompanied by histomorphological dedifferentiation (increased tumour grading) and loss of thyroid-specific functions, e.g. reduced or missing expression of thyrotropin receptor or reduced ability to accumulate iodine. Thus these tumours may no longer be amenable to standard treatment protocols, including TSH suppression and radioiodine therapy.

Patients included in this study had dedifferentiated tumours. However, survival in this selected group of patients with a poor prognosis in terms of thyroid cancer can be as long as many years. Therefore, response to therapy as reflected by arrest of tumour growth and radioiodine uptake were the parameters of interest, rather than survival. This rationale is supported by the fact that no deaths occurred during the course of the study. In those 13 patients in whom additional follow-up for a mean of 20 months was possible, one tumour-related death was documented. Furthermore, comparison with historical controls would suffer from bias owing to differences in the therapeutic approach, such as the radioiodine dosage and rhTSH application.

The molecular basis of dedifferentiation in thyroid carcinomas is not yet very clear. Various genetic alterations are known to occur in the development of thyroid cancer. Unlike in the Vogelstein model of colon carcinoma, genetic alterations do not clearly delineate either benign from malignant thyroid lesions or stepwise tumour progression from dedifferentiation. p53 mutation is the only genetic change that clearly correlates with poor prognosis and loss of differentiation, showing a frequent association with anaplastic carcinoma of the thyroid. However, there are some well-known markers of differentiation in thyroid carcinoma that can be correlated with retinoid effects. Loss of TSH receptor explains the insensitivity to TSH and the lack of effectiveness of TSH suppressive therapy with L-thyroxine [24]. Reduction or loss of sodium-iodine symporter (NIS) expression corresponds to the clinical phenomenon of loss of radioiodine uptake [25]. In addition, tumour dedifferentiation is accompanied by more aggressive tumour growth, leading to extensive and infiltrative local tumour growth or, much more frequently, to distant metastatic spread, so that surgical removal is neither sensible nor feasible.

Function of retinoic acids and experimental results

Retinoids are the natural, biologically active derivatives of vitamin A. They are involved in the regulation of growth, differentiation and morphogenesis in vertebrates and are known to be teratogenic (causing limb malformation) [2, 3, 10, 26]. In adults, retinoids maintain the functional integrity of lung epithelium, the testes, skin and eyes [9, 12, 27]. Differentiating effects have been shown in various cell culture models of promyelocytic leukaemia, phaeochromocytoma (PC12), neuroblastoma and others [28, 29, 30, 31, 32, 33].

RA signal transduction takes place via specific RA receptors, RARs and RXRs. These belong to the superfamily and nuclear receptors for small lipophilic ligands such as T3, vitamin D_3, glucocorticoids, steroid hormones and others [5, 6], which act as ligand-dependent transcription factors modulating gene expression. The retinoid receptors are specific for natural RA isoforms, i.e. RARs bind all-*trans*-RA and 9-*cis*-RA, while RXRs bind to RXRs only.

Redifferentiation therapy of thyroid cancer with RAs requires intact receptor pathways in the tumour tissues. The presence of RARs and RXRs and their subgroups has been studied in tumour cell lines and tissues by Northern blot, RT-PCR, electrophoretic mobility supershift and ligand binding assays [34]. RA receptor mRNAs, high-affinity binding sites for RA and functional DNA-binding RA receptors were demonstrated in thyroid tumour cell lines, and mRNAs for most receptor subtypes were seen in thyroid tumour tissues. There were two exceptions: RAR-beta mRNA was strongly reduced in a follicular thyroid tumour cell line and RXR-beta mRNA was undetectable in most of the examined tissues. However, given the known redundancy in the RA receptor signalling pathways [35], the RA receptor complement of thyroid carcinoma cells should enable them to transduce RA signals required for RA redifferentiation.

According to these considerations, thyroid differentiation markers or specialized thyroid functions, whose expression is reduced or lost in thyroid carcinomas, could be re-induced by RA in thyroid cancer model cell lines. Type I 5'-deiodinase (5'-DI), which deiodinates T_4 to its biologically active form T_3, is a differentiation marker in thyroid carcinomas [21, 24]. 5'-DI enzyme activity and the corresponding mRNA are stimulated by RA in well-differentiated follicular thyroid carcinoma cell lines [18]. Furthermore, the expression of the mRNA coding for NIS was enhanced in these two cell lines [36]. NIS mediates uptake of (radioactive) iodide into the thyrocyte and, therefore, plays a central role not only in iodide metabolism but also in the diagnosis and treatment of thyroid cancer [37]. As NIS expression and function are often lost in thyroid carcinomas [38], this molecule is a major target for redifferentiation therapy. E-cadherin is a well-established differentiation marker in various epithelial tumours, and loss of E-cadherin expression correlates with dedifferentiation, increased invasiveness and poor prognosis. This has also been shown in thyroid carcinoma [39]. In thyroid cell culture, RA is able to induce E-cadherin expression [40]. In one patient, induction of E-cadherin expression in a lymph node metastasis could be demonstrated after RA treatment (unpublished data). RAs also have antiproliferative effects. Follicular thyroid carcinoma cells (FTC-133) showed a 33% reduction in their number after 3 days of treatment with RA [39, 40]. Induction of expression of fas protein in the thyroid carcinoma cell line FTC-236 might indicate exertion of RA via apoptotic pathways [41]. Finally, tumourigenicity of follicular thyroid carcinoma cells (FTC-133) was found to be reduced after pretreatment with all-*trans*-RA prior to xenotransplantation onto nude rats.

The convincing results in clinical studies with other tumours and the above-mentioned promising experimental results have prompted various clinical studies on the administration of 13-*cis*-retinoic acid in patients with advanced thyroid cancer [42, 43, 44, 45]. A preliminary report was published on 20 patients, who are also included in this series [45]. A protocol was developed for retinoid therapy of thyroid cancer. This pilot study was performed in a multicentre fashion and represents the largest series of RA-treated patients with thyroid cancer. The approach is a palliative one based on the discussed theoretical and experimental results. The theoretical therapeutic implications are as follows:

1. Thyroid tumour cells regain former thyroid-specific properties such as iodine uptake, consequently allowing re-application of radioiodide therapy.
2. Pro-apoptotic pathways are restored, reflecting antiproliferative activity, or an improved immune response via cytotoxic activity is obtained.
3. TSH receptors are re-established and TSH responsiveness is re-induced.

Clinical results

The results of this clinical pilot study in a series of 50 patients clearly demonstrate a response to RA treatment in some of the patients. Enhanced radioiodine uptake and reduction or stabilization of tumour size and TG levels were the aims of the study. At this early stage of investigation, the final benefit for the patient in terms of the mentioned parameters is not always clear.

As could be demonstrated, increased radioiodine uptake does not always correspond to response of the disease. Despite improved iodine uptake, tumour progression was seen in some of the patients. Quantification was not available at the beginning of the study. Therefore evaluation was done by comparison of tumour lesion with background and physiological activity. With the advent of rhTSH application, a protocol for dosimetry was

developed. Thus dosimetry according to this protocol was performed in six patients only, in whom increased radioiodine accumulation was achieved. At least in some of the patients, the increase in radioiodine uptake was not sufficient to be effective in achieving tumour reduction. The doses measured in this small series of six patients were between 5 and 50 Gy, which are below the expected effective radiation doses. This explains why there was no tumour reduction in some patients with increased iodine uptake. Intracellular iodine trapping by inhibition of iodine efflux might offer a means of improving radioiodine uptake [46, 47]. NIS seems to play a key role in iodine uptake. However, the experimentally proven increased mRNA expression is not necessarily associated with increased iodine uptake. This dissociation of experimental data and clinical results may indicate that the promoter activity is involved in this mechanism.

Interpretation of serum TG levels is difficult in some patients. Serum TG level is accepted as a marker of tumour relapse, and increase in TG suggests an increase in the tumour mass. However, in the setting of redifferentiation, an increase in serum TG might be interpreted as reflecting both an increase in tumour mass and an increase in tumour differentiation, with subsequently enhanced production and release of thyroid-specific protein. In another study with long-term application of retinoids, TG levels showed a significant increase in patients who demonstrated increased radioiodine uptake [42, 43]. In our own experience, TG levels always increased when there was a growing tumour mass, whereas both increases and decreases in TG were observed when there was no detectable change in tumour mass. Among six cases with a reduction in tumour size, the TG level decreased correspondingly in five cases. Therefore in our evaluation increasing TG levels seemed to be primarily an indicator of tumour progression rather than redifferentiation. When tumour size was unchanged, we therefore classified as responders only those patients in whom a reduction in TG level occurred in conjunction with induction of radioiodine uptake; patients with stable TG levels were classified as having stable disease.

The most relevant parameter in assessing therapeutic success would be the tumour size. We expected increased radioiodine uptake to be the mechanism of tumour reduction. Thus, increased iodine uptake was the primary goal of RA therapy. However, we found that an increase in iodine uptake was not always associated with tumour reduction and, conversely, that when tumour regression occurred it was not always accompanied by increased iodine uptake. Experimental studies have shown that retinoids exert other effects in addition to induction of iodine transport. They also have antiproliferative effects that are mediated via pro-apoptotic pathways or direct actions on the cell cycle [45, 48, 49, 50]. In this context, synthetic RAs might play a future role, with their specific effects on growth regulation and apoptosis [22, 51].

Summarizing the data, 19 patients (38%), including nine with stable disease, showed a response to RA treatment. Inclusion of patients with stable disease after a mean follow-up of 16 months (median 18 months, range 5–46 months) seems justified bearing in mind that all of these patients had documented tumour progression before RA therapy. No deaths occurred during the observation period of the study. During further follow-up there was one tumour-related death.

In a significant number of the patients (13/50; 26%) the “hard” criterion of tumour size was not assessable owing to diffuse metastases which are not amenable to quantification by means of CT scan or MRI. There was no correlation between RA response and histological subtype.

Until now, only advanced tumour stages have been treated with retinoids. Suppression of NIS in normal thyrocytes and differentiation-dependent effects of RAs in experimental studies might offer opportunities for the application of retinoids in less advanced and dedifferentiated tumours [34, 36]. The encouraging results of recent studies and the good tolerability of retinoids, with a low rate of side-effects, warrant further studies with altered inclusion criteria, employment of other redifferentiating agents or combinations of agents, and other imaging techniques. A multicentre trial has been initiated to study whether the direct antiproliferative action of retinoids or the increased radioiodine uptake is responsible for the tumour response.

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