The effect of radioiodine treatment on the diseased thyroid gland

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Introduction and background

In health, thyroidal iodine metabolism involves absorption oxidation and organification of iodide ions (I\(^{-}\)) to form the thyroid hormones triiodothyronine \(\text{T3}\) and thyroxine \(\text{T4}\) (Fisher 1996). The unique avidity of thyroid tissue to absorb iodide offers a highly targeted means of treating thyroid disease. In the context of benign thyroid disease, radioiodine \(\text{I}^{131}\) therapy is the treatment mainstay for several benign and malignant thyroid disorders, however \(\text{I}^{131}\) is known to cause DNA damage and liberation of thyroidal self-antigens inducing secondary immunoreactivity. The exact mechanisms underpinning cellular death and subsequent induction of autoimmune thyroid disease following \(\text{I}^{131}\) treatment have not yet been fully elucidated. This manuscript aims to review the literature concerning the effects of \(\text{I}^{131}\) on the thyroid gland.

Conclusion: The effects of \(\text{I}^{131}\) on malignant thyroid cells appears to depend on absorbed dose with the literature demonstrating a clear initial delay in the triggering of apoptosis in response to \(\text{I}^{131}\)-mediated cellular damage. Some studies also observed necrotic cellular death following high-dose \(\text{I}^{131}\) treatment. Liberation of thyroidal self-antigen following \(\text{I}^{131}\) treatment helps to explain phenomena such as the subsequent induction of autoimmune thyroid disease. The clinical utility of cytokines and autoantibodies for prognostication of hypothyroidism and treatment failure following \(\text{I}^{131}\) remains uncertain and further appropriately-powered studies are required to clarify their role. The potential role of other cell death mechanisms activated after treatment with \(\text{I}^{131}\) should also be explored in order to fully delineate the thyroidal response.

Abbreviations: \(\text{I}^{131}\): radioactive iodine; DNA: deoxyribonucleic acid; TPO: thyroid peroxidase; TSH: thyroid stimulating hormone; TSHR: thyroid stimulating hormone receptor; GD: graves’ disease; \(\text{T3}\): triiodothyronine; \(\text{T4}\): thyroxine; MeV: mega electronvolt; PS: phosphatidylserine; ATM: ataxia telangiectasia mutated; ATR: ataxia telangiectasia and Rad3-related; GBq: giga-bequerel; MDM2: murine double minute 2 homolog; TG: thyroglobulin; CDK2: cyclin-dependent kinase; RRTC: radioiodine-refractory thyroid carcinoma

J\(^{131}\) is a systematically administered radioactive nuclide which decays to xenon 131 by emitting both beta and gamma particles. The principal beta particles (89.3%) exhibit an energy of 606 keV and can be used for radiotherapy. In contrast, the principal gamma particles (81.2%) exhibit a 364 keV photopeak and are used for clinical imaging (Wyszomirska 2012). Beta-irradiation has a maximal tissue penetration range of 2.2 mm, achieving cytotoxicity by direct and indirect DNA damage of thyroid cells which subsequently triggers cell death (Wyszomirska 2012).

It has previously been postulated that cell death in response to \(\text{I}^{131}\) occurs through both apoptosis and necrosis. Apoptosis, first described by Kerr and colleagues, is an energy dependent, programmed form of cell death first associated with cellular elimination during biological events such as embryonic development and healthy tissue turnover (Kerr et al. 1972). The process of apoptosis is strictly controlled in order to minimize local inflammation; cells are ‘marked’ for phagocytosis by translocation of the phospholipid phosphatidylserine (PS) onto the outer leaflet of the cellular plasma.
membrane and retain both plasma membrane integrity and some degree of metabolic activity until they are cleared by phagocytic cells (efferocytosis; Green et al. 2016). The immunological quiescence of apoptosis is further maintained by the release of anti-inflammatory cytokines, however in vitro studies have demonstrated a transition of end-stage apoptotic cells toward a necrotic morphotype in the absence of cells with phagocytic abilities in a process termed ‘secondary necrosis’ (Vanden Berghe et al. 2010). Necrosis is a passive, unprogrammed form of cell death that results from environmental perturbations causing loss of membrane integrity and thus the uncontrolled release of inflammatory cellular contents (Galluzzi et al. 2018). These components, also known as damage-associated molecular patterns (DAMPs), represent a heterogeneous group of cellular molecules which, according to the ‘danger model’ proposed by Matzinger (1994), are immuno-stimulatory. Furthermore, the immune-stimulatory effects characterizing necrotic cell death are also evident with secondary necrosis, underpinned by the release of DAMPs (Sachet et al. 2017).

The downstream effects of apoptosis are initiated by either the intrinsic or extrinsic pathway (Figure 1), the former precipitated by activation of the DNA damage response (DDR) pathway (Szondy et al. 2017). The DDR pathway safeguards genomic integrity and maintains cellular homeostasis by sensing damage to the DNA molecule and inhibiting cellular division until DNA repair or cellular elimination has occurred (Negrini et al. 2010). The initial steps of the DDR involve ataxia-telangiectasia mutated (ATM) protein kinase and ataxia telangiectasia and Rad3-related (ATR) kinase. Both ATM and ATR proteins detect DNA damage and activate downstream proteins by phosphorylation (Abraham 2001). Phosphorylation of the tumour suppressor protein p53 by ATM in response to DNA damage leads to its dissociation from MDM2 and consequent nuclear accumulation. p53 then initiates the production of p21 which complexes with the cell division stimulating protein cyclin-dependent kinase (cdk2) halting cell division at the G1 or G2 phase (Zilfou and Lowe 2009). Furthermore, during the intrinsic pathway of apoptosis,
nuclear accumulation of p53 stimulates the transcription of pro-apoptotic genes such as PUMA and NOXA. The resulting proteins function to inactivate the anti-apoptotic Bcl2-family of proteins and activate pro-apoptotic proteins Bax or Bak triggering mitochondrial outer-membrane permeabilisation (MOMP; Borges et al. 2008). MOMP precipitates apoptotic cell death by irreversibly permitting the release of the pro-apoptotic factor cytochrome c from the mitochondria. Once liberated, cytochrome c induces formation of the apoptosome, a multimeric complex constituting the adapter molecule Apaf-1 and dATP (Li et al. 1997). Apaf-1 oligomerization induces the recruitment of caspase-9 which in turn activates effector caspases 3 and 7 thus initiating the ‘execution phase’ of apoptosis (Movassagh and Foo 2008). Caspase cleavage of various cellular substrates then leads to the morphological features of apoptotic cell death (Degterev et al. 2003). Furthermore, cleavage of ICAD (inhibitor of caspase-activated DNase) permits cleavage of DNA into classical oligomeric fragments by caspase-activated DNase whilst cleavage of cytoskeletal proteins such as actin and lamin leads to loss of gross cellular shape and nuclear shrinkage respectively (Fulda and Debatin 2006). Although p53 functionality is vital for the successful induction of intrinsic apoptosis, the extrinsic pathway of apoptosis relies on the interaction of so-called death ligands such as Fas, tumor necrosis factor-α (TNF-α) and TNF-related apoptosis inducing ligand (TRAIL) with their respective cell-membrane bound receptors (Movassagh and Foo 2008). This ligand–receptor binding leads to the eventual formation of the death inducing signaling complex (DISC) and subsequent proteolytic activation of caspases 8 and 10 whose activation of downstream effector caspases lead to the execution phase of apoptosis (Yang et al. 1998).

Elucidating the mechanisms by which thyroid cancer cells undergo death in response to treatment with ¹³¹I is important to identify the lowest effective ¹³¹I dose and develop successful adjuvant therapies and assays capable of predicting treatment outcome. Furthermore, outlining the pathways through which radioiodine treatment causes cell death may identify molecular targets which can act as radiosensitizers. This paper aims to discuss the intricate biological interactions concerning thyroid cell death and subsequent immune modulation which occur following treatment of thyroid disease with ¹³¹I.

**Thyroid cancer treatment using ¹³¹I**

Differentiated thyroid carcinomas (DTC) make up approximately 90% of all thyroid cancers (Yan et al. 2017). Most DTCs retain basic features of differentiation such as expression of the sodium iodide symporter (NIS). The NIS mediates active uptake of iodide across the basolateral membrane, essential for the successful downstream effects of ¹³¹I in the treatment regimen of DTC (Luster et al. 2008). ¹³¹I treatment of metastatic thyroid lesions was first described in the 1940s by Seidlin (reviewed by Siegel 1999) and is now an integral part of DTC management. ¹³¹I treatment for DTC aims to eradicate remnant or disseminated DTC material following surgical removal of the primary lesion, thus allowing the subsequent monitoring of thyroglobulin (Tg) production as a specific marker of recurrence. Detectable Tg in serum is highly suggestive of recurrent or metastatic disease (Luster et al. 2008; Jia et al. 2017). Patients with a pT1/2 tumor without nodal metastases and no microscopic residual disease (R0) receive 1.1 GBq ¹³¹I whilst pT3 and/or N1 tumors potentially receive 3.7 GBq dependent on the presence of other prognostic factors (Perros et al. 2014). The effective half-time for the total body clearance of radiiodine is approximately 132 h, with an initial rapid clearance due to urinary excretion and secondary clearance by catabolism of organified radioiodine (Tabei et al. 2012). Tumor resection followed by ¹³¹I treatment is considered best practice in high-risk thyroid cancers (Mitchell et al. 2016).

Though DTC typically remains localized, up to 15% of patients develop distant metastasis, which is the most important prognostic marker of poor long-term survival (Mazzaferri and Massoll 2002). Two thirds of patients with distant metastases progress to develop radioiodine-refractory thyroid carcinoma (RRTC), becoming unresponsive to ¹³¹I treatment. In these cases, 10-year survival declines to 10%, although the prevalence of RRTC is relatively low, with an incidence of 4–5 cases per year per million people (Elisei et al. 2010; Schmidt et al. 2017). Though the ability to absorb ¹³¹I is totally lost in some patients, such as those suffering RRTC, it is always somewhat reduced in malignant thyroid tissue. Furthermore, the efficiency of accompanying mechanisms governing the organification of iodide are effected in the context of malignancy, compounded by the relatively short (8.02 days) effective half-life of ¹³¹I (Thomas-Morvan et al. 1974; Robbins and Schlumberger 2005).

Some studies conducted previously have sought to elucidate iodide processing mechanisms in the context of thyroid malignancy. Lazar et al. (1999) investigated expression of functional genes encoding NIS and thyroid peroxidase (TPO) in healthy and malignant thyroid tissue using real-time PCR and found that median NIS mRNA expression was 100-fold lower in malignant thyroid tissue, whilst TPO mRNA expression was 5- to 500-fold lower. Furthermore, mRNA expression of these genes was negatively correlated with advanced tumor stage which is indicative of accumulation of genetic defects and thus dedifferentiation with disease progression. This is concordant with other studies employing immunohistochemistry to assess NIS expression (Caillou et al. 1998; Jiang et al. 1998). Moreover, Min et al. (2001) found that NIS expression in thyroid cancer predicted ¹³¹I uptake with 100% specificity, although it offered a sensitivity of just 50%. In their study of 28 tissue samples extracted from patients showing positivity for ¹³¹I uptake, 50% stained positive for NIS expression whilst the other 50% exhibited no NIS expression. Moreover, all 12 samples retrieved from patients with negative ¹³¹I uptake scans showed negative NIS expression.

Given the prognostic advantage to retaining NIS expression, attempts have been made to reverse de-differentiation using novel agents (Nagarajah et al. 2016). Limited clinical
efficacy was found using existing agents such as isoretinoin and rosiglitazone (now withdrawn from the market). These agents demonstrated restoration of NIS expression and measurable $^{131}$I uptake, although few patients demonstrated tumor mass or TG level reduction (Simon et al. 2002; Tepmongkol et al. 2008). There is growing interest in novel tyrosine kinase inhibitors (TKIs) including selumetinib dabrafenib and CH512676 (known as CKI) as through selective inhibition of the mitogen-activated protein kinase (MAPK) pathway, these agents can stimulate radiiodine uptake in RRTC in patients with BRAF and NRAS mutations (Ho et al. 2013; Rothenberg et al. 2015; Nagarajah et al. 2016). Phase 3 clinical trials are awaited for selumetinib and dabrafenib, although preclinical results using CKI in the murine model demonstrated complete disappearance of tumor compared to selumetinib. However, it is unclear whether CKI is tumoricidal or a highly potent redifferentiating agent (Nagarajah et al. 2016).

There has been a general trend towards treatment de-escalation of low risk DTC with active surveillance a recommended option for papillary microcarcinoma with low-risk features according to American Thyroid Association guidelines (Haymart et al. 2017). In UK clinical practice, lobectomy is offered for papillary microcarcinoma with no other risk factors. Following this, postsurgical radiiodine remnant ablation (RRA) may also be offered according to histological features such as tumour size (Perros et al. 2014). Data on cancer survival and recurrence following RRA is largely retrospective and clear recommendation cannot always be made for certain patient groups of patients therefore individualization of treatment is required (Perros et al. 2014). Furthermore, the need for RRA may indeed decline if the trend towards de-escalation in low-risk cancer continues (Kovatch et al. 2018).

**DNA damage response to $^{131}$I**

Beta-particles from $^{131}$I pass directly through the tertiary DNA structure causing molecular ionization and subsequent damage of the DNA molecule directly. In addition, cellular water can become ionized by the aforementioned beta-particles in a process termed radiolysis, first described by Louis Harold Gray (Gray 1953). This process leads to the formation of a highly reactive hydroxyl (OH•) radical. Free radicals are characterized by the presence of a highly reactive unpaired electron within their structure which causes oxidative degradation of plasma membrane lipids or structural damage (i.e. damage to the DNA double helix configuration; Russo et al. 2013). Although the radiation dose absorbed by the cell nucleus is considered the main parameter, there is evidence of cell death being initiated by damage to other cellular compartments (Shao et al. 2004; Hei et al. 2009). OH• mediated DNA damage can result in base or sugar-phosphate backbone damage leading to both single strand (SSB) and double strand (DSB) DNA breaks or DNA cross-linkage all of which have the potential to activate apoptosis (Elmore 2007; Maier et al. 2016). As radiation damage to healthy tissue is unavoidable in radiotherapy, the reduced efficiency of DNA repair mechanisms in cancer cells is one of the key factors in the success of radiotherapy for the treatment of malignant disease (Sutherland et al. 2000; Jeggo and Lobrich 2015; Pajonk et al. 2010). Repair of DNA damage, Redistribution of cells in the cell cycle, Repopulation, and Reoxygenation (Mitchell 2013).

DNA damage resolution is dependent on the cell type affected, the extent of DNA damage and potentially the abundance of p53 within injured cells. With low levels of DNA damage, p53 initiates cell cycle arrest, regulates DNA damage repair and induces other pro-survival pathways. With high levels of DNA damage, p53 initiates the apoptotic response (Helton and Chen 2007). Furthermore, the differential presence of p53 responsive genes in active chromatin between different cell types can predispose a cell to either apoptosis or cell cycle arrest in response to DNA damage (Eriksson and Stigbrand 2010). p53 is one of the most commonly mutated genes in malignant tumors and is corrupted in over 50% of human cancers, leading to a higher level of resistance in these malignancies (Brosh and Otter 2009) although its aberration is seldom seen in well-differentiated thyroid cancer (Manzella et al. 2017). In contrast, 50–80% of undifferentiated, anaplastic thyroid carcinomas (ATC) possess mutations in TP53 (Manzella et al. 2017). It has been previously postulated that ATC develops from BRAF-mutated (V600E) papillary thyroid cancer (PTC) due to p53 mutation and consequent loss-of-function; >98% PTCs possess a functional p53 gene (Ito et al. 1992). Therefore, thyroid carcinoma appears not to develop according to the Vogelstein model whereby p53 aberration is a crucial activating mutation transforming adenoma to carcinoma (Arends 2000). In fact, p53 mutation in thyroid carcinogenesis appears to be a late-stage occurrence allowing the progression (and de-differentiation) of the thyroid tumor occurring alongside a marked increase in the rate of cellular proliferation (Zou et al. 1993). In the context of $^{131}$I treatment, Zhang et al. (2015) found that apoptosis following $^{131}$I exposure may be p53-independent as they did not find increased levels of p53 mRNA or protein product after treating cells with $^{131}$I. However, more recently, Zhao and Pang (2017) demonstrated that $^{131}$I did increase the expression of p53 in a dose-dependent manner, which was correlated with increased apoptosis and downregulation of Bcl-2. In addition, tumor cells which retain an intact p53 gene frequently possess aberrant downstream apoptotic mechanisms. These mutations include increased expression of anti-apoptotic (e.g survivin and BCL-2) and reduced expression of pro-apoptotic (e.g BAX CD95 death receptor) genes (Igney and Kramer 2002). The incongruence in the findings of Zhang et al. and Zhao and Pang demonstrate the need for further clarification of the role of p53 in this setting. Furthermore, the role of the p53-like protein p73, which belongs to the same family and possesses high homology with the p53 protein itself has been investigated in the context of thyroid...
The metabolic activity of cells treated with a range of I\(^{131}\) on the thyroid

The role of the p73 protein in thyroid cancer is not yet fully clear. The TAp73\(x\) and ΔNp73 p73 isoforms have been detected in thyroid cancer cell lines but not in normal cultured thyrocytes. Moreover, the biological activity of these p73 isoforms was found to be impaired in thyroid cancer cells, potentially suggesting that functional impairment of p73 occurs during malignant transformation (Frasca et al. 2003). Interestingly, a more recent study conducted by Malaguerna et al. found that the TAp73\(x\) isoform exerted a synergistic effect on p53 tumour suppression by disrupting Mdm2-mediated p53 degradation in thyroid cancer cells (Malaguerna et al. 2008). The delicate ratio of pro- and anti-apoptotic factors and its modulation of thyroid apoptosis and survival (Shi et al. 2010) has been investigated in multiple studies. Gunda et al. (2014) investigated apoptotic resistance in thyroid cells and found that the anaplastic cell line 8505c possessed very high basal expression of the anti-apoptotic protein BCL-X\(_L\) coupled with low expression of BAX. Furthermore, treatment of the cells with the TRAIL-R2 death receptor agonist lexatumumab was associated with a significant rise in the anti-apoptotic protein PAXT in the 8505c cells compared to other sensitive cell lines where PAXT was suppressed. A separate study found that innate overexpression of the transcription factor TWIST1 in anaplastic thyroid cells led to upregulation of microRNA 584 which negatively regulates the pro-apoptotic protein TUSC2 (tumor suppressor candidate 2; Orlandella et al. 2016). Nuclear factor kappa-B (NFκB) is also implicated in modulation of the anti-apoptotic cascade following both intrinsic and external stimuli (Barkett and Gilmore 1999). Amplified NFκB activity has been linked to thyroid carcinogenesis as evidenced by increased mRNA and protein expression of p65 (one of five known NFκB members) in thyroid cancer cells versus normal cells (Visconti et al. 1997).

Studies investigating the effect of I\(^{131}\) on the thyroid

Zhang et al. (2015) studied the effect of I\(^{131}\) on the human cell line HTori-3, previously characterized as a non-tumorigenic differentiated thyrocyte cell line (Caudill et al. 2005). The metabolic activity of cells treated with a range of I\(^{131}\) activities over various time points demonstrated a time- and dose-dependent decrease in reduction of MTT to formazan, indicating a reduction in mitochondrial activity. Interestingly, Marx et al. (2006) detected an initial upsurge in the metabolic activity in poorly-differentiated B-CPAP thyroid cells immediately following a 'low dose' (1–10 MBq ml\(^{-1}\)) I\(^{131}\) treatment compared to a 50% reduction following a 'high dose' (10–100 MBq ml\(^{-1}\)) I\(^{131}\) treatment. The authors postulate that this initial rise in metabolic activity is perhaps due to increased activity of mitochondrial dehydrogenases as part of a gross cellular response to accrued DNA damage. Further evidence for this notion is supported by a 50% reduction in viability after continued incubation with activity-free medium representing a delayed induction of cellular death (Marx et al. 2006). Furthermore, Zhang and colleagues measured apoptosis by annexin-V-fluorescein isothiocyanate staining using flow cytometry observing dose-dependent apoptosis in response to I\(^{131}\). Cells treated with 7.4 14.8 and 22.2 MBq ml\(^{-1}\) I\(^{131}\) for 48 h were 20.3, 35.4 and 53.6% positive for apoptosis, respectively. The data also illustrate a time-dependent apoptotic response: apoptosis was only marginally increased after 24 h treatment however the apoptotic index increased to 35.4% after 48 h and 50.6% after 72 h (Zhang et al. 2015). This slow initiation of apoptosis was also seen in a study by Del Terra et al. (2001) whereby DNA fragmentation, consistent with late stage apoptosis, was only detectable after 48 h of UV light exposure in FRTL-5 (rat) thyroid cells. Additionally, Marx et al. (2006) had previously found that exposure of B-CPAP cells to I\(^{131}\) resulted in a significant induction of apoptosis after 48 h treatment with highest levels observed after treatment with 5MBq ml\(^{-1}\) I\(^{131}\). These authors suggested that delayed apoptosis in B-CPAP may be due to point mutation of the p53 gene, causing prevention of early apoptosis (Marx et al. 2006). However, the authors concede that the actual number of apoptotic cells may have been under-represented in the study as their assay of choice was incapable of detecting specific early or late stage apoptotic events such as loss of membrane asymmetry. Interestingly however, significant initiation of apoptosis only occurred after 48 h treatment in all studies using thyroid derived cell lines, regardless of their p53 status, demonstrating an innate delay in the initiation of apoptosis.

Russo et al. (2013) investigated the response of ex vivo benign thyroid tissue fragments (1mm\(^2\)) to either 3.7 or 37MBq I\(^{131}\), quantifying apoptosis by measurement of the neo-epitope M30 produced during apoptosis-mediated caspase cleavage of intracellular cytokeratin. Both 3.7 and 37MBq I\(^{131}\) were capable of inducing an apoptotic response with M30 fragments detectable in supernatant after 72 h treatment (Russo et al. 2013). This outcome conflicts with the hypothesis by Marx et al. (2006) that p53 corruption is the culprit for delayed induction of apoptosis as the benign thyroid tissue used in their experiments would likely possess an intact p53 gene, although p53 expression was not evaluated within the study. In contrast, the same group report that 10\(\mu\)M staurosporine, an inducer of apoptosis, was capable of producing a significantly increased level of apoptosis within thyroid tissue fragments after just 24h treatment. Ohnishi et al. (2009) quantified phosphorylation of histone H2AX, a process triggered in response to DNA DSBs by immunostaining. They found that 90 min treatment with 0.37MBq I\(^{131}\) induced a significant induction of DSBs in FRTL-5 cells (91% versus 4.3% in the control group). Continued culture of treated cells within activity-free medium revealed a partial recovery of the DNA DSBs after 24 h, however flow cytometric evaluation of cellular viability after a further 6 days by 7-AAD uptake revealed no significant difference from control cells (Hershman et al. 2011). Overall it appears that both malignant and benign thyroid cells sensitive to I\(^{131}\) treatment are subject to cell death by apoptotic mechanisms in both a dose- and time-dependent.
manner. The significance of the p53 protein in the induction of thyr
cocyte apoptosis is still not fully elucidated. Interestingly, Zhang et al. (2015) observed an upregulation of Fas ligand expression following $^{131}$I treatment of thyroid cells, indicating that both the intrinsic and extrinsic pathways are involved in apoptotic signal transmission. This phenomenon has also been observed in other cell types such as leukemia (Friesen et al. 2003) and breast cancer (Wang et al. 2011).

Furthermore, studies have explored the involvement of necrosis in response to $^{131}$I treatment. A study carried out by Turgut et al. (2006) aimed to distinguish between apoptotic and necrotic cell death following $^{131}$I treatment. Wistar rats were subjected to a mean $^{131}$I activity of 34.59 MBq before thyroidal DNA was extracted and damage analyzed by DNA electrophoresis. Interestingly, the majority of rat DNA (17/18) was hyper-fragmented consistent with necrotic damage, with 72% of those rats’ DNA showing apoptotic fragmentation. Furthermore, in one of the 18 treated rats no DNA damage was detected, whilst four rats possessed partially healthy DNA alongside necrotic and apoptotic damage suggesting an individual (epi) genetic resistance to radiation damage. In addition, Marx et al. (2006) noted a clear dose-dependency in terms of cell death mode in their experiments using B-CPAP cells; 10MBq ml$^{-1}$ $^{131}$I ($\sim$40–50 Gy) induced a primarily necrotic response whereas necrosis was rarely observed at an activity of $<1$ MBq ml$^{-1}$ $^{131}$I ($\sim$5Gy).

**Thyroid immune cells, grave’s disease and $^{131}$I**

In addition to causing thyroid cell death as desired against malignant cells $^{131}$I can have local and systemic effects on the immune system causing additional effects on the thyroid gland $^{131}$I is used to treat Graves’ disease (GD) – an autoimmune thyroid disorder characterized by the production of autoantibodies and thyrotoxicosis (Smith and Hegedus 2016). GD represents the failure of tolerance to thyroid self-antigen and the consequent production of autoantibodies most commonly against the thyroid-stimulating hormone receptor (TSHR-Ig; Smith and Hegedus 2016).

The link between $^{131}$I therapy and thyroid autoimmunity appears to occur through thyroid cell death, whereby self-antigens are liberated from the thyroid gland following $^{131}$I exposure. In 1997, DeGroot hypothesized that $^{131}$I treatment of GD causes a biphasic change in immunoreactivity in keeping with antigen exposure (DeGroot 1997). Firstly, an initial exacerbation of GD and its extrathyroidal complications occurs, with the most common being Graves’ ophthalmopathy. Secondly, antigen exposure immunoreactivity and clinical severity are reduced with progressive thyroid ablation. $^{131}$I-induced cell injury could release genomic double-stranded DNA (dsDNA) into the cytosol which is recognized by extrachromosomal histone H2B in thyroid cells. Furthermore, non-infective, non-radioactive cell injury was shown to induce the production of type I interferon inflammatory cytokines and chemokines in cultured thyroid cells; dsDNA can also induce antigen presentation molecules and suppress the expression of the sodium/iodide symporter and subsequent $^{131}$I uptake (Kawashima et al. 2011). T cell activation combined with an increase in the activity of inhibitors of suppressor T cells (contra-suppressor T cells) may also be involved (Teng et al. 1990). These findings demonstrate the role of cell injury on inducing or augmenting immunoreactivity and exacerbating existing autoimmune processes or inducing GD in previously non-autoimmune disease.

The thyroid gland, like all tissues, contains antigen presenting cells (APCs sometimes known as thyroid dendritic cells; Purnamasari et al. 2015). Specialised APCs also exist in draining lymph nodes where they take up and process antigen for presentation to T lymphocytes (Hassan et al. 2013; Figure 2). This process involves several checkpoints (e.g. co-stimulation) and regulatory elements (i.e. immunosuppressive regulatory cells) important in both controlling normal immunity against pathogens and preventing autoimmunity (Chen and Flies 2013; Kristensen 2016). Genetic and/or epigenetic susceptibility likely facilitates immune dysregulation allowing autoreactivity to progress against liberated self-antigens following $^{131}$I therapy (Wang et al. 2017). The risk of radiation-induced GD when $^{131}$I is used to treat functional thyroid autonomy is increased in patients with existing immunopathy, hypeochogenicity on ultrasound, elevated anti-thyroid peroxidase (TPO-Ig) or borderline positive TSHR-Ig (Dunkelmann et al. 2004). These variables for autoimmunity predisposition provoke the notion of an ‘autoimmune threshold’ that must be reached to establish clinically significant autoimmunity (Blyuss and Nicholson 2015). This is one of the leading hypotheses of autoimmunity initiation whereby genetic predisposition (e.g. polymorphisms in major histocompatibility complex genes and cytokine signaling components) and environmental factors (e.g. infection and the microbiome) interact which leads to autoreactive T cell formation in the context of defective regulation by T regulatory cells (Rosenblum et al. 2015).

The GD thyroid gland exhibits a predominantly CD4+ T cell infiltrate with greater numbers of B-cells forming germinal centers than would be found in healthy individuals. In GD, thyroid infiltrating lymphocytes were found to demonstrate a T helper (T$_h$) 2 profile of secreted cytokines: interleukin (IL)-4 IL-5 IL-10 and IL-13 which can activate B lymphocytes and cause autoantibody production (Gianoukakis et al. 2008). However, it appears that $^{131}$I can shift this profile to favor a T$_h$1 response. Jones et al. (1999) studied the cytokine profile of peripheral blood mononuclear cells in GD patients treated with $^{131}$I. They found that $^{131}$I therapy normalized IL-4 and IFN-$\gamma$ from low levels pre-treatment as compared to healthy control subjects. Furthermore, $^{131}$I therapy elevated IL-6, IL-10 and tumor necrosis factor-alpha (TNF-$\alpha$) from normal levels on post-treatment day 17. Although the evidence for IL-10 is varied, this profile would favor cell-mediated immunity (T$_h$1) rather than autoantibody production (T$_h$2; Jones et al. 1999).

Similar findings have been observed in cancer treatment with $^{131}$I. For example, $^{131}$I therapy for DTC was found to reduce peripheral blood cell secretion of T$_h$2 cytokines (IL-4...
IL-5 and IL-13) although the significance of this is uncertain given that the Th1-to-Th2 ratios were not different to healthy control patients (Simonovic et al. 2015). The apparent inadequacy of a strict Th1/Th2 balance suggests the idea of a spectrum of possible T helper and effector cell responses which is governed by the secreted cytokine profile. The apparent shift toward a Th1 response would fit with CTLs targeting thyroid cells within the autoimmune milieu. A similar effect of I131 may be seen in the tumor microenvironment of thyroid cancer and this represents an interesting area for future research which is currently lacking. The interaction between I131 and immune mediators could also be critical in GD pathophysiology and with greater understanding it may be possible to use them as prognostic markers. The prototypical CXC chemokine ligand 10 (CXCL10) is known to be dysregulated in GD. The elevated baseline serum levels of CXCL10 in GD patients are reduced after 6 months of I131 treatment which destroys the thyroid tissue and most probably attenuates the local immune response (Antonelli et al. 2007). Conversely, intercellular adhesion molecule 1 (ICAM-1) levels were shown to increase and then stabilize after 12 months (Jurgilewicz et al. 2002; Dong et al. 2011; Leite et al. 2011). As ICAM-1 elevation is a marker of endothelial cell and fibroblast activation, this may represent the activation of repair processes within the inflamed thyroid gland (Crescioli et al. 2007).

Despite the clinical efficacy of I131 treatment, a secondary immune response can be observed through both reduced serological remission and increased post-treatment TSHR-Ig levels relative to medical therapy (antithyroid drugs) or surgery (Lauberberg et al. 2008). Conflicting data exists on whether pre- or post-I131 serological titers of TSHR-Ig have prognostic value in predicting hypothyroidism or treatment failure. Studies have suggested that the presence of TSHR-Ig before or after I131 treatment predicts post-I131 hypothyroidism (Chiovato et al. 1998; Ahmad et al. 2002) although others indicate that pre-I131 treatment serology is not significantly associated with post-I131 hypothyroidism (Andrade et al. 2001; Gomez-Arnaiz et al. 2003). However, complete ablation of thyroid tissue with I131 therapy causes progressive disappearance of pre-existing autoantibodies (TSHR-Ig TPO-Ig and anti-thyroglobulin) in patients with autoimmune thyroid disease (Chiovato et al. 2003). Together these findings support the concept that autoantibody production relies on ongoing autoantigen presence and that I131 treatment appears to liberate self-antigens until complete ablation has been achieved. It is possible that variance in the degree of ablation of thyroid tissue by I131 explains these disparities.
Summary and conclusions

I\textsuperscript{131} induces thyroidal cell death as a result of both direct and indirect damage to thyrocyte DNA largely through apoptosis. Some studies however have detailed findings of necrotic cell death following treatment using I\textsuperscript{131}. The effects of I\textsuperscript{131} on malignant thyroid cells appears to depend on absorbed dose with the literature demonstrating a clear initial delay in the triggering of apoptosis in response to I\textsuperscript{131}-mediated cellular damage. Further studies should investigate the potential role of other cell death mechanisms activated after treatment with I\textsuperscript{131} in order to fully delineate the physiological thyroidal response. An additional and distinct indirect effect of I\textsuperscript{131} treatment on the thyroid is the induction of secondary immunoreactivity by liberated thyroidal self-antigens which augment intra-thyroidal inflammation. Serological changes are induced following I\textsuperscript{131} treatment with a shift in the immune profile from autoantibody production towards a chemokine response to attract immune cells to inflamed thyroid tissue. The clinical utility of cytokines and autoantibodies for prognostication of thyroid function in Graves' disease or toxic nodular goiter. J Clin Endocrinol Metab. 92(4):1485–1490.

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