Immune responses in the thyroid cancer microenvironment: making immunotherapy a possible mission

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Abstract

The incidence of thyroid cancers has been steadily increasing worldwide over the past few decades. Although five-year survival rates for differentiated thyroid cancers are upwards of 90%, clinical outcomes for patients with undifferentiated, recurrent and/or metastatic disease are often dismal despite conventional interventions. As such, there is a demand for novel treatment options. Cancer immunotherapy represents the ultimate form of personalized medicine by leveraging the specificity and potency of a patient's immune system to kill their tumor. The thyroid cancer microenvironment is rich in immunological cells, making it a reasonable candidate for immunotherapy. This review maps out the immunological features of thyroid cancers and how these can be modulated. There are surprising immunological consequences of conventional therapies that demand attention. Also, hormonal modulation of the immune system is highlighted as a unique and confounding feature of thyroid cancers. A variety of cutting-edge immune-based therapies are discussed, with an emphasis placed on how these can be integrated with the current standard of care. Several high priority areas in need of research are also highlighted.

Key Words

- thyroid
- thyroid cancers
- immune system
- immunotherapy

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Introduction

The most common endocrine cancers are those that originate in the thyroid gland (Kilfoy *et al.* 2009). Although the reported incidence of thyroid cancers has increased steadily over the past few decades, this may be due, in part, to improved detection as a result of technological advancements in diagnostic imaging (Kent *et al.* 2007). However, it is thought that societal increases in risk factors such as obesity are also a contributing factor (Han *et al.* 2013, Morris *et al.* 2013). The vast majority of thyroid cancers (i.e. ~90% of diagnosed cases) are derived from differentiated follicular cells and can be subdivided into two main types: papillary and follicular, which are

the most and second most common subtypes, respectively (Cabanillas *et al.* 2016, Howlader *et al.* 2016). Papillary and follicular thyroid cancers are generally associated with good prognoses (up to a 97% five-year survival rate) if conventional treatments are implemented (Cabanillas *et al.* 2016). The medullary form represents only ~5% of thyroid cancers, originates from the parafollicular cells, is usually moderately differentiated and associated with a five-year survival as high as 86%. Poorly differentiated thyroid carcinomas and the anaplastic (undifferentiated) types account for less than 5% of cases. Although rare, these cancers often present with metastases.

Disconcertingly, metastatic anaplastic thyroid carcinomas are virtually untreatable and, therefore, almost universally fatal, with median survival of less than six months after diagnosis (Cabanillas *et al.* 2016, Howlader *et al.* 2016). The size, stage and subtype of thyroid cancer influence the course of therapy. Typical standard of care treatment includes thyroidectomy followed by radioiodine therapy and suppression of thyroid-stimulating hormone (TSH) (Mazzaferri & Jhiang 1994). In cases of advanced or metastatic disease, where survival rates drop precipitously, additional interventions such as chemotherapy, tyrosine kinase inhibitors and radiation therapy are often implemented (Howlader *et al.* 2016). The most pressing need for improving outcomes for patients is the development of novel ways to effectively treat recurrent and metastatic thyroid cancers. To this end, cutting-edge immunotherapies show promise for providing oncologists with a novel array of therapeutic tools in the near future (Couzin-Frankel 2013). However, nuances will need to be taken into consideration when applying these in the context of thyroid cancers.

Immunosurveillance is a process that allows the immune system to detect potentially dangerous neoplasias in the body and typically prevents the manifestation of cancerous growths (Dunn *et al.* 2004). However, if neoplastic cells are not eliminated at this early stage of development, a state of equilibrium can become established between transformed cells and the immune system. At this point, acquisition of mutations that facilitate evasion from immunological effector mechanisms can lead to uncontrolled growth, resulting in the development of clinical cases of cancer. The concept of immunological pressure driving the development of immunoevasive phenotypes in cancers is known as immunoediting (Dunn *et al.* 2002). Immunotherapies aim to potentiate a patient's immune system, while exploiting its inherently exquisite specificity, to kill cancer cells with minimal side-effects. The potential for immunological pressure to kill neoplastic cells but also edit tumors has resulted in two approaches to immunotherapy. One is to quantitatively and/or qualitatively increase tumor-specific immune responses. The other is to counteract the immunosuppressive potential of cancers. The most efficacious immunotherapies will likely combine both aspects.

Immunological cell subsets in thyroid cancers: identifying friends and foes

The immunosurveillance and immunoediting concepts provide a framework for understanding

how immunological effector cells can mediate tumor regression, while immunosuppressive cells can promote the growth of cancers. Indeed, there is a growing body of literature to support correlations of the number and type of tumor-infiltrating leukocytes with either positive or negative clinical outcomes in patients (Gooden *et al.* 2011, Gentles *et al.* 2015). Several subsets of leukocytes have well-defined roles in thyroid cancers prior to therapeutic interventions.

Myeloid cells

Macrophages, dendritic cells and neutrophils are mature cell types that develop from the myeloid lineage during hematopoiesis. In contrast, myeloid-derived suppressor cells (MDSCs) are phenotypically heterogeneous myeloid cells that have not fully matured (Gabrilovich & Nagaraj 2009). Although MDSCs are rare in healthy individuals, they are found in higher numbers in cancer patients, where they have potent immunosuppressive potential (Marvel & Gabrilovich 2015). As such, an increased number of MDSCs is often associated with a poor prognosis. In thyroid cancers, the presence of MDSCs may even be a useful biomarker for distinguishing benign nodules from cancerous lesions. Specifically, there seems to be an association between higher preoperative levels of circulating MDSCs and thyroid cancers as compared to patients with benign nodules or other non-cancerous thyroid diseases (Suzuki *et al.* 2013, Angell *et al.* 2016). There also seems to be a correlation between numbers of circulating MDSCs and aggressive growth characteristics of differentiated thyroid cancers (Angell *et al.* 2016). Prognoses could potentially be improved in thyroid cancer patients by either differentiating MDSCs into mature myeloid cells or depleting or functionally inhibiting them with various treatments, including some chemotherapeutics, nitric oxide inhibitors, tyrosine kinase inhibitors (e.g. sunitinib) and/or bisphosphonates, amongst others (Najjar & Finke 2013). Notably, sunitinib has recently demonstrated anti-tumor activity in advanced differentiated thyroid cancer (Bikas *et al.* 2016). Although the benefit seen with sunitinib treatment has not been definitively attributed to MDSC depletion, it is known to augment MDSCs, which correlates with enhanced tumor-specific responses generated by cancer vaccines in preclinical models (Wesolowski *et al.* 2013).

The density of tumor-associated macrophages (TAMs) varies in the different pathologic subtypes of thyroid cancers (Jung *et al.* 2015). In particular, anaplastic thyroid cancers were found to have the most TAMs, with their

numbers correlating with poorer prognoses (Jung *et al.* 2015). Although the average number of TAMs tends to be lower in papillary thyroid cancers, there is a similar correlation with clinical outcomes, including more lymph node metastases, larger tumors and reduced survival (Ryder *et al.* 2009, Kim *et al.* 2013, Fang *et al.* 2014, Jung *et al.* 2015). *In vitro* data also suggested that TAMs could promote invasiveness of a human thyroid cancer cell line through interleukin (IL)-8 secretion (Fang *et al.* 2014). This mechanistic link was further corroborated by enhanced metastasis of human papillary thyroid cancer cells following treatment of immunodeficient mice with IL-8 (Fang *et al.* 2014). However, a retrospective study of patients with thyroid cancers found a favorable association between the number of tumor-infiltrating macrophages and improved disease-free survival (Cunha *et al.* 2012). Additional and more detailed research will be required to resolve these apparently conflicting results. Specifically, efforts should be made to differentiate inflammatory type 1 vs suppressive type 2 TAMs because a high ratio in favor of the former subset usually confers better outcomes in other cancer types (Zhang *et al.* 2014a, Yuan *et al.* 2015).

Dendritic cells (DCs) are central players in the induction of immunity, as they are critically involved in the role of antigen presentation and regulation of immune cell function via secretion of cytokines. Under normal homeostatic conditions, DCs are rarely found in the thyroid gland. However, the number of DCs is increased in human papillary thyroid cancers (Hilly *et al.* 2013). Despite their role in the induction and regulation of immune responses, DC density in tumors was not found to be associated with disease-free survival. This may be the result of tumor-infiltrating DCs often adopting an immature or paralyzed phenotype, with low expression of costimulatory molecules, high expression of regulatory molecules, resulting in impaired antigen presentation (Harimoto *et al.* 2013, Tran Janco *et al.* 2015). Immature DCs are not only poor inducers of T cell and natural killer (NK) cell-mediated responses, but they can also inhibit immune responses via the production of suppressive cytokines such as IL-10 and TGF- β (Scouten & Francis 2014). Regulatory T cells and DCs appear to collaborate in the immunosuppressive conditioning of the tumor microenvironment. In a model of pancreatic ductal adenocarcinoma, regulatory T cells were found to suppress the function of tumor-infiltrating DCs, by preventing expression of costimulatory ligands necessary for the activation of CD8⁺ T cells (Jang *et al.* 2017). Although similar mechanistic data is lacking in the case of thyroid cancers, regulatory T cells and DCs

are enriched in human papillary thyroid cancers (Yu *et al.* 2013). Therefore, interruption of regulatory T cell and DC interactions in thyroid tumors would likely be of benefit. Several strategies to directly restore the functions of tumor-infiltrating DCs could be employed, including blockade of immunosuppressive pathways such as those associated with PD-1, secretion of IL-10 and production of lactic acid (Tran Janco *et al.* 2015).

Neutrophils are typically the first responders during inflammation. Interestingly, the neutrophil-to-lymphocyte ratio (NLR) has been explored as a prognostic indicator in thyroid cancers. A high NLR correlated with more aggressive forms of thyroid cancers (e.g. poorly differentiated type such as anaplastic) and represented a poor prognostic marker for these cancers (Cho *et al.* 2015). Similarly, higher NLR was strongly associated with larger tumor sizes (Liu *et al.* 2013). These results are in agreement with a recent report that identified neutrophils as the leukocyte subset with the strongest negative correlation with patient outcomes across cancer types (Gentles *et al.* 2015). Unlike MDSCs, the NLR could not be used to distinguish between benign and malignant lesions (Liu *et al.* 2013).

Natural killer cells

Natural killer (NK) cells can spontaneously kill cells deemed to be dangerous to the host, including cancer cells and are, therefore, presumed to be important in cancer immunosurveillance (Guillerey *et al.* 2016). NK cells differentiate their targets from 'healthy self' by integrating signals from multiple inhibitory and activating receptors. Nevertheless, few solid tumors have shown to be responsive to NK cell-mediated immunotherapy due to resistance to NK cell-induced lysis, as well as poor homing and infiltration of NK cells into tumors (Wennerberg *et al.* 2014). However, anaplastic thyroid cancer cell lines are known to be sensitive to NK cell-mediated cytotoxicity *in vitro*, suggesting thyroid cancers could benefit from immunotherapies that incorporate the recruitment of activated NK cells into the tumor microenvironment (Wennerberg *et al.* 2014). Additionally, the cells secreted CXCL10 when stimulated with IFN- γ and demonstrated an ability to attract CXCR3⁺ NK cells (Wennerberg *et al.* 2014). If this observation could be confirmed in an *in vivo* animal model of anaplastic thyroid cancer, where many other key cell types and factors may impact NK cell functions, then adoptive transfer of *ex vivo*-expanded NK cells may be a useful treatment option.

One major barrier to effective immunotherapy with NK cells is tumor immunosuppression. Intratumoural NK cells display a suppressed phenotype when compared to blood-derived NK cells. It has been demonstrated that anaplastic thyroid cancer cells expressing high levels of COX2 co-cultured with NK cells resulted in downregulation of NKG2D expression on NK cells, when compared with co-culture of COX2-negative cell lines (Wennerberg *et al.* 2014). NKG2D is an activation receptor for NK cells, which potentiates the lysis of tumor cells. This downregulation was rescued by administration of neutralizing antibodies to prostaglandin E2. Others have also shown NK dysfunction in tumor-bearing mice. Pahar *et al.* demonstrated reduced splenocyte-mediated cytotoxicity in thyroid tumor-bearing *LSL-Braf^{V600E}/TPO-Cre* mice compared to normal *LSL-Braf^{WT}/TPO-Cre* mice (Parhar *et al.* 2016). NK and CD8⁺ T cells were important mediators of this cytotoxicity, and the reduced cytotoxic capacity of splenocytes in tumor-bearing mice was partially restored by treatment with exogenous IL-12 and anti-TGFβ (Parhar *et al.* 2016).

NK cell therapy relies on a high number of functional NK cells. Many studies have explored the use of cytokines to improve NK cell proliferation and cytotoxicity. In particular, IL-15 is critical for NK cell maturation and survival (Cooper *et al.* 2013). Stimulation with IL-15 enhances NK cell functions and cytotoxicity against pancreatic duct adenocarcinoma *in vitro* (Audenaerde *et al.* 2017). *Ex vivo* supplementation of IL-15 and IL-21 enhanced NK cell proliferation and cytotoxicity against rhabdomyosarcoma cells, and adoptive transfer of these *ex vivo*-expanded NK cells even reduced tumor growth in a xenograft model when used in combination with radiation therapy in a proof-of-concept study (Wagner *et al.* 2017). Both studies also demonstrated an increase in expression of NKG2D with IL-15 stimulation (Audenaerde *et al.* 2017, Wagner *et al.* 2017). Others have sought methods to supply IL-15 *in vivo*; for instance, genetically modifying NK cells to express IL-15 in order to support NK cell survival and proliferation (Liu *et al.* 2017). Genetic alterations have also been used to boost NK cell activity, such as expression of chimeric antigen receptors to redirect their specificity against cancer cells (Liu *et al.* 2017). These strategies present the opportunity to overcome the resistance of cancer cells toward NK cell-mediated killing. Further research is warranted to understand the full extent of NK cell dysfunction within thyroid tumors to facilitate the rational design of effective therapeutic strategies to circumvent this suppressive phenotype.

T cells

Lymphocytic infiltration is associated with favorable outcomes in numerous types of cancers including metastatic melanomas (Clemente *et al.* 1996), ovarian (Zhang *et al.* 2003, Sato *et al.* 2005), colorectal (Galon *et al.* 2006, Teng *et al.* 2015) and breast cancers (Wang *et al.* 2016). In human papillary thyroid cancers, lymphocyte density is also associated with better overall survival and lower recurrence of tumors (Matsubayashi *et al.* 1995, Kuo *et al.* 2017). In another study, actively replicating lymphocytes, which were identified based on expression of the proliferation-associated nuclear antigen Ki-67, were identified as a particularly strong predictor of extended disease-free survival in both children and young adults (Gupta *et al.* 2001). CD8⁺ T lymphocytes are a key focus in the field of immunotherapy due to their dominant natural role in eliminating cancer cells (Schreiber *et al.* 2011). Infiltration of CD8⁺ T cells into thyroid tumors was associated with improved disease-free survival (Cunha *et al.* 2012). In the same study, both CD8⁺ and CD4⁺ T cells, as well as B cells were shown to be favorably correlated with reduced tumor sizes (Cunha *et al.* 2012). In contrast, increased infiltration of CD8⁺ T cells was found to be associated with a higher risk of relapse in another study (Cunha *et al.* 2015). However, the majority of the T cell infiltrates lacked expression of granzyme B, which tends to identify cells with cytolytic capacity. A higher resolution biomarker for positive prognoses in thyroid cancers may be quantification of CD8⁺ granzyme B⁺ T cells. The expression of cytolytic proteins granzyme and perforin is regulated by IL-2 and IL-15 (Janas *et al.* 2005, Tamang *et al.* 2006). A therapy that could induce the expression of, or deliver IL-2/IL-15 to the thyroid cancer microenvironment may arm T cells with cytotoxic granules required to kill neoplastic cells. Systemic delivery of IL-2 can be toxic, but novel ways of preferentially getting IL-2 to a tumor has been explored such as IL-2 linked to a tumor-associated ligand or having it encoded by an oncolytic virus (Dasgupta *et al.* 2003, Kang *et al.* 2012).

Generally, tumor-infiltrating T lymphocytes tend to adopt an exhausted or dysfunctional phenotype due to the immunosuppression exerted by the cancer microenvironment. Inhibitory receptors like programmed death ligand-1 (PD-1) represent immunological checkpoints and provide a natural mechanism to dampen T cell responses via induction of anergy or apoptosis (Chen *et al.* 2015), to reduce the risk of autoimmunity following induction of protective responses. In the case of cancers,

autoreactive T cells specific for 'cancerous self' are precisely what are required to eliminate tumors. Unfortunately, tumors tend to promote upregulation of inhibitory receptors such as PD-1 on T cells while simultaneously expressing high levels of cognate ligands like PD-L1 and PD-L2. This results in a decreased production of interferon (IFN)- γ and concomitant reduction in cytopathic potential, compared to extra-tumoral lymphocytes from the same patient (Ahmadzadeh *et al.* 2009). Notably, expression of PD-1 seems to be a characteristic of thyroid tumor-infiltrating CD8⁺ and CD4⁺ T cells (Bastman *et al.* 2016). This suggests that checkpoint inhibitor therapies may be an effective way to reinvigorate cytotoxic T cell responses in thyroid cancers.

Regulatory T cells are involved in shutting down immune responses and their presence correlates with disease progression and may encourage metastases to lymph nodes in various cancers (Halvorsen *et al.* 2014). Regulatory T cells are known to be present in papillary thyroid cancers and may be involved in promoting more aggressive disease (French *et al.* 2010). Other regulatory subsets of CD4⁺ T lymphocytes include T helper 17 cells and follicular helper T cells, but their roles in thyroid cancers have not been studied thoroughly. Due to a paucity of data, the prognostic value of CD4⁺ T cells in thyroid cancers remains unclear. Arguably, evaluating the CD8⁺ cytotoxic:regulatory T cell ratio in thyroid cancers would be in important future direction of research, since this has proven to be a strong prognosticator in other cancers (Gooden *et al.* 2011). A recent paper identified a population of T lymphocytes in thyroid cancers that did not express CD4 or CD8 (Imam *et al.* 2014). These double-negative T cells were found in much higher numbers in thyroid tumors as compared to the glands of patients suffering from autoimmune diseases of the thyroid (Imam *et al.* 2014). In this study, the intratumoural double-negative T cells appeared to reduce the proliferation and cytokine production of neighboring activated effector T cells (Imam *et al.* 2014). As such, reducing the number of these cells in thyroid cancers might assist immune-mediated therapies.

Taken together, the milieu of thyroid cancers appears to be amenable to immunomodulatory treatments. Exploring treatment modalities to remove or convert cells of myeloid origin from immunosuppressive to immunostimulatory phenotypes appears to be warranted in the context of thyroid cancers. Moreover, enhancing the activation and infiltration of effector NK and T cells and preventing their shutdown could potentiate the

regression of thyroid cancers. While substantial progress has been made in charting an immunological map for thyroid cancers, better differentiation of immunological subsets such as TAMs and cytotoxic T cells with functional characteristics is still required to resolve some apparently conflicting results.

Hormones T₃, T₄ and TSH: confounding immunomodulatory factors that are unique to thyroid cancers

Thyroxine (T₄) which gets converted to 3,5,3'-triiodo-L-thyronine (T₃) are two hormones produced by the thyroid gland and are involved in the regulation of numerous mammalian metabolic processes. Of the two, T₃ is, by far, the most potent stimulator of metabolism. Several studies have suggested that these hormones also have an impact on the immune system. For example, B cell development was shown to be suppressed in thyroid hormone-deficient mice (Foster *et al.* 1999). Also, high concentrations of T₃ were associated with an increase in the amount of complement proteins and the number and phagocytic activity of monocytes (Hodkinson *et al.* 2009). Moreover, physiological levels of T₃ were demonstrated to induce the maturation of murine DCs (Mascanfroni *et al.* 2008). Similarly, T₄ was found to be associated with higher concentrations of complement proteins and also higher neutrophil counts (Hodkinson *et al.* 2009). T₄ concentration was also inversely correlated with the ratio of naïve to memory cytotoxic T cells (Hodkinson *et al.* 2009). Taken together, these results suggest that higher concentrations of thyroid hormones could enhance innate and adaptive immunity, via maintenance of specific subsets and increased responsiveness to immune stimuli (Hodkinson *et al.* 2009). In another line of research, immunological parameters have been assessed in scenarios of non-cancer-related hyperthyroidism and hypothyroidism, in which thyroid-derived hormones are increased and decreased, respectively. In these disease settings, a wide variety of immunological functions are impacted, including antibody production, trafficking of leukocytes, proliferation of lymphocytes and generation of reactive oxygen species, as summarized in Table 1 (and reviewed by De Vito *et al.* 2011). Thyroid-stimulating hormone (TSH) is a pituitary hormone that stimulates the thyroid to produce T₃ and T₄. TSH can also have direct effects on the immune system. TSH enhanced antibody responses *in vitro* in a dose-dependent manner (Blalock *et al.* 1984, Klein 2006). It was also reported that TSH could induce

Table 1 The effects of hyper- vs hypothyroidism on the immune system.

Immune function	Hyperthyroidism	Hypothyroidism	References
Antibody production	↑ serum concentrations of IgG, IgA and IgE	↓ serum concentrations of IgG and IgA	Jafarzadeh <i>et al.</i> (2010), Bittencourt <i>et al.</i> (2007)
Monocyte migration	↓	No effect	Rosa <i>et al.</i> (1995)
Production of hydrogen peroxide by activated macrophages	↓	↑	Rosa <i>et al.</i> (1995)
Phagocytic capacity	↑ (polymorphonuclear granulocytes; mediated by T ₃) ↑ (monocytes; mediated by T ₃)	↑ (macrophages)	Balázs <i>et al.</i> (1980), Rosa <i>et al.</i> (1995), Hodkinson <i>et al.</i> (2009)
NK cell activity	<i>In vitro</i> spleen cells pre-incubated with thyroxine increased NK cytotoxic activity induced by IFN Mice injected with T4 had increased peritoneal NK cell activity	No data	Sharma <i>et al.</i> (1982), Provinciali <i>et al.</i> (1992)
Inflammatory markers (C3 and C4)	Association between higher T3 and T4 and higher C3 and C4 No difference	↑ C3; no differences in serum C4	Hodkinson <i>et al.</i> (2009), Jafarzadeh <i>et al.</i> (2010)
Memory T lymphocytes	↑ (associated with elevated T4)	No data	Hodkinson <i>et al.</i> (2009)
Splenic T cell #	↑	↓	Watanabe <i>et al.</i> (1995)
Splenic B cell #	↓	↑	Watanabe <i>et al.</i> (1995)
Splenic NK cell #	↓	↓	Watanabe <i>et al.</i> (1995)

NK cell activity and increase IL-2-induced proliferative responses of murine splenic lymphocytes (Provinciali *et al.* 1992). How TSH augments cells of the immune system in the thyroid tumor microenvironment remains an understudied area that requires further investigation. Patients with thyroid cancers that receive thyroidectomies often receive synthetic T₄ to compensate for hormonal deficiency from the resulting hypothyroidism. This is also known as TSH suppression therapy, since elevated levels of T₄ cause a negative feedback loop that reduces production of TSH. Importantly, suppression of TSH is associated with a reduced incidence of recurrent disease (Biondi & Cooper 2010). However, given the immunostimulatory potential of TSH, perhaps this adds to the debate of whether TSH suppression therapy should be used less often in low-risk-of-recurrence patients, since it can also induce adverse events such as osteoporosis (Wang *et al.* 2015a). Currently, there is a body of literature highlighting immunological effects of non-cancer-related hypo- and hyper-thyroidism. A separate set of studies evaluating the neuroendocrine-immune axis have clearly demonstrated that thyroid-related hormones are immunomodulatory. However, there appears to be a large gap in knowledge where these fundamental biologies intersect in the context of thyroid cancers. Many patients with thyroid cancers will experience disturbances in thyroid hormone homeostasis. Specifically, full/partial thyroidectomies can cause hypothyroidism. This is often alleviated with the administration of exogenous T₄, which restores levels of this hormone, but suppresses TSH. As mentioned above,

TSH suppression may not be indicated as a treatment modality, particularly in patients with a low risk of cancer recurrence. Moreover, patients with Hashimoto's thyroiditis (HT) often develop hypothyroidism from chronic inflammation of the thyroid gland. This autoimmune disease has interesting implications for papillary thyroid cancers. A meta-analysis of patients with papillary thyroid cancers revealed that concurrent HT was significantly associated with a lack of lymph node involvement, an absence of extrathyroidal extension and prolonged recurrence-free survival, particularly among women (Lee *et al.* 2013). Paradoxically, the authors arrived at the conclusion that HT may actually contribute to the development of papillary thyroid cancers, despite simultaneously conferring a better prognosis (Lee *et al.* 2013). Importantly, this very unique case of immune-cancer cell interplay could be an invaluable resource for discerning which immune cell infiltrates are tumourigenic and which ones are tumouricidal. Although rare, thyroid cancers can occur in patients with hyperthyroidism as well, which can complicate diagnoses, and may have implications for immune system function and clinical outcomes (Gabriele *et al.* 2003, Gulcelik *et al.* 2006, Yeh *et al.* 2013). We can extrapolate what is known in the fields of endocrinology and immunology to speculate on the immunomodulatory effects of thyroid cancers and subsequent clinical interventions (Fig. 1). In light of the interplay between TSH, T₃, T₄, and the immune system, integration of immunotherapy with standard of care hormone replacement therapy may have unexpected

Figure 1

Predicted hormonal effects on the immune system of patients with thyroid cancers. Several immunological effects of hyper- and hypothyroidism have been defined in non-cancer-related scenarios. Similarly, thyroid gland-related hormones have known immunomodulatory functions. Although never directly assessed, these findings can be extrapolated to speculate on what might happen to the immune system of patients with thyroid cancers. Some patients experience hyperthyroidism concurrently with a growing tumour (Box 1). Hypothyroidism induced by partial or total thyroidectomy, or with concurrent Hashimoto's thyroiditis (HT) (Box 2), can be followed by a unique hormone profile following administration of thyroxine (T₄) to suppress thyroid-stimulating hormone in an effort to reduce the risk of disease recurrence (Box 3). NK, natural killer cell; PMN, polymorphonuclear cell; ROS, reactive oxygen species.

outcomes. This is unique to thyroid cancers and should be highlighted as a high priority area for research.

Perioperative considerations: minimizing surgery-induced immunosuppression

Recurrent thyroid cancers can be notoriously difficult to treat, as neoplastic nodules can present in local soft tissues, nerves, lymph nodes and as distant metastases (Kim *et al.* 2017). Patients with recurrent thyroid cancers have a treatment history, following the standard of care framework, which can impact the immunological environment. For example, stage I, II and III patients usually have surgical removal of cancerous margins or the entire thyroid gland (Mazeh & Chen 2011). Importantly, surgery and accompanying anesthesia are generally immunosuppressive, resulting in postoperative minimization of immunological cell numbers and functions critical to anticancer responses; especially T and NK cells (Hogan *et al.* 2011). The ratio of effector T to regulatory T cells can be altered in favor of the latter, which is linked to the development of metastases, since regulatory

T cells are capable of orchestrating shutdown of innate and adaptive immune responses (Takeuchi & Nishikawa 2016). The direct impact of surgery on the pre-existing anticancer cytotoxic T cell repertoire was demonstrated through an elegant cancer vaccine model (Ananth *et al.* 2016). Oncolytic adenovirus encoding a transgene for the melanoma-associated antigen dopachrome tautomerase was delivered to mice bearing B16-F10 lung metastases to induce tumor-specific cytotoxic T cells. Abdominal nephrectomy was performed to mimic major surgery, which resulted in direct suppression of dopachrome tautomerase-specific T cells. A reduction in both the number and functionality of cancer-specific T cells correlated with increased tumor burden and decreased survival, simulating clinical recurrence such as that observed following thyroid resection (Ananth *et al.* 2016).

Surgery-induced immune dysregulation is an under-appreciated driver of recurrent disease, making it an important treatment point to consider introducing corrective immunotherapies. Several strategies have been implemented to diminish the immunosuppression from surgery and reduce risk of metastases. The use of

preoperative non-steroidal anti-inflammatory drugs can decrease physiological stress and relieve suppression of leukocytes, leading to increased anti-tumor responses in patients, as demonstrated by greater numbers of tumor-infiltrating CD4⁺ and CD8⁺ effector T cells and a decrease in intratumoural gene expression of the regulatory T cell transcription factor FOXP3 (Lönnroth *et al.* 2008). Recombinant cytokine therapy can be delivered preoperatively as an immunostimulant, as in the case of IL-12 (Schwartz *et al.* 2008). Insufficient IL-12 is associated with limited IFN- γ production by NK and T cells, leading to weak adaptive anti-tumor cytotoxic T cell responses. In preclinical models of breast cancer, recombinant IL-12 has been shown to improve anti-tumor responses by synergizing with an anti-HER2 monoclonal antibody (Jaime-Ramirez *et al.* 2011). In addition to IL-12, recombinant IL-2 has mediated moderate improvement in survival for patients with operable colorectal cancers (Brivio *et al.* 2006). The administration of systemic cytokines is often a double-edged sword, where efficacy is matched with toxicity, as in the case of IL-2 (Dhupkar & Gordon 2017). To circumvent this issue, researchers have explored the use of vector-based therapeutics such as recombinant oncolytic viruses (OVs) to focus transgene expression inside the cancer microenvironment.

Oncolytic virotherapy

Oncolytic viruses have emerged in the clinic as promising vectored immunotherapeutics. OVs preferentially infect and replicate in tumor cells, where they can induce a broad inflammatory reaction by producing immunostimulatory viral proteins, lipids and nucleic acids that serve as pathogen-associated molecular patterns that can be sensed by sentinel cells in the immune system. Direct viral oncolysis also elicits secretion of damage-associated molecular patterns and release of tumor antigens. Therefore, OVs can provide a source of tumor antigens and danger signals required to promote antigen presentation to T cells. As such, OVs can function as endogenous personalized cancer vaccines (Chiocca & Rabkin 2014). Further, leading OV platforms harbor immunomodulatory transgenes, such as the cytokine granulocyte macrophage colony-stimulating factor (GM-CSF), which can expand and mature dendritic cells, enhancing their immunotherapeutic potential (reviewed by Kaufman *et al.* 2015). T-VEC is a herpes virus that expresses GM-CSF that has received approval from the United States Food and Drug Administration

for treating advanced melanomas (Andtbacka *et al.* 2016). OV transgene expression is preferentially driven in tumors, which can potentially avoid acute toxicities associated with systemic delivery of a recombinant cytokine. Indeed, T-VEC performed better than GM-CSF alone in terms of increasing overall survival in melanoma patients, indicating the advantages of cytokine delivery via an OV (Andtbacka *et al.* 2015). Recently, OVs have been administered perioperatively to target postoperative metastases (Tai & Auer 2014). In this setting, there is striking evidence that OVs provide a multimodal counter to postoperative metastases by first directly infecting and destroying metastatic nodules and second, by acting as an adjuvant to stimulate the patient's immune system, mainly through activation of NK cells. Oncolytic poxviruses, such as vaccinia virus (VACV) and Orf virus (ORFV), when administered during the perioperative window in a murine melanoma lung metastasis model dramatically increased the number of activated NK cells (Tai *et al.* 2013, 2014). Moreover, a greater proportion of NK cells produced IFN- γ in mice treated with VACV or ORFV with surgery than compared to surgery alone, which correlated with a decrease in tumor burden. Antibody-mediated depletion of NK cells in mice treated perioperatively with VACV or ORFV, proved their role in mediating efficacy. Another study that used Maraba virus showed similar results (Zhang *et al.* 2014b). OVs may be broadly applicable as perioperative immune-potentiating therapies and should, therefore, be given consideration as a way to counteract thyroidectomy-induced immunosuppression.

Multiple OVs have been studied both pre-clinically and in clinical trials for thyroid cancers (Guan *et al.* 2014). An oncolytic adenovirus, dl922-947, has demonstrated anti-thyroid cancer activity in a preclinical murine xenograft model (Passaro *et al.* 2016). In addition to its cytolytic activity, dl922-947 modulated the tumor microenvironment through decreased production of tumourigenic IL-8 and an increase in IFN- γ , coupled with an increase in anti-tumoral type 1 TAMs. Other OVs, including VACV, Newcastle disease virus and measles virus have been investigated pre-clinically for the treatment of anaplastic thyroid carcinomas. Unfortunately, however, these studies used xenograft murine models that, by definition, exclude the impact of the host immune system. Importantly, OV vectors based on adenovirus have progressed into clinical trials, with one phase II study nearing completion (ClinicalTrials.gov Identifier: NCT01229865, VB-111). In this study, 44% of patients receiving a therapeutic dose of virus experienced

a reduction in tumor size. Preclinical studies to support more human trials would benefit from exploring OV in orthotopic, immunocompetent murine models of thyroid cancer (Vanden Borre *et al.* 2014, Kirschner *et al.* 2016).

Thyroid cancer vaccines

Cancer vaccination is a rapidly growing field of immunotherapy used to invoke tumor-specific immune responses. New insights into how antigens are processed, presented and how cell-mediated immune responses are generated has paved the way for the development of many novel cancer vaccines (Guo *et al.* 2013). Moreover, a growing understanding of how tumors employ immunosuppressive cells and pathways to avoid their demise has reinvigorated the field with promising combination therapies (Guo *et al.* 2013). Designing a vaccine to specifically target thyroid cancers, or any cancer for that matter, can be a challenge. Various vaccine platforms have been developed, including whole cells, cancer-derived lysates, peptides and recombinant viral vectors, including OVs. Many of these immunization strategies rely on the identification of antigens that are exclusively or overexpressed in or on tumor cells. Lists of high-priority tumor antigens are an invaluable resource (Cheever *et al.* 2009) for the design of new vaccines. Very few immunogenic thyroid cancer-specific antigens with minimal risk of provoking off-target pathological autoimmunity have been identified, making this an understudied area with the potential to improve immunotherapies. Survivin has been shown to be differentially expressed in various subtypes of malignant human thyroid cancers when compared to benign and healthy thyroid tissues via reverse transcriptase-PCR (Chen *et al.* 2012, Waligórska-Stachura *et al.* 2014). Indeed, peptide-based survivin vaccines are being explored in various human solid cancer clinical trials (Miyazaki *et al.* 2011, Becker *et al.* 2012, Lennerz *et al.* 2014), and may be aptly suited for thyroid cancers. In one study, human epidermal growth factor receptor-2 was found to be overexpressed in 44% of follicular thyroid tumors ($n=45$) and 18% of papillary thyroid tumors ($n=45$) (Ruggeri *et al.* 2016). Telomerase reverse transcriptase (TERT) expression and activity in thyroid cancers are quite variable, but were estimated to be present in approximately 50% of samples across all subtypes of thyroid cancers from a meta-analysis (Capezone *et al.* 2009). Additionally, mutations in the TERT promoter region occurred in approximately 10–14% of papillary and follicular thyroid cancers,

which correlated with increased expression and more aggressive clinical features (Muzza *et al.* 2015, Liu *et al.* 2016). Notably, distant thyroid cancer metastases had an increased prevalence (i.e. 52%) of mutations in the TERT promoter (Melo *et al.* 2017). Recently, an adenoviral vector was made that expressed both TERT and VEGF-R2 and was found to successfully induce T cells that recognized and were cytolytic in differentiated thyroid carcinomas (Wang *et al.* 2015). Importantly, simultaneous targeting of tumor cells and neovasculature appeared to have potent synergy. However, the authors noted a loss of fertility in mice vaccinated with their vector and suspected the VEGF-R2-specific response was the culprit. Several clinical studies of TERT vaccine vectors have not shown autoimmune toxicities (Yan *et al.* 2013). Mutated BRAF genes are also common in thyroid cancers, occurring in approximately 45% of the papillary subtype (Xing 2005). Thyroid cancers with BRAF mutations typically have poorer outcomes for many unknown reasons, though it has been demonstrated that papillary thyroid cancers with constitutively active BRAF (V600E) express more PD-L1 compared to those with wild-type BRAF (53% vs 12.5%) (Angell *et al.* 2014a). In addition, tumors with mutated BRAF had lower CD8⁺:regulatory T cell ratios and greater infiltration of type 2 macrophages, suggesting this particular mutation correlates with an immunosuppressive microenvironment (Angell *et al.* 2014). Considering its prevalence, association with immunosuppression and poor outcomes, BRAF mutations appear to be a reasonable target for thyroid cancer vaccines. Interestingly, endogenous BRAF V600E-specific T cells have been found in some melanoma patients (Andersen *et al.* 2004, Somasundaram *et al.* 2006). Notably from this study, metastatic lesions had lost the BRAF mutant genotype in two patients with BRAF mutant-specific T cells, suggesting selective pressure for wild-type BRAF tumor cells. Considering the V600E mutation's association with poorer prognoses, antigen escape-driven wild-type gene selection may be beneficial in thyroid cancers that have BRAF V600E mutations. Gene expression analysis found NY-ESO-1, a cancer-testis antigen, in 15 of 23 (65%) of medullary thyroid carcinoma samples (Maio *et al.* 2003). In another study, NY-ESO-1 was not expressed in several thyroid cancer cell lines. However, epigenetic modification of these cells with the hypo-methylating agent 5-aza-2'-deoxycytidine forced the expression of NY-ESO-1 in both *in vitro* and *in vivo* experiments (Gunda *et al.* 2014). In a similar study, another cancer-testis antigen, MAGE-A4, was evaluated in the same human cell lines and expression was also

induced or enhanced by hypo-methylating agents (Gunda *et al.* 2013). Immunohistochemical analysis by another group failed to reveal expression of MAGE-A4 or MAGE-C1 in 50 differentiated thyroid tumors (Melo *et al.* 2011). Conversely, in another study, 31/85 (36.5%) of papillary thyroid cancers had evidence of high levels of expression of MAGEA1-6 genes, although it was unclear whether this was reflected at the level of transcribed protein (Lee *et al.* 2013). Epigenetic modification may represent a useful tool to induce or enhance the expression of otherwise silent antigens in thyroid cancers, thereby making them more amenable to treatment with cancer vaccines. Another barrier to T cell-centric cancer vaccines, in addition to antigen scarcity, is loss of expression of MHC class I (Garrido *et al.* 2016). It was recently shown that 29/33 (87.9%) of papillary thyroid cancers either failed to express MHC class I or expressed it at relatively low levels. Notably, expression of MHC class I could be upregulated on papillary thyroid cancer-derived cell lines when treated with selumetinib, a MEK1/2 inhibitor, and/or interferon (Angell *et al.* 2014). There is evidence from other cancer types that radiation therapy can increase the expression of MHC class I (Reits *et al.* 2006, Son *et al.* 2016). Unfortunately however, radiation therapy of thyroid cancer cell lines failed to increase MHC class I expression (Angell *et al.* 2014). CD8⁺ T cell-centric cancer vaccines should benefit from combination treatments that enhance the expression of MHC class I on neoplastic cells.

The generation of a tumor-specific adaptive immune response is reliant on antigen processing and presentation, which are two features that DCs do particularly well (Steinman 1991). As such, the development of DC-based vaccines has become an area of interest for the field of cancer immunotherapy. DC vaccines use a patient's progenitor cells, which get differentiated into DCs in the presence of cytokines such as GM-CSF and IL-4. The DCs can then be stimulated to initiate their maturation, loaded with tumor antigens and then injected back into patients. Proof-of-principle studies for using DCs to treat medullary thyroid cancers have been conducted using xenogenic calcitonin peptides (in mice) and allogeneic tumor cell lysates (in humans) (Papewalis *et al.* 2008, Bachleitner-Hofmann *et al.* 2009). Additionally, a phase I trial evaluated the safety of an autologous tumor lysate-pulsed DC vaccine (Kuwabara *et al.* 2007). Notably, all studies reported little-to-no adverse reactions to the DC vaccines. Interestingly, it was recently demonstrated that the thyroid hormone T₃ could enhance the activation, maturation and antigen-presenting capacity of DCs in mice (Alamino *et al.* 2015).

Thyroid cancer patients can have dysregulated thyroid hormone levels. Therefore, administration of DC-based vaccines may be contraindicated when patients have low levels of T₃. The interplay between thyroid hormones and DCs is an area that could be explored further to inform future clinical trials. The DC vaccine concept has been clinically validated by the United States Food and Drug Administration's approval of Sipuleucel-T in 2010, where the vaccine was able to extend median survival by approximately four months in men with metastatic castration-resistant prostate cancers (Kantoff *et al.* 2010). The field of DC vaccination is in its infancy, but this complex therapeutic strategy is being refined and improved each year (Butterfield 2013). Cost will continue to be a caveat for DC vaccines for the foreseeable future, but should not hinder the pursuit of this treatment modality as it has proven to be another useful tool in the cancer immunotherapy toolbox.

The primary aim of most cancer vaccines is to generate robust tumor-specific CD8⁺ T cells. However, the therapeutic benefit of generating or having CD8⁺ T cells in a tumor is not always linearly correlated with survival, despite a wealth of data suggesting they are the primary effector cell subset responsible for tumor elimination. Decades of research have contributed to the elucidation of strategies utilized by tumors to induce potentially immunosuppressive microenvironments (Drake *et al.* 2006). At the forefront of efforts to counteract tumor immunoevasion, are immune checkpoint blockade strategies. Immunological checkpoints rely on receptor-ligand interactions and can, therefore, be blocked with antibodies (Pardoll 2012). Cytotoxic T lymphocyte-associated protein-4 (CTLA-4) and programmed cell death protein-1 (PD-1) are the most studied targets of immune checkpoint blockade therapy in various cancers. CTLA-4 is a receptor expressed on activated T cells that competes with CD28, for ligation of the costimulatory molecules CD80 and CD86 that are expressed on antigen-presenting cells. CTLA-4 ligation is inhibitory, while CD28 is stimulatory. Thus, blocking CTLA-4 can prevent T cell inactivation. PD-1 on, the other hand, is transiently expressed by activated T cells and constitutively expressed on T cells with an exhausted phenotype. As such, anti-PD-1 therapy can restore the effector function of exhausted T cells (Jiang *et al.* 2015). PD-L1, the ligand for PD-1 is expressed by several types of thyroid cancers, albeit at low frequencies. Notably, out of 407 primary thyroid cancers, there was low-to-modest expression of PD-L1 in 6.1, 7.6 and 22.2% of papillary, follicular and anaplastic thyroid carcinomas, respectively

(Ahn *et al.* 2017). Similarly, a relatively higher frequency of expression on anaplastic thyroid cancers was found by another group (Zwaenepoel *et al.* 2017). Yet, another study noted very low expression, or lack thereof, of PD-L1 in medullary thyroid cancers (Bongiovanni *et al.* 2017). Therefore, it would appear that techniques for PD-L1 interference may only be applicable to a small subset of thyroid cancers. Notably, anaplastic thyroid cancers, for which there is the greatest need for new treatments, may be most amenable to PD-L1 blockade. CTLA-4 blockade therapy is poorly studied in the context of thyroid cancers. However, anti-CTLA-4 and anti-PD-1 are currently being tested in several clinical trials for various solid tumor types. Results from these immunotherapies were encouraging in the treatment of melanomas (Larkin *et al.* 2015). In general, combining checkpoint blockade inhibitors with conventional or experimental therapies shows great promise (Duraismamy *et al.* 2013, Engeland *et al.* 2014, Farkona *et al.* 2016). With the slow accrual of patients in clinical trials for mixed cancer types, time will discern the applicability of checkpoint inhibition for thyroid cancers.

Adoptive cell therapy

Adoptive T cell therapy (ACT) typically involves *ex vivo* expansion of blood or tumor-derived T cells followed by infusion back into a patient (Rosenberg & Restifo 2015, Baruch *et al.* 2017). Once isolated, the T cells can either be selected for tumor specificity naturally or they can be genetically modified to recognize a particular tumor antigen. ACT has shown promise in hematological malignancies, but requires further optimization to see similar efficacy in solid cancers (Rosenberg & Restifo 2015). A hurdle for ACT in the context of thyroid cancers will likely be the identification of T cells that can recognize highly expressed tumor antigens, that are rarely, or ideally, never expressed in healthy tissues. An alternative to acquiring naturally occurring tumor-associated antigen-specific T cells is to engineer T cells to express TCRs (chimeric antigen receptors) that recognize rationally selected targets with high affinity. One study demonstrated that adoptive transfer of hTERT₈₆₅₋₈₇₃-specific TCR-engineered T cells into leukemia-bearing mice was able to inhibit tumor progression and prolong overall survival (Sandri *et al.* 2017). Since TERT is also overexpressed in thyroid cancers, adoptive transfer of T cells engineered to be TERT-specific may be an effective therapeutic candidate. Unfortunately, the enthusiasm toward ACT has declined due to the

significant toxicities associated with them. For example, while patients treated with T cells engineered to express MAGE-A3-specific TCRs had tumors regress, they also experienced unexpected and severe neurological toxicities due to cross-reactivity to MAGE-A12, whose expression in the human brain was previously unrecognized (Morgan *et al.* 2013). One method that has shown potential in mitigating these unwanted adverse effects of T cells with chimeric antigen receptors is the incorporation of a 'safety switch', such as an inducible caspase 9 (iCas9) or suicide gene (Straathof *et al.* 2005, Tey 2014). The use of chimeric antigen receptor-expressing T cells expands antigen targets beyond peptide-MHC restricted specificity to include tumor-associated carbohydrates. Recently, a glycosphingolipid, Globo H, was found to be expressed on some thyroid cancers (Cheng *et al.* 2016). More research into thyroid cancer-specific antigens would be particularly valuable for immunotherapies in general, but especially ACT. A recent study used a murine model of anaplastic thyroid cancer to establish a sophisticated clinical monitoring technique (Vedvyas *et al.* 2016). Although ACT has not been sufficiently explored in the context of thyroid cancers, the therapy is robust enough to have broad applications, limited only by the availability of target antigens. Iterations of ACT for thyroid cancers will undoubtedly develop alongside ACTs targeting solid tumors in general, given the overlap in expression of antigens amongst tumors from various tissues.

Immunogenic radiotherapy

Iodine radiotherapy (RAI) has been used to treat patients with thyroid malignancies for decades with considerable success when combined with surgical resection for primary treatment, but does not improve the overall survival compared to surgery alone for recurrent disease (Coburn *et al.* 1994). RAI has historically been applied with other clinical assessments to detect recurrent disease, as RAI amasses in some recurrent metastatic lesions. In principle, radiation therapy can destroy local malignancies and distant metastases via an abscopal effect, which can release tumor antigens into an immunogenic microenvironment. The mechanism underlying the abscopal effect has been ascribed to the broad term immunogenic cell death (ICD) (Galluzzi *et al.* 2013). ICD classifies a diverse, yet overlapping consortium of cell death pathways that together culminate in the release of critically immunogenic damage-associated molecular patterns.

These damage-associated molecular patterns, referred to as the hallmarks of ICD, are endogenous proteins and metabolites that undergo temporally scripted translocation to abnormal cellular compartments and are then recognized by innate immune cells, particularly DCs (Galluzzi *et al.* 2016). Expression of damage-associated molecular patterns by dying tumor cells increases the uptake of tumor antigens and provides maturation signals to professional antigen-presenting cells, thereby facilitating the activation of cancer-specific T cells. Thus, a strong ICD response is hypothesized to improve anticancer immune responses and improve clinical outcomes. Indeed, a retrospective study analyzing the outcomes of patients with breast cancers harboring a Toll-like receptor (TLR)-4 mutation, the main signaling receptor for the damage-associated molecular pattern high-mobility group box 1 (HMGB1), had reduced survival following chemotherapy with an ICD-inducer than patients with a fully functioning receptor (Apetoh *et al.* 2007, Ladoire *et al.* 2015).

The hallmarks of ICD include, but are not limited to, the exposure of the endoplasmic reticulum-resident chaperone calreticulin (CRT) on the outer plasma membrane leaflet, the extracellular translocation of adenosine triphosphates, and the passive diffusion of non-histone DNA-binding protein HMGB1 (Apetoh *et al.* 2007, Obeid *et al.* 2007, Michaud *et al.* 2011). Cell surface-expressed CRT is a ligand for CD91, offering a pro-phagocytic signal to antigen-presenting cells (Gardai *et al.* 2005). Adenosine triphosphates are recognized by the purinergic receptors P2Y2 and P2X7, which promote recruitment and maturation of DCs, respectively (Ghiringhelli *et al.* 2009, Junger 2011). Passive release of HMGB1 signals through TLR4, TLR9 and the receptor for advanced glycation end-products (RAGE) to promote further maturation of DCs (Kang *et al.* 2013). However, the molecular translocation and signaling mechanisms that surround HMGB1 in ICD are complex and incompletely elucidated. An abundance of preclinical data and retrospective analyses support research to develop methods to accentuate ICD for clinical therapies, especially using protocols that are compatible with current standards of care (Garg & Agostinis 2016). ICD has been underexplored in thyroid cancers; current studies are limited to evaluating hallmark damage-associated molecular patterns as prognostic markers or drivers of disease (Netea-Maier *et al.* 2008, Mardente *et al.* 2015, Guan *et al.* 2017). BRAF V600E mutations in papillary thyroid cancers appears to downregulate the expression of HMGB1, adding further evidence to suggest this mutation is immunosuppressive in nature

(Guan *et al.* 2017). ICD may be a critical mechanism for the success of radiotherapy, especially due to the high mutational load in thyroid cancers, which creates neoantigens; a prerequisite for robust T cell responses in immunotherapies like immune checkpoint blockade (Rizvi *et al.* 2015, Bailey *et al.* 2016). OV may be well-suited for the treatment of thyroid cancers because many have been shown to elicit ICD through divergent cell death pathways (Donnelly *et al.* 2013, Koks *et al.* 2015) and have synergistic effects when used in combination with standard of care such as radiotherapy (Harrington *et al.* 2010, Markert *et al.* 2014, Wilkinson *et al.* 2016) and chemotherapy (Kuryk *et al.* 2016, Binz *et al.* 2017). OVs may not only provide additional inflammatory stimuli in the form of pathogen-associated molecular patterns, but also limit the escape of tumors with mutations in key ICD pathways. The never-ending molecular arms race between eukaryotic cells, and viruses has endowed cells with the ability to succumb to multiple forms of death, especially when infected with viruses that manipulate those same pathways. This may be exploited in the context of OVs that can stimulate cells to die by an alternative immunogenic fashion, thus rescuing ICD and establishing and anti-tumor immune responses. A recent study demonstrated that an adenovirus-based oncolytic (dl922-947) synergized with radiotherapy both *in vitro* and in an *in vivo* xenograft murine model of anaplastic thyroid carcinoma (Passaro *et al.* 2013). Interestingly, this study demonstrated that enhanced killing of cancer cells was only seen when irradiation preceded viral infection. This was attributed to irradiation arresting cells in stages of the cell cycle that are more permissive to viral replication (G2/M) (Passaro *et al.* 2013). Conversely, in another study, pretreatment with an adenovirus enhanced the susceptibility of several human cancer cell lines to irradiation. The mechanism involved the adenoviral protein E1B55kDa, which is able to inhibit DNA repair machinery involved in sensing double-strand breaks in DNA (Kuroda *et al.* 2010). Though the outcomes of these studies differ in mechanisms of action, they provide the rationale to test combinations of OVs and irradiation in the context of thyroid cancers.

Despite its clinical success, radiotherapy is viewed as a double-edged sword; it can be attributed to the development of secondary cancers later in life. This is especially impactful for thyroid cancers, where radiation has been intimately linked to its development. Retrospective studies of testicular and Hodgkin lymphoma patients unveiled risk of thyroid cancers years following radiation therapy (Travis *et al.* 2005, Castellino *et al.* 2011).

Limiting the negative impacts of radiotherapy may also be achievable through radiovirotherapy, using OV's engineered to carry the sodium iodide symporter gene NIS (Trujillo *et al.* 2013, Galanis *et al.* 2015). NIS is normally expressed on thyroid follicular cells and mediates uptake of radioactive iodine during RAI. OV-driven expression of NIS functions to concentrate radioisotopes with the additional advantages of specific targeting to cancer cells and immune stimulation by viral infection. Although mainly used to extend the use of radiovirotherapy to other solid cancers that do not have basal NIS expression, this method has been applied to thyroid cancers pre-clinically using a VACV carrying the human NIS (Gholami *et al.* 2011). This strategy could be used clinically to reduce the off-target effects of standard-of-care radiotherapy and offer potential to eliminate drug-resistant metastases that mediate disease recurrence.

Conclusions and future directions

Immunotherapy for thyroid cancers is indeed a possible mission. Various immunological cell subsets naturally infiltrate the thyroid tumor microenvironment. These leukocytes have prognostic value and play roles in tumor progression and elimination. Compared to other tumor types, there is a relative lack of research focused on the development of immunotherapies specifically for thyroid cancers. However, patients with thyroid tumors could feasibly benefit from the extensive development of immunotherapeutics targeting other solid tumors. Notably, many of the standard-of-care treatments already being used for thyroid cancers have previously unappreciated immunomodulatory effects. The effect of thyroid-derived hormones on immune responses in patients being treated with thyroid cancers remains a particularly understudied area. Developing a better understanding of this particular endocrine-immune axis will be essential to tailor immunotherapies for thyroid cancers. Perioperative treatments can help reduce the impact of surgery-related immunosuppression, potentially lightening the impact of thyroid tumor resection on any integrated immunotherapies. Radiation therapy, while inherently precarious, can induce tumors to become quite immunogenic, offering utility if used in combination with immunotherapy. Cancer vaccine-induced and/or adoptively transferred T cells targeting thyroid tumors hold the potential to be potent therapies, provided appropriate antigens can be identified. Developments involving checkpoint blockade inhibitors

are also an exciting potential treatment modality for thyroid cancer and would be particularly amendable to potentiating methods designed to expand the repertoire of tumor-specific T cells. Moreover, vector-based vaccines such as oncolytic viruses, when armed with iodine symporter or cytokine transgenes could add an additional angle of attack. Overall, immunotherapies represent a particularly promising cutting-edge approach to treating thyroid cancers and will undoubtedly become effective components in the toolbox of future oncologists that treat this disease.

Declaration of interest

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