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The impact of elective cervical lymph node treatment on the tumour immune response in head and neck squamous cell carcinoma: time for a change in treatment strategy?

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The elective ablation of cervical lymph nodes, via surgery or irradiation, is a mainstay in the treatment of head and neck squamous cell carcinoma (HNSCC). In this setting, the decision to treat the clinically node negative neck is based upon risk analysis of various factors, primarily derived from tumour features. However, the impact of ablation of tumour-draining lymph nodes upon the tumour-immune response and immunocompetence is largely unknown. In this review we highlight recent evidence of the communication between tumour and tumour-draining lymph nodes and the fundamental importance of this axis. We will provide a perspective of how recent cancer biology discoveries may juxtapose with current treatment pathways, with potential translational line of site for future research. In particular, neo-adjuvant therapy or biomarkers from tumour-draining lymph nodes may present opportunities to preserve lymphatics and harness improved immunocompetence in HNSCC patients.

BJC Reports; https://doi.org/10.1038/s44276-024-00095-1

INTRODUCTION

Ablation of regional lymph nodes is a key component of the treatment strategy for head and neck squamous cell carcinoma (HNSCC), either via surgery or irradiation. In surgically treated HNSCC's, surgical technique has evolved from a morbid radical neck dissection procedure to selective nodal removal in the majority of cases [1]. In addition, although multiple level neck irradiation remains in most cases the mainstay in head and neck radiotherapy, there is a growing interest in selective and super-selective neck dosimetry with the aim of reducing toxicity whilst maintaining oncologic outcomes. However, it should be noted that the management of the clinically node negative (cN0) neck and methods to stratify the need for regional treatment are still debated [2, 3]. At the other end of the treatment pathway, in the recurrent and/or metastatic (R/M) setting, despite success in several cancer types, immunotherapy with anti-PD-1 immune checkpoint inhibitors (ICI) has largely underperformed initial expectations yielding only modest outcomes in HNSCC [4]. Furthermore, anti-CTLA-4 in combination with anti-PD-1 ICI therapy has failed to demonstrate benefit for HNSCC patients [5]. In contrast to tumours such as melanoma, with a higher tumour mutational burden, where combination ICI therapy is now standard practice [6]. Additionally, in locally-advanced disease, adding anti-PD-1 ICI to standard chemoradiation has failed to show any benefit in either progression-free or overall survival. Collectively, these findings raise the possibility that standard-of-care oncologic treatment for HNSCC may compromise host immunity and the ability to respond to ICI. In this review we will outline evidence regarding the pivotal role of tumour draining lymphatics and immune function, juxtaposed with the concept of elective lymphatic ablation in HNSCC. We will highlight the axis of communication via both tumour cell and immune cell transport that occurs between the primary tumour and regional lymph nodes. Furthermore, throughout this perspective review we will ask key questions to consider that may allow a paradigm shift in the treatment planning and surgical management of the cervical lymph nodes in HNSCC patients.

RATIONALE FOR CERVICAL LYMPH NODE TREATMENT

Single modality primary treatment is indicated for the treatment of early stage HNSCC, with treatment to the neck mirroring that of the primary. For the majority of oral (anterior) cancers this involves surgical treatment, but for posterior tumours radiotherapy is often favoured. Several large cohort clinical trials demonstrate a clear benefit for surgical elective neck dissection (END) in early oral cancer to increase both disease-free and overall survival compared to watchful wait [2, 7]. Accepted wisdom is to perform an END when the risk of occult neck metastases is greater than 20% [8], however the stratification of such risk is open to interpretation and largely based upon data relating to tumour depth of invasion. While up to 25% of patients with a clinically N0 neck may have occult neck metastases [9], there is a clear counter argument that up to 75% of N0 patients do not require elective treatment. Improved detection of occult metastasis may hold the answer, with convincing evidence for the role of surgical staging by sentinel lymph node biopsy (SLNB) [3]. The effectiveness of elective neck irradiation (ENI) for treating occult metastatic lymph

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Received: 1 July 2024 Revised: 17 August 2024 Accepted: 21 August 2024
Published online: 09 September 2024

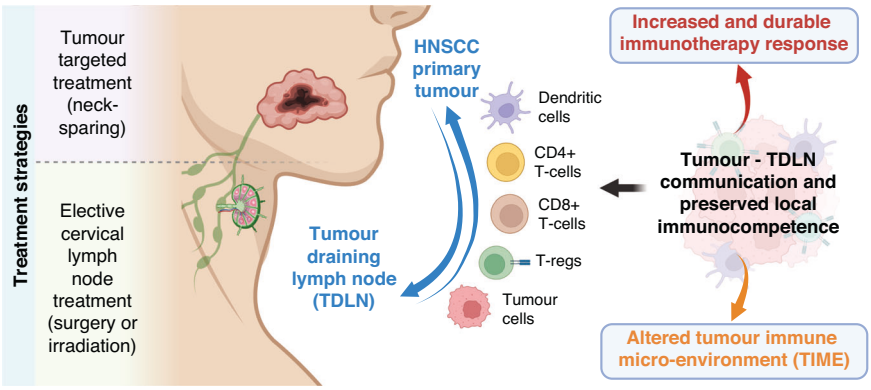

Fig. 1 Picture representation of how treatment strategies of standard elective neck node ablation or tumour-specific neck-sparing treatment can influence the axis of communication between the primary tumour and tumour-draining lymph nodes. Important cell-types are listed, and how they can alter the tumour immune microenvironment and impact immunotherapy response.

nodes, first demonstrated by Fletcher [10] and later supported by others, led to its routine administration for clinically node-negative necks. In ENI of HNSCC, determining the appropriate irradiation volume is crucial, since insufficient radiation can lead to poor tumour control, whilst excessive radiation can lead to increased toxicity. Currently, the guidelines proposed by Grégoire et al. in 2000 [11], which were updated in 2018 [12] and 2019 [13], are widely used for selecting the ENI volume. However, these guidelines have some limitations, since they do not account for some clinical and pathological factors (i.e. tumour size, grade and subsite) or any other biomarker-led selection. Additionally, despite evidence showing that the lymph node metastasis rate is lower in the contralateral neck compared to the ipsilateral neck, the guidelines recommend the same irradiation scheme for both sides. In some cases this may be contradictory to surgically treated patients who receive a unilateral END. Hence, to achieve more personalized and precise lymphatic ablation for HNSCC patients, it is essential to gain further and deeper understanding of the cancer biology, interplay between tumour and tumour-draining lymph nodes and anti-cancer immune response.

The oncological rationale behind neck node treatment is clear—oral cancer primarily metastasises to regional lymph nodes, which when present instantly drops survival by half, *so why not remove the LNs that may harbour occult metastases?* In contrast, perhaps an additional question that should be asked is—*what role do tumour-draining neck LN's play in immune-mediated tumour suppression and how can we improve the selection of patients that require ablation of the neck?*

METASTATIC SPREAD VIA LYMPHATIC VESSELS

Malignant cells mainly disseminate via the blood and lymphatic vessels. Conceptually, cancer cells invade into the lymphatics and passively move to collecting lymphatic vessels, migrating to tumour-draining lymph nodes (TDLNs). The sentinel lymph node (SLN) of a tumour is defined as the first TDLN with direct lymphatic flow from the primary tumour. In the clinical setting, micro-metastasis to the SLN is a key determinant associated with reduced distant metastasis-free survival in different tumour types [14], including HNSCC [15]. Prior to metastasis, cancer cells release specific soluble factors (including vascular-endothelial growth, platelet-derived growth factors and fibroblast growth factors) that modulate the microenvironment in the SLN to establish a beachhead for successful colonisation [16]. After colonisation, cancer cells inhibit anti-cancer immunity by inducing the recruitment of regulatory T cells (Tregs) and myeloid-derived suppressor cells—thus suppressing the function of dendritic cells (DCs) and $CD8^+$ T-cells, and promoting the release of immuno-suppressive cytokines, all of which contribute to the establishment of a pre-metastatic microenvironment [17, 18]. Indeed, a reduction in lymphocyte infiltration and interaction between exhausted $CD8^+$ T-cells with high *CXCL13* expression with tumour cells to acquire more aggressive phenotypes of extra-nodal expansion, have been reported in HNSCC [19]. Furthermore, a population of immunosuppressive Tregs ($CD4^+$, $CD25^{hi}$, $FOXP3^+$) has shown to be significantly elevated in tumour-cell positive lymph nodes in different malignancies [20]. The diversity of T-cells in the primary tumour and SLN is very significant and only a very small proportion of expanded T-cell clones have been found in SLNs [21]. Collectively, existing data suggest that the immune micro-environment in the SLN is conditional prior to cancer cell arrival, and that reactivation of anti-cancer immunity in the SLN may prevent tumour metastases and may play a crucial role in the response to immunotherapies.

COMMUNICATION BETWEEN TUMOUR AND TUMOUR-DRAINING LNS

The relationship between the tumour immune micro-environment (TIME) and tumour draining lymph nodes (TDLNs) in HNSCC is a recent topic of growing interest, as outlined in Fig. 1. Much research has concentrated on identifying predictive biomarkers in the blood or TIME [22]. However, the critical role of TDLNs as sources of tumour-specific $CD4^+$ and $CD8^+$ T-cells, and as sites for essential anti-cancer immunological processes—including antigen presentation, immune cell activation, priming, proliferation, and differentiation—requires more understanding. As discussed above, growing evidence highlights the crucial role of TDLNs (in particular the SLN) in orchestrating anti-cancer immune responses. Taken together, the trajectory of T-cell populations between tumour and LN, and the impact of lymphatic ablation upon this tumour-LN axis and subsequent efficacy of anti-cancer therapy is poorly understood.

When considering the impact upon immune checkpoint inhibitor (ICI) therapy, in a tobacco-signature oral squamous cell carcinoma preclinical mouse model, Saddawi-Konefka et al. observed that lymph node dissection prior to ICI therapy (anti-CTLA-4) significantly reduced response rates and overall survival [23]. In this seminal paper, increased $CD8^+$ T-cells were observed in TDLNs with a key role for interferon type-I (IFN-I) mediated stimulation, among other cytokines and chemokines. In addition, they highlighted the role of antigen-presenting DCs (conventional type I DCs), which were increased twofold in TDLNs when lymphatic ablation was avoided. Crucially, DC mediated tumour immunity via tumour-specific antigen T-cells priming decreased fivefold in primary tumours when a lymph node dissection was performed. This preclinical study strongly supports the rationale for upfront lymphatic-preserving therapy, particularly to harness

the endogenous IFN-I- and DC-driven anti-tumour response in the immediate period following delivery of ICI, which ultimately leads to regional and systemic host anti-tumour immunity. With regard to anti-PD-1/PD-L1 treatment, multiple studies have described how T-cell activation by ICI therapy, defined as an increase in $CD8^+$ T cells and activated $CD8^+$ T-cells, takes place in TDLNs but *not* in n-TDLNs or in lymph nodes from tumour-free mice [24]. Indeed, interactions between PD-$L1^+$ T-cells and PD-$L1^+$ DCs in TDLNs seem crucial to induce an enhanced anti-tumour T-cell immunity by seeding the tumour site with progenitor-exhausted T-cells, resulting in improved tumour control [25].

The impact of lymphatic ablation upon the TIME is also critical. In one study, a distinct immune signature was observed in mice receiving tumour irradiation with preserved nodal basins, in comparison to those also receiving elective nodal irradiation. Marciscano et al. described an 'intra-tumoural T-cell chemoattractant signature' present in those mice which received nodal sparing irradiation, with increased chemokines (CXCR3 and CCR5) and infiltration of tumour-antigen specific $CD8^+$ T-cells and regulatory T-cells (Tregs) [26]. As above, survival following ICI therapy was increased in node-sparing populations [26]. Tregs are observed to be trafficked between tumour and TDLNs, and tumour invasion in TDLNs is associated with Treg accumulation in breast cancer patients. Moreover, Tregs in TLDNs and primary tumour present with a high suppresive phenotype (with elevated levels of CTLA-4, ICOS, GITR and OX40 expression) [27]. Clearly the cross-talk between tumour and TDLNs is complex and remains to be elucidated. But the above data should demonstrate how functional neck LNs are critical to enable infiltrating immune cells to mount an effective anti-tumour response.

The use of T-cell receptor sequencing (TCRseq) has further illuminated this niche relationship between the TIME and TDLNs. Research in TCRseq has globally indicated trafficking of T-cells between TDLNs and tumours with shared TCR clonotypes of PD-1^+ $CD8^+$ T-cells in lymph nodes and tumour-infiltrating lymphocytes in different tumour types [27–29]. Recently, Suzuki et al. showed in a small cohort of HNSCC patients, that TCR repertoire diversity was higher in cancer tissue and metastatic TDLNs compared with non-metastatic TDLNs, and that TCR repertoire similarities in Tregs and $CD8^+$ T-cells between metastatic TDLNS and tumours were higher when compared with those at other sites [30]. These findings suggest that cancer-reactive T-cell clones are enriched in both, when metastatic positive TLDNs are present. In addition, the detection of shared TCR CDR3βs between tumour-expanded T-cell clones and TDLN T-cells underscores the potential contribution of TLDNs as source of tumour-specific T-cells that might be modulated by ICIs. As suggested by Zemmour et al, Tregs with the same TCRs and same CDR3β share their antigen specificity and may also share an early imprinted specific programme that persists after priming and has driven them to specific anatomical locations with common environmental cues [31]. Furthermore, inter-tissue and TLDN sharing of Tregs clones might suggest a loco-regional suppression mechanism. The fact that a certain proportion of tumour-Treg CDR3β are found to be shared with conventional T-cells, may indicate that a proportion of these Tregs could arise through peripheral or TLS induction or conversion of $CD4^+$ conventional T-cells. The role of specific TCR characteristics in the TDLNs and their contribution to anti-cancer immune response or prevention from further cancer dissemination to other lymph nodes or distant organs remains unknown. Indeed, TCRseq of both the tumour and the TDLNs may serve as a way to reconcile conflicting reports that support either clonal replacement (recruitment of novel T-cells into the tumour) or clonal revival (reinvigoration of pre-existing tumour-infiltrating T-cells) to ICI or other therapies, including radiation.

ALTERING TREATMENT PROTOCOLS

Herein lies the core question of this perspective review. At first glance, the historical concept of elective nodal ablation and recent evidence demonstrating the role of TDLNs in immune-mediated tumour suppression appear to be diametrically opposed—*how can these be reconciled in an oncologically safe but immunologically effective treatment protocol?*

Currently, one might argue that the above considerations only apply to early stage (T1/T2) tumours which are clinically N0, since clearly patients with positive nodal disease will require some form of lymphatic ablation. Despite this, there may be potential for "window of opportunity" neo-adjuvant treatment that can down-stage disease to levels amenable to a more conservative approach. Reported response rates to neoadjuvant PD-1 ICI therapy in resectable HNSCC vary, in the region of 10–30% [32–34]. A recent meta-analysis demonstrated significantly higher response rates when ICI therapy was combined with chemo or radiotherapy, upwards of 75% [32]. Of note, in sub-group analysis, three studies demonstrated a favourable nodal pathological response, with opportunity to down stage the neck. A recent trial by Leidner et al., which evaluated neo-adjuvant (neck sparing) tumour radiotherapy and anti-PD-1 immunotherapy yielded particularly promising results, with 90% of patients being down staged from clinical to pathological staging [35]. When one looks more closely at this data, of interest is the number of patients who were N-positive and down staged to pathological N0 following neo-adjuvant immuno-radiotherapy. In total, of the 18 clinically N-positive patients, 16 of those were down staged to N0. If we apply current treatment protocols to these down staged patients, i.e. those with a cN0 neck, they would potentially be eligible for SLNB staging. In this scenario the SLNB would allow pathological assessment of the neck response and allow lymphatic preservation of negative, in patients that would ordinarily receive lymphatic ablation. It should be noted that a pathological response in the primary tumour following neo-adjuvant treatment is not necessarily a surrogate marker for neck response—further emphasising the importance of accurate staging of the neck. The IMCISION trial (which evaluated neo-adjuvant combination nivolumab and ipilimumab vs single agent nivolumab) reported that all four N+ patients who demonstrated a primary tumour major pathological response exhibited residual nodal tumour deposits, with a partial nodal response in two patients [36]. Thus, accepting the above logic of the importance of regional (neck) lymphatic function in immune-mediated tumour suppression—*in future should we be considering the opportunity to preserve neck LNs in advanced tumours with the aid of neo-adjuvant therapy?*

From a clinical viewpoint, future-proofing treatment protocols for immunotherapy use is clinically less pertinent in early stage disease—where survival is around 80% at five-years and rates of recurrence are generally low. In contrast, advanced stage disease has a high incidence of local recurrence up to 60% with greater risk of distant treatment failure. Therefore, these are patients who will likely benefit from neo/adjuvant ICI therapy, or the option of improved ICI therapy response rates at time of R/M by the preservation of the TDLNs—a so-called "planning for failure" approach. The timing of immunotherapy and lymphatic ablation is shown to be critical to enable durable anti-tumour immunity. In the aforementioned study, Saddawi-Konefka et al. demonstrated not only that employing a 'late' lymphatic dissection (6 days after ICI therapy) resulted in a more effective anti-tumour response, but also the response to ICI therapy prevailed in mice who had an initial complete response followed by lymphatic ablation and a further tumour challenge and subsequent ICI therapy [23]. Thus, the TDLNs are required for a priming action to provide lasting durable immunity i.e. *one can still perform elective neck lymphatic ablation and not diminish efficacy of future ICI therapy if treatment is sequenced correctly*.

BIOMARKER STRATIFICATION

Finally, it remains to be seen which biomarkers will best predict a durable response from neo-adjuvant ICI therapy that survives lymphatic ablation. Whether this will be driven by clinical response or immune markers will be the topic of future research. As evidenced from multiple trials, tumour expression of immune checkpoint markers, for example PD-L1, are poorly predictive of ICI treatment response in HNSCC—in the region of 20–30% [37, 38]. In general, HNSCC remains poorly served by predictive biomarkers, despite great impetus in the literature to answer this question [22]. However, rapidly advancing multi-omic tumour analytical strategies that combine high-depth proteomic cellular phenotype interrogation with spatially orientated genomic/transcriptomic analysis herald a new era of biomarker discovery. In addition to static or pre-treatment biomarkers (i.e. tumour intrinsic factors such as genomic instability and mutational load), dynamic and peripheral biomarkers may have an important role in selecting patients for elective ablation or lymph node sparing treatment - these include circulating tumour DNA [39] and peripheral TCRseq [40]. Furthermore, the assessment of TDLNs (in particular the SLN) is a potential rich source of biomarker stratification for ICI therapy [41, 42], which could be elegantly combined with established SLNB procedures when staging the neck. A novel avenue of future research will be the multi-omic assessment of tumour and TDLN heterogeneity in HNSCC [43, 44] and how this relates to high-dimensional liquid biopsy data outputs [45, 46].

CONCLUSION

In summary, we have highlighted the importance of the tumour-LN axis in immune mediated tumour suppression and the impact that lymphatic ablation has upon the TIME. We have described how future treatment protocols in HNSCC may seek to leverage the relationship between SLNB and lymphatic preservation combined with ICI therapy in the neo-adjuvant setting. Adding ICI therapy to standard of care treatment of HNSCC has not yielded the clinical benefit seen in other cancers. Therefore, treatment protocols need reconsidering with a paradigm shift in rationale for surgery and radiotherapy, such as elective nodal treatment, and a greater understanding of the biological significance this has upon the innate and adaptive anti-tumour response [47]. As was our aim, we have provided more questions than answers in a contentious and fast-evolving landscape concerning neck LN management in current HNSCC treatment. Evidence that seeks to change practice in the management of the neck in HNSCC will need to be rigorous and robust to alter long established treatment protocols. Clinical trials that combine ICI therapies with novel multi-omic analysis for risk stratification will undoubtedly hold the answer.

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AUTHOR CONTRIBUTIONS

KP—conceptualisation. KP, PN, CS—writing, original draft. KP, PN, CS—writing, review and editing. All authors approved the final version.

COMPETING INTERESTS

KP is an Associate Editor for BJC Reports. He was not involved in any aspect of handling of this manuscript or any editorial decisions. The authors declare no other competing interests.

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