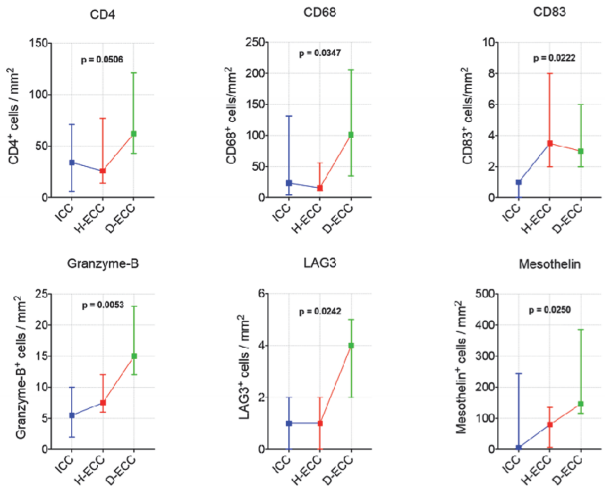
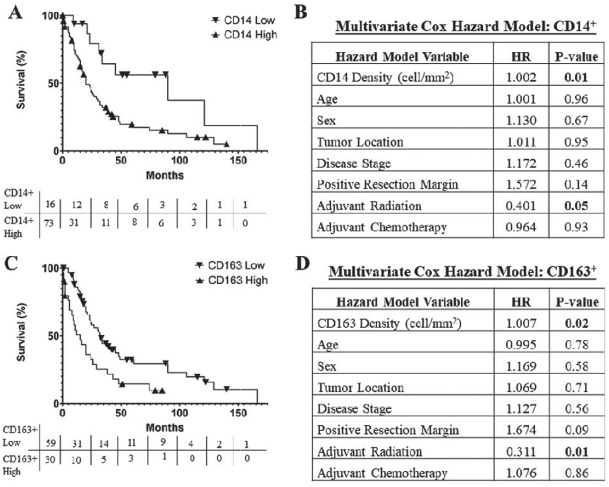


Abstract 748 Figure 2 Immune infiltration based on primary tumor location
Increase in immune infiltrate in primary tumors as distance from liver increases. P-values determined by Jonckheere-Terpstra Test with FDR corrections



Hazard Model Variable	HR	P-value
CD14 Density (cell/mm²)	1.002	**0.01**
Age	1.001	0.96
Sex	1.130	0.67
Tumor Location	1.011	0.95
Disease Stage	1.172	0.46
Positive Resection Margin	1.572	0.14
Adjuvant Radiation	0.401	**0.05**
Adjuvant Chemotherapy	0.964	0.93

Hazard Model Variable	HR	P-value
CD163 Density (cell/mm²)	1.007	**0.02**
Age	0.995	0.78
Sex	1.169	0.58
Tumor Location	1.069	0.71
Disease Stage	1.127	0.56
Positive Resection Margin	1.674	0.09
Adjuvant Radiation	0.311	**0.01**
Adjuvant Chemotherapy	1.076	0.86

Abstract 748 Figure 3 CD14 and CD163 Correlate with OS
A+C) Kaplan Meier Curve of OS for (A) CD14 (Median OS: 20 vs. 90 months, log-rank p-value <0.01) and (C) CD163 (Median OS: 15 vs. 32 months, log-rank p-value<0.01). B+D) Multivariate Cox Hazard Models. Assumptions of Cox Hazard Model were checked with Schoenfeld residual values, significance level <0.01

Conclusions The TIME of CC varies significantly by primary tumor location and between primary and metastatic lesions. D-ECC has a favorable immune profile compared to ICC and H-ECC, with a better milieu for antigen presentation including increased mesothelin and less suppressive macrophages, which may support better response to checkpoint blockade. The data supported the hypothesis that higher densities of intra-tumoral M2 macrophages and myeloid cells correlated with worse OS, even after controlling for clinical variables, suggesting that these cell populations may represent promising immunotherapeutic targets in CC.

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749 SPATIAL PROFILING OF THE TUMOUR MICROENVIRONMENT IN HEAD AND NECK CANCER TO IDENTIFY BIOMARKERS OF RESPONSE TO THERAPY

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Background Immune checkpoint inhibitors (ICI) have shown durable and long-term benefits in a subset of head and neck squamous cell carcinoma (HNSCC) patients. To identify patient-responders from non-responders, biomarkers are needed which are predictive of outcome to ICI therapy. Cues in the tumour microenvironment (TME) have been informative in understanding the tumour-immune contexture.
Methods In this study, the NanoString GemoMx™ Digital Spatial Profiling (DSP) technology was used to determine the immune marker and compartment specific measurements in a cohort of HNSCC tumours from patients receiving ICI therapy.
Results Our data revealed that markers involved with immune cell infiltration (CD8 T-cells) were not predictive of outcome to ICI therapy. Rather, a number of immune cell types (CD4, CD68, CD45, CD44, CD66b) were found to correlate with progressive disease.
Conclusions This study, to our knowledge, represents the first spatial analysis of HNSCC tumours.
Ethics Approval The study was approved by the Queensland University of Technology Ethics Board.

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750 MALAT1 LNCRNA CONTROLS METASTATIC REACTIVATION OF DORMANT BREAST CANCER BY IMMUNE EVASION

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Background Long non-coding RNAs (lncRNAs) are involved in various biological processes and diseases. Malat1 (metastasis-associated lung adenocarcinoma transcript 1), also known as Neat2, is one of the most abundant and highly conserved nuclear lncRNAs. Several studies have shown that the expression of lncRNA Malat1 is associated with metastasis and serving as a predictive marker for various tumor progression. Metastatic relapse often develops years after primary tumor removal as a result of disseminated tumor cells undergoing a period of latency in the target organ.[1–4] However, the correlation of tumor intrinsic lncRNA in regulation of tumor dormancy and immune evasion is largely unknown.
Methods Using an in vivo screening platform for the isolation of genetic entities involved in either dormancy or reactivation of breast cancer tumor cells, we have identified Malat1 as a positive mediator of metastatic reactivation. To functionally uncover the role of Malat1 in metastatic reactivation, we have developed a knock out (KO) model by using paired gRNA CRISPR-Cas9 deletion approach in metastatic breast and other cancer types, including lung, colon and melanoma. As proof of concept we also used inducible knockdown system under in vivo models. To delineate the immune microenvironment, we have used 10X genomics single cell RNA-seq, ChIRP-seq, multi-color flowcytometry, RNA-FISH and immunofluorescence.
Results Our results reveal that the deletion of Malat1 abrogates the tumorigenic and metastatic potential of these tumors

and supports long-term survival without affecting their ploidy, proliferation, and nuclear speckles formation. In contrast, overexpression of Malat1 leads to metastatic reactivation of dormant breast cancer cells. Moreover, the loss of Malat1 in metastatic cells induces dormancy features and inhibits cancer stemness. Our RNA-seq and ChIRP-seq data indicate that Malat1 KO downregulates several immune evasion and stemness associated genes. Strikingly, Malat1 KO cells exhibit metastatic outgrowth when injected in T cells defective mice. Our single-cell RNA-seq cluster analysis and multi-color flow cytometry data show a greater proportion of T cells and reduce Neutrophils infiltration in KO mice which indicate that the immune microenvironment playing an important role in Malat1-dependent immune evasion. Mechanistically, loss of Malat1 is associated with reduced expression of Serpinb6b, which protects the tumor cells from cytotoxic killing by the T cells. Indeed, overexpression of Serpinb6b rescued the metastatic potential of Malat1 KO cells by protecting against cytotoxic T cells.

Conclusions Collectively, our data indicate that targeting this novel cancer-cell-initiated domino effect within the immune system represents a new strategy to inhibit tumor metastatic reactivation.

Trial Registration N/A

Ethics Approval For all the animal studies in the present study, the study protocols were approved by the Institutional Animal Care and Use Committee(IACUC) of UT MD Anderson Cancer Center.

Consent N/A

REFERENCES

1. Arun G, Diermeier S, Akerman M, *et al*., Differentiation of mammary tumors and reduction in metastasis upon Malat1 lncRNA loss. *Genes Dev* 2016 Jan 1;**30**(1):34–51.
2. Filippo G. Giancotti, mechanisms governing metastatic dormancy and reactivation. *Cell* 2013 Nov 7;**155**(4):750–764.
3. Gao H, Chakraborty G, Lee-Lim AP, *et al*., The BMP inhibitor Coco reactivates breast cancer cells at lung metastatic sites. *Cell* 2012b;**150**:764–779.
4. Gao H, Chakraborty G, Lee-Lim AP, *et al*., Forward genetic screens in mice uncover mediators and suppressors of metastatic reactivation. *Proc Natl Acad Sci U S A* 2014 Nov 18; **111**(46): 16532–16537.

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752 THE IMPACT OF GRADE OF DIFFERENTIATION AND BRAF MUTATION STATUS ON NEOANTIGEN AND IMMUNE LANDSCAPE IN PAPILLARY THYROID CANCER

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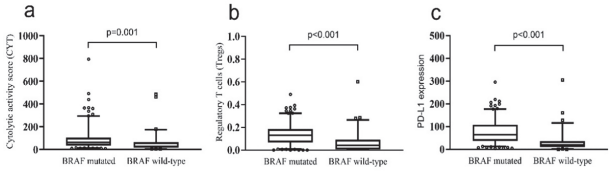
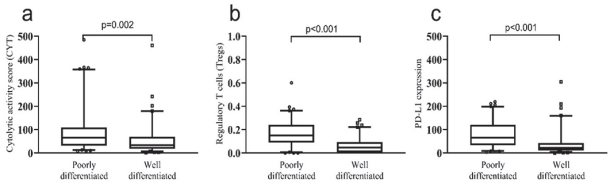
Background The use of immune checkpoint inhibitors (ICIs) in cancer treatment has been approved by the FDA, but its application is experimental in the treatment of papillary thyroid cancer (PTC). Induction of immune response via recognition of neoantigens is considered to be the basis for the treatment mechanism of ICIs.[1] However, the neoantigen landscape has not been explored in PTC. Our aim is to investigate the immune landscape of PTC in relation to neoantigens, taking into account the BRAF mutation status and grade of differentiation as contributing factors.

Methods BRAF V600E mutation status and thyroid differentiation scores (TDSs) were gathered from the PTC cohort of The Cancer Genome Atlas (TCGA). TDS was derived from the mRNA expression levels of 16 thyroid function genes to quantify the grade of differentiation. Tumors with TDSs in the 1st quartile and 4th quartile were defined as poorly differentiated and well differentiated, respectively. The neoantigen burden for each sample was predicted using CloudNeo pipeline. The infiltration of immune cells was calculated through CIBERSORT.

Results Among 400 patients with predicted neoantigen data, 187 (47%) had BRAF mutations. The BRAF mutated tumors showed increased cytolytic activity score (CYT, p=0.001), increased infiltration of regulatory T cells (Treg, p<0.001), and higher PD-L1 expression (p<0.001) compared to BRAF wild-type tumors (figure 1). In regard to grade of differentiation, poorly differentiated tumors showed increased CYT (p=0.002), increased infiltration of Treg (p<0.001), and higher PD-L1 expression (p<0.001) compared to well differentiated tumors (figure 2). However, BRAF mutation status or grade of differentiation did not correlate with the neoantigen burden. Also, the neoantigen burden did not show any correlations with immune landscape features such as infiltration of CD8+ T cells or Treg, CYT, and PD-L1 expression.



Abstract 752 Figure 1 Immune traits according to BRAF mutation status. (a) Cytolytic activity score(CYT). (b) Infiltration of regulatory T cells(Tregs). (c) PD-L1 expression.



Abstract 752 Figure 2 Immune traits according to grade of differentiation. (a) Cytolytic activity score(CYT). (b) Infiltration of regulatory T cells(Tregs). (c) PD-L1 expression.

Conclusions Increased CYT and higher expression of PD-L1 in the BRAF mutated or the poorly differentiated tumors imply the possible role of ICI use in these subgroups of patients. However, the immune response to these subgroups does not seem to be mediated through the increase in neoantigen formation. Further studies are warranted to explore markers for immunotherapy implication.

REFERENCES

1. Schumacher TN, Schreiber RD, Neoantigens in cancer immunotherapy. *Science* 2015; **348**:69–74.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0752