common ICIs were pembrolizumab, nivolumab, followed by ipilimumab-nivolumab, ipilimumab, and durvalumab. 81 patients of 218 received antibiotics within 6 months of receiving checkpoint inhibitors. Of antibiotics administered, the most common classes were cephalosporins (86%), fluoroquinolones (28%), and glycopeptides (23%) with substantial overlap. Overall survival and progression-free survival was improved for those who did not receive antibiotics prior to ICI therapy (median OS 6.5 vs. 2.3 years, HR 0.36, $p < 0.0001$; median PFS 1.1 vs 0.5 years, HR 0.6, $p = 0.0027$) (figure 1 and 2 respectively). Linear regression showed no significant association between antibiotic use prior to ICI use and age, sex, race, ICI type, or ECOG status.

Conclusions This data adds to the growing body of knowledge that the use of antibiotics prior to ICI treatment leads to inferior overall and progression-free survival.

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RARE CASE REPORTS ON THYMIC CARCINOMA PATIENTS TREATED WITH PEMBROLIZUMAB

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Background Thymomas and thymic carcinomas (TC) are intrathoracic malignancies that, although rare, represent the most common anterior mediastinal tumors in adults (comprising approximately 0.2–1.5% of all malignancies).¹ Recent accumulating evidence on immune checkpoint pathways suggests that immunotherapy might be a promising therapeutic option for refractory TCs.² We herein report two cases of a pembrolizumab-treated TC patient.

Methods Immunotherapy is accessible through individual permission for the treatment of TC in Hungary. We retrospectively collected data of two TC patients treated with pembrolizumab in one institute.

Results The first patient was a 66-year-old woman. Squamous cell TC was diagnosed in her left parasternal region. She was

a former smoker, had no history of autoimmune disorders, and had no associated symptoms at the time of diagnosis. She underwent open thymectomy. The histology proved type C (WHO classification) TC with a pathological TNM of T1aN0 and microscopic-positive margins. Consequently, the patient received 60 Gy of postoperative radiotherapy. Nine months after the surgery local recurrence and multiple hepatic metastases appeared. Six cycles of chemotherapy (ADOC regimen) were introduced as first-line systemic therapy which resulted in stable disease (SD) for 8 months. Pembrolizumab was administered as second-line treatment (200 mg every 3 weeks) for 6 cycles. The best result was SD. Due to progression, third-line docetaxel treatment was initiated. Shortly after, ptosis and diplopia developed. Myasthenia gravis was diagnosed, and third-line chemotherapy was judged ineffective. The second patient was a 63-year-old man. He was diagnosed with unresectable TC and treated with chemotherapy (ADOC regimen) up-front. After six cycles the tumor regressed, and surgery was performed with R2 result. Postoperatively, the patient was given six cycles of chemotherapy (cisplatin/etoposide) and radiotherapy. Six months later local progression was detected and pembrolizumab was commenced. Eight cycles of pembrolizumab produced SD as best response. No immune-related adverse effects (irAEs) were detected. After progression Sunitinib therapy was started. In both cases, additional immunohistochemistry investigations were performed.

Conclusions In the literature, there is no phase 3 trial on immune-checkpoint inhibitor (ICI) therapy of TC. Phase 2 trials reported promising results with pembrolizumab.^{3–4} However, there are conflicting results with other ICIs.⁵ Before starting, it is important to rule out autoimmune disorders to evade serious, even life-threatening immune-complications. The high likelihood of irAEs in TC also underpin the importance of predictive biomarkers. Further studies are required to evaluate the efficacy and safety of immunotherapy in TC.

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A ROLE FOR IMMUNE CHECKPOINT BLOCKADE TO ENHANCE T CELL-MEDIATED RESPONSES IN COMBINATION WITH CHEMOTHERAPY IN OESOPHAGEAL ADENOCARCINOMA

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Background Combining immune checkpoint inhibitors (ICIs) with immunogenic chemotherapies is a promising approach in

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oesophageal adenocarcinoma (OAC) to convert 'cold' tumours to 'hot' tumours expanding the efficacy of ICIs to a greater spectrum of patients.¹ However, there is a vast array of immune checkpoints (ICs) expressed by T cells and the effect of ICIs in combination with chemotherapy regimens is largely unknown.²

Methods The expression profile of a range of ICs on circulating and tumour-infiltrating T cells was assessed using flow cytometry prior to and post-neoadjuvant treatment and correlated with clinical parameters (n=20). PBMCs isolated from OAC blood were treated with single agent ICIs alone (single agent anti-PD-1, anti-PD-L1, anti-A2aR and anti-TIM-3 inhibition) and in combination with FLOT (5-Fluorouracil, oxaliplatin and docetaxel) and CROSS (carboplatin and paclitaxel) chemotherapy regimens. The production of anti-tumour cytokines by T cells was assessed in vitro by flow cytometry (n=6).

Results In the treatment-naïve and post-treatment setting, a range of ICs were expressed by circulating T cells and were significantly increased on tumour-infiltrating T cells, which included PD-L1, PD-L2, CD160, PD-1, CTLA-4, TIGIT, TIM-3, LAG-3, A2aR and ICOS (p<0.05) (figure 1). Pre-treatment circulating PD-1+ T cells positively correlated with pathological nodal status (p<0.05), (figure 2). Whereas tumour-infiltrating CD3+CTLA-4+ cells positively correlated with nodal metastasis and lymphovascular invasion (p<0.05). The percentage of tumour-infiltrating CD3+CTLA-4+ and CD3+ICOS+ cells was significantly lower post-neoadjuvant treatment (p<0.05) (figure 3). However, post-neoadjuvant treatment circulating CD3+PD-1+ cells and CD3+CD4+TIGIT+ cells positively correlated with a better treatment response, determined by PET/CT (p<0.05), (figure 4). ICIs enhanced T cell production of anti-tumour cytokines IL-2 and IFN-γ alone and in combination with chemotherapy in vitro from treatment-naïve OAC patients (p<0.05).

Abstract 776 Figure 1

The percentage of T cells expressing PD-1 was significantly increased in tumour tissue compared with peripheral blood circulation in the treatment-naïve setting of OAC patients CD3+, CD3+ CD4+ and CD3+ CD8+ cells were screened for the surface expression of PD-1 in OAC patient blood pre-treatment blood and tumour tissue (n=20). Mann Whitney test **<0.001.

Abstract 776 Figure 2

Pre-treatment circulating PD-1+ T cells positively correlated with pathological nodal status and tumour-infiltrating CD3+CTLA-4+ cells positively correlated with nodal metastasis and lymphovascular invasion. The percentage of CD3+, CD3+CD4+ and CD3+CD8+ cells expressing ICs in peripheral circulation and infiltrating OAC tissue in the treatment-naïve setting was correlated with each other, patient demographics and clinical features of the tumour. Patient demographics and clinical features included gender (female=0, male=1), age, tumour type (OAC=0 and OGJ=1), neo-adjuvant treatment received (CROSS=0 and FLOT=1), treatment response (determined by radiographic features using PET/CT), tumour regression grade (TRG), clinical tumour stage and nodal involvement, pathological tumour stage and nodal involvement, body mass index (BMI kg/m²), peri-neural invasion, serosal invasion and lymph-vascular invasion. BMI and weight measurement was recorded post-treatment. Spearman correlation. Only significant data shown.

Conclusions T cells expressing ICs in circulation and infiltrating OAC tissue were adverse prognostic markers in the pre-treatment setting, perhaps due to their role in enabling tumour immune evasion and subsequent tumour progression. In contrast, T cells expressing ICs post-chemotherapy treatment in peripheral circulation were favorable prognostic markers. ICs are typically expressed by 'hot' tumours² therefore, the presence of ICs in the post-treatment setting may be

Abstract 776 Figure 3

The percentage of tumour-infiltrating T cells expressing CTLA-4 and ICOS were significantly lower post-neoadjuvant therapy in OAC tumour tissue. CD3+, CD3+ CD4+ and CD3+ CD8+ cells were screened for the surface expression of CTLA-4 and ICOS infiltrating OAC tissue pre-treatment and post-treatment (n=20). Mann Whitney test *<0.001.

Abstract 776 Figure 4

The percentage of circulating CD3+PD-1+ cells and CD3+CD4+TIGIT+ cells positively correlated with a better treatment response in the post-treatment setting. The percentage of CD3+, CD3+CD4+ and CD3+CD8+ cells expressing ICs in peripheral circulation and infiltrating OAC tissue in the post-neoadjuvant treatment setting was correlated with each other, patient demographics and clinical features of the tumour. Patient demographics and clinical features included gender (female=0, male=1), age, tumour type (OAC=0 and OGJ=1), neo-adjuvant treatment received (CROSS=0 and FLOT=1), treatment response (determined by radiographic features using PET/CT), tumour regression grade (TRG), clinical tumour stage and nodal involvement, pathological tumour stage and nodal involvement, body mass index (BMI kg/m²), peri-neural invasion, serosal invasion and lymph-vascular invasion. BMI and weight measurement was recorded post-treatment. Spearman correlation. Only significant data shown.

as a result of an induced-anti-tumour immune response following immunogenic chemotherapy/chemoradiotherapy treatment and may be a useful strategy for stratifying patients into chemotherapy/chemoradiotherapy responders or non-responders. A therapeutic rationale is also highlighted for combining ICIs with chemotherapy regimens in OAC patients to enhance anti-tumour T cell-mediated responses and potentially boost response rates to chemotherapy treatment.

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Ethics Approval The work was performed in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving human samples.

Consent Patients provided informed consent for sample and data acquisition, and the study received full ethical approval from the St. James's Hospital/AMNCH Ethical Review Board.

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PRECLINICAL STUDY USING A GLUTAMATERGIC SIGNALING AND IMMUNE-CHECKPOINT INHIBITORS IN A SPONTANEOUS MELANOMA PRONE MOUSE MODEL

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Background Much progress has been made in understanding melanoma pathogenesis within the last few years through targeted therapies and immunotherapies. However, resistance to small molecule inhibitors remains an obstacle. Immunotherapies such as checkpoint inhibitors against PD-1/PD-L1 lead to durable responses but only in a subset of melanoma patients. Mouse models reflecting human cancers provide invaluable tools towards the translation of basic science discoveries to clinical therapies, but many of these in vivo studies are short-term and do not accurately mimic patient circumstances. Our lab has a melanoma-prone transgenic mouse model which is driven by ectopic expression of a normal neuronal receptor, metabotropic glutamate receptor 1 (GRM1). This mouse model recapitulates melanoma development and progression frequently associated with melanoma patients, where aberrant GRM1 expression is detected. We have shown that in >90% of late-stage melanoma patients, there is atypical GRM1 mediated signaling and expression.

Methods In this study, we are using these mice, TGS, to determine the long-term, 18-week, therapeutic consequences of troriluzole, a prodrug for riluzole, which is an inhibitor of glutamatergic signaling plus anti-PD-1, an immune-checkpoint inhibitor. Tumor burden is monitored every 6 weeks for 18 weeks using a small imaging system, IVIS and tumor burden is quantified using ImageJ software. Blood, lymphoid, and tumor samples were collected at several time points during the study for molecular, and immune analyses.

Results Preliminary results suggest a gender-biased treatment response and that the combination of troriluzole and anti-PD-1 is more efficacious than either agent alone. In males, a 43.9% reduction in tumor burden was observed while in females there was a 29.6% increase in tumor burden in the combination group compared to vehicle. In concordance, after the removal of the treatment modality, the male mice in the combinatorial group survived 42 days longer compared to vehicle controls with sustained tumor reduction by 68.3%. In female mice no significant advantage in survival or reduction in tumor burden was noted.

Conclusions N/A

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IMMUNE CHECKPOINT BLOCKADE IMPACTS THE SUPPRESSIVE PHENOTYPE AND FUNCTION OF REGULATORY T CELLS IN AN ENDOGENOUS MOUSE LYMPHOMA MODEL

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Background Antibodies against programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) have become established part of anti-cancer therapy.