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.

Acknowledgements We would like to thank all the patients who kindly donated their samples to our research

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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Abstract 779 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.
However, the mechanisms contributing to the therapeutic success have not been entirely uncovered by now. Here we focus on the impact of PD-1/CTLA-4-blocking antibodies on regulatory T cells (Tregs), which are known to be involved in tumor immune evasion in many cancer types.

**Methods** To evaluate how Tregs are affected by anti-PD-1/CTLA-4 therapy, we used a MYC-transgenic mouse model of spontaneously arising B-cell lymphoma, which can be effectively treated by immune checkpoint inhibition. Data were acquired by flow cytometry.

**Results** As earlier shown, Tregs were involved in immune escape of MYC tumors. The Treg to effector T cell (Teff) ratio was elevated within the CD4-positive cell compartment. Tumor-infiltrating Tregs were predominantly thymic Tregs, which recognized overexpressed tumor-derived self-peptides in an MHC class II-restricted manner and showed upregulated expression of activation markers, Foxp3, CD25 and IL-10. To examine whether these phenotypic alterations correlated with the immunosuppressive capability of Tregs, an in vitro suppression assay was established. In this setting, MYC Tregs turned out to suppress proliferation and IFN-γ release from Teff cells more effectively than wildtype Tregs. The suppression observed in vitro was mediated by cell contacts and IL-10. Further suppressive mechanisms are likely to play a role, such as competition for MHC-II ligands and a consumption of IL-2. To investigate if immune checkpoint blockade interferes with these Treg-dependent immunosuppressive pathways, MYC mice were treated with a combination of anti-PD-1 and anti-CTLA-4 antibodies. Tregs from treated MYC mice showed decreased expression of CD69, CD137, Foxp3, CD25 and IL-10 as compared to Tregs from untreated MYC mice. This correlated with a lower suppressive capacity of Tregs from treated animals in the in vitro suppression assay.

**Conclusions** Taken together, the data show that immune checkpoint blockade impairs the development of the suppressive phenotype of intratumoral Tregs. Thus, apart from the initially described Teff reactivation, other mechanisms are also relevant for unfolding the therapeutic effect of immune checkpoint inhibitors.

**Ethics Approval** All animal experiments were approved by Regierung von Oberbayern, approval number 55.2-1-54.

This has resulted in broadening of immunotherapy research programs to target additional co-inhibitory (e.g. LAG-3, TIM-3) and co-stimulatory (e.g. 4-1BB, GITR, OX40, ICOS) receptors individually and in combination.

A major challenge in the development of antibody-based biologics is access to quantitative and reproducible functional bioassays. Existing methods rely on primary cells and measurement of complex functional endpoints. These assays are cumbersome, highly variable and fail to yield data required for drug development in a quality-controlled environment.

**Methods** To address the need of access to a robust and reliable functional assay for immunotherapy drug development programs, we have developed a suite of cell-based functional bioassays to interrogate modulation of immune checkpoint receptors individually (e.g. PD-1, CTLA-4, LAG-3, TIM-3, GITR, 4-1BB,OX40, CD40) and in combination (e.g. PD-1+CTLA-4, PD-1+4-1BB). These assays consist of stable cell lines that express luciferase reporters driven by response elements under the precise control of mechanistically relevant intracellular signals. Thus, the bioassays reflect mechanisms of action for the drug candidates designed for each immune checkpoint receptor and demonstrate high specificity, sensitivity and reproducibility. Here we describe the application of MoA-based immune checkpoint receptor bioassays as tools for biologics drug discovery, development, potency and stability studies.

**Results** We demonstrate that these bioassays measure response and inhibition with blocking drugs or potency changes from stressed samples.

**Conclusions** In summary, these reporter-based bioassays provide valuable tools for the development, stability testing, and potency determination in the manufacture of biologics that are targeted to immune checkpoint.

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