Results Combination of HLA peptidomics with 16S rDNA sequencing of 17 melanoma metastasis derived from 9 different patients, lead us to the unbiased identification of an intracellular bacterial peptide repertoire presented on HLA-I and HLA-II molecules. We were able to validate these results by co-culturing the bacterial species identified by 16S sequencing with the patient derived melanoma cells, further validating the peptide's presentation by preforming HLA peptidomics on the infected cells. Importantly, we were able to identify common bacterial peptides from different metastases of the same patient as well as from different patients. Some of the common bacterial peptides, as well as others, were able to elicit an immune response by the autologous tumor infiltrating lymphocytes (TILs), suggesting potential therapeutic implications of these peptides.

Conclusions The insights gathered through this study elucidate the effect of intra-tumor bacteria on the immune response and so, may lead to the development of novel clinical applications.

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673 PRECISION MICROBIOME MAPPING IDENTIFIES A MICROBIOME SIGNATURE PREDICTIVE OF IMMUNE CHECKPOINT INHIBITOR RESPONSE ACROSS MULTIPLE RESEARCH STUDY COHORTS

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Background The gut microbiome of cancer patients appears to be associated with response to Immune Checkpoint Inhibitor (ICIs) treatment.[1–4] However, the bacteria linked to response differ between published studies.

Methods Longitudinal stool samples were collected from 69 patients with advanced melanoma receiving approved ICIs in the Cambridge (UK) MELRESIST study. Pretreatment samples were analysed by Microbiotica, using shotgun metagenomic sequencing. Microbiotica's sequencing platform comprises the world's leading Reference Genome Database and advanced Microbiome Bioinformatics to give the most comprehensive and precise mapping of the gut microbiome. This has enabled us to identify gut bacteria associated with ICI response missed using public reference genomes. Published microbiome studies in advanced melanoma,[1–3]renal cell carcinoma (RCC) and non-small cell lung cancer (NSCLC)[4] were reanalysed with the same platform.

Results Analysis of the MELRESIST samples showed an overall change in the microbiome composition between advanced melanoma patients and a panel of healthy donor samples, but not between patients who subsequently responded or did not respond to ICIs. However, we did identify a discrete microbiome signature which correlated with response. This signature predicted response with an accuracy of 93% in the MELRESIST cohort, but was less predictive in the published melanoma cohorts.[1–3] Therefore, we developed a bioinformatic analytical model, incorporating an interactive random forest model and the MELRESIST dataset, to identify a microbiome signature which was consistent across all published melanoma studies. This model was validated three times by accurately predicting the outcome of an independent cohort. A final microbiome signature was defined using the validated model on MELRESIST and the three published melanoma cohorts. This was very accurate at predicting response in all four studies combined (91%), or individually (82–100%). This signature was also predictive of response in a NSCLC study and to a lesser extent in RCC. The core of this signature is nine bacteria significantly increased in abundance in responders.

Conclusions Analysis of the MELRESIST study samples, precision microbiome profiling by the Microbiotica Platform and a validated bioinformatic analysis, have enabled us to identify a unique microbiome signature predictive of response to ICI therapy in four independent melanoma studies. This removes the challenge to the field of different bacteria apparently being associated with response in different studies, and could represent a new microbiome biomarker with clinical application. Nine core bacteria may be driving response and hold potential for co-therapy with ICIs.

Ethics Approval The study was approved by Newcastle & North Tyneside 2 Research Ethics Committee, approval number 11/NE/0312.

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674 LATE ANTIBIOTIC ADMINISTRATION DURING DURVALUMAB TREATMENT MAY BE ASSOCIATED WITH CLINICAL BENEFIT

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Background Early concurrent antibiotic usage in patients receiving immune checkpoint inhibitors (ICIs) is linked to

decreased progression-free survival (PFS) and overall survival (OS), likely mediated by lower gut microbial diversity and skewed taxonomic abundance. The relationship of late antibiotic exposure to ICI efficacy has not been explored.

Methods Data from a single-arm, Phase 1/2 study (Study 1108) were retrospectively analyzed in 945 patients with metastatic disease in 18 tumor types (largest subsets: non-small-cell lung cancer [NSCLC], 31.5% and urothelial cancer [UC], 20.8%) receiving durvalumab 10 mg/kg Q2W between 8/2012–6/2017. Early antibiotic exposure was defined as 30 days prior to until 30 days after durvalumab initiation. Late exposure was any time >30 days after durvalumab initiation. Demographics, infection type, and antibiotic data (route, duration, class) were collected. Median PFS and OS were compared between no antibiotics (n=525), early antibiotics (n=239), and late antibiotics (n=181) by Kaplan-Meier methods and log-rank tests. The Cox proportional hazards model was used for multivariable adjustments. In a translational in vivo analysis, Balb/c mice implanted with CT26 tumors were prospectively treated with oral levofloxacin 1 mg/mL for 7 days either 1 week prior to, concurrently with, or 1 week after initiating anti-PD-L1 or control IgG therapy (n=40 per group). The primary endpoint was tumor growth kinetics.

Results ß-lactams (51.3%) and fluoroquinolones (39.3%) were most commonly prescribed overall. Early antibiotic exposure was associated with reduced mOS compared to no exposure (7.2 vs 9.8 months, p=0.049). Unexpectedly, patients with late antibiotic exposure had markedly improved mOS versus those with no exposure (19.8 vs 9.8 months, p<0.001), which persisted after adjusting for baseline tumor volume, demographics, treatment-induced immune-related adverse events, and neutrophil to lymphocyte ratio (table 1). In NSCLC and UC cohorts, these results were preserved. To account for time-on-treatment bias, an independent model using antibiotic start time as a time-dependent covariate also showed improved benefit with late antibiotics (HR 0.91, 95% CI 0.87–0.95, p<0.001). Compared to control IgG, mice receiving prior or concurrent levofloxacin with anti-PD-L1 had similar survival, while mice receiving late levofloxacin with anti-PD-L1 had improved survival (p=0.004).

Abstract 674 Table 1 mPFS and mOS by antibiotic exposure

Groups (n)	mPFS (95% CI), months	mOS (95% CI), months
All patients (945)	1.6 (1.5–1.8)	10.8 (9.3–12.4)
No antibiotics (525)	1.4 (1.4–1.6)	9.8 (7.8–11.4)
Early antibiotics* (239)	1.5 (1.4–2.2)	7.2 (5.4–9.4)
Late antibiotics** (181)	4.4 (2.8–5.6)	19.8 (15.5–23.0)

* Early antibiotics defined as 30 days prior to until 30 days after durvalumab initiation
** Late exposure was any time greater than 30 days after durvalumab initiation

Conclusions This large retrospective analysis is consistent with previous studies showing associations of early antibiotics with shorter survival during ICI therapy. For the first time, we report that late antibiotics do not negatively impact survival and may actually benefit survival. Future studies will evaluate immune phenotyping and microbiome characterization to clarify mechanistic underpinnings. These findings will require confirmation in prospective clinical studies.

Trial Registration clinicaltrials.gov NCT01693562

Ethics Approval This study was conducted according to the Declaration of Helsinki and approved by the independent ethics committee/institutional review board at each participating center.

Consent Informed consent was obtained from all patients.

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675 OXYGEN CONCENTRATION ALTERS NATURAL KILLER CELL PHENOTYPE AND FUNCTION IN THE SOLID TUMOR MICROENVIRONMENT

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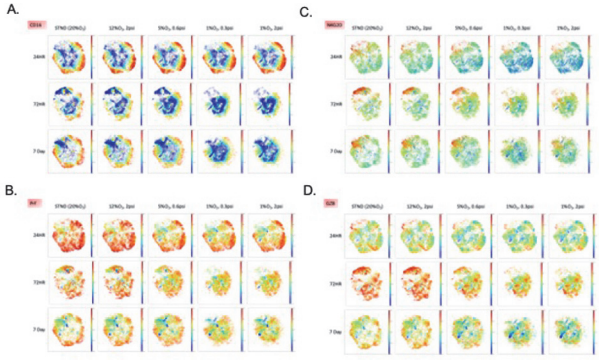
Background Natural Killer (NK) cells can eliminate cancer cells through the release of cytotoxic granules triggered by interactions with natural ligands or through antibody-dependent cellular cytotoxicity (ADCC).[1–3] NK cell-based treatments have had therapeutic success for hematological malignancies but strategies to treat solid tumors have been limited due to immunosuppression within the tumor microenvironment (TME).[4–6] An important and understudied aspect of NK cell immunosuppression is the low oxygen (hypoxia) environment created by proliferating tumor cells. We used the novel AVATAR incubator system to model oxygen levels of three key tissues that NK cells inhabit in vivo: the peripheral blood (12% O2), the bone marrow (5% O2) and the TME (1% O2).

Methods NK cells were incubated in the AVATAR incubators for 24 hours, 72 hours and 7 days. We conducted a mass cytometry (CyTOF) analysis to assess phenotype, flow cytometry-based assays to assess proliferation and an IncuCyte machine and immunofluorescent imaging to measure cytotoxicity of NK cells incubated at different oxygen conditions. We evaluated NK cell metabolism using Seahorse assays, gene expression using RNA-Seq and are in the process of evaluating epigenetic regulation using ATAC-Seq.

Results NK cells from the 1% O2 condition express fewer activating receptors (CD16, NKG2D, Nkp30, Nkp46, DNAM-1) and less perforin and granzyme than NK cells from the higher oxygen conditions (figure 1). NK cells in the 1% O2 condition also have decreased aggregation of perforin and granzyme granules at the immune synapse. This translates to reduced natural cytotoxicity and ADCC responses against tumor targets (figure 2). We also observe a sharp decrease in proliferation in the NK cells at 1% O2 (figure 3). This is



Abstract 675 Figure 1 Oxygen concentration alters NK cell phenotype
300,000 enriched NK cells were incubated for 24 hrs, 72 hrs and 7 days at noted incubator conditions with 1 ng/ml IL-15. At the end of the incubation, cells were barcoded and stained with a custom panel for CyTOF evaluation. Data is show here for CD16 (A), NKG2D (B), Perforin (C) and Granzyme B (D). N=3 (data concatenated).