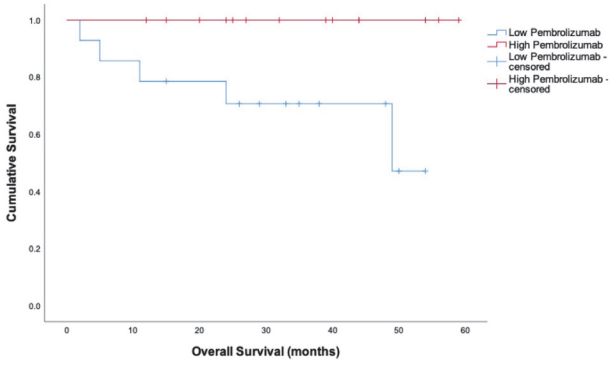
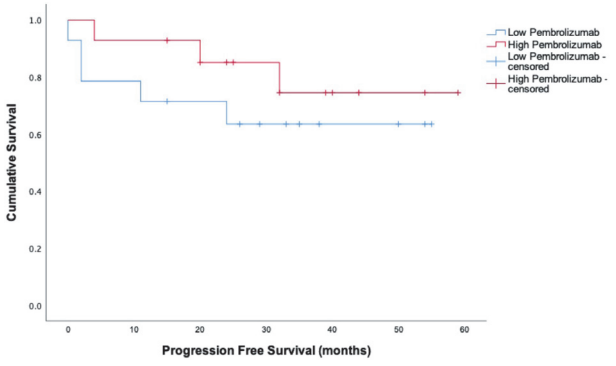
Background Despite the paradigm shift heralded by immune checkpoint blockade (ICB), only a small proportion of patients have a meaningful response. Dose selection of ICB agents was significantly based on in-silico modelling.[1] Trial data has shown that clearance of these agents varies over time, with a reduction in clearance associated with improved best overall response (BOR).[2, 3] Real world data has shown patients with higher exposure to ICB, manifested as higher plasma trough concentrations, experience improved BOR and longer survival.[4] This study aimed to determine the relationship between longitudinal ICB exposure and BOR, progression free survival (PFS) and overall survival (OS) in patients with metastatic melanoma receiving pembrolizumab monotherapy.

Methods 28 patients with metastatic melanoma receiving weight based pembrolizumab (2 mg/kg Q3w) had serial pharmacokinetic trough draws prior to their next scheduled dose, up to a maximum of 22 cycles. BRAF mutation positive patients were pre-treated with BRAFi/MEKi therapy, otherwise pembrolizumab was given first line. Plasma trough levels were determined using the Abcam® pembrolizumab ELISA kit.The cohort was split by best overall response (BOR), determined by iRECIST. No statistically significant differences were determined, using one-way ANOVA. The cohort was stratified into high versus low pembrolizumab trough concentrations, split by the median. Trough is an established surrogate for drug exposure.[5] Kaplan-Meier survival analysis for progression-free and overall survival was performed based on pembrolizumab drug exposure groups.



Abstract 243 Figure 1 OS Kaplan-Meier survival curves stratified for the group with the 50% highest trough concentrations (red) and 50% lowest trough concentrations (blue). The median OS for high pembrolizumab exposure group was not reached, which was significantly longer than the low pembrolizumab exposure median of 48 months (p=0.021)



Abstract 243 Figure 2 PFS Kaplan-Meier survival curves stratified for the group with the 50% highest trough concentrations (red) and 50% lowest trough concentrations (blue). The mean PFS for the high pembrolizumab exposure group was 49.2 months, which was not significantly longer than low pembrolizumab exposure mean PFS of 37.9 months. The median PFS was not reached in either group.

Results Median follow up was 32.5 months. Complete responders (CR) (n=11) had 29.8% higher geometric mean pembrolizumab trough levels (90.8 mcg/mL) than partial responders (PR) (n=9) (63.7 mcg/mL, p=ns). CR patients had 16.1% higher trough levels than patients with progressive disease (PD) (n= 6) (76.2 mcg/mL, p=ns). 2 patients with stable disease had mean trough pembrolizumab levels of 106.4 mcg/mL.The high pembrolizumab exposure group experienced significantly longer median OS (not reached versus 48 months, p=0.021) (figure 1), than the low exposure group. No significant difference was found in mean PFS between the groups (49.2 versus 37.9 months, p=ns) (figure 2). The median PFS was not reached in either group.

Conclusions A positive exposure survival relationship for pembrolizumab in metastatic melanoma is described in a real world setting. Whether this relationship indicates a true causal effect of variation in drug exposure on clinical outcomes remains to be determined. Further pharmacokinetically driven dosing studies are required to identify whether therapeutic drug monitoring of pembrolizumab in the clinic is a necessity.

Acknowledgements This study was funded by a Hunter Medical Research Institute Project Grant (HMRI983) - Hunter Cancer Biobank Serial Blood Collection Project.

Ethics Approval This study was approved by the Hunter New England Health Local Health District (14/12/10/4.02) and University of Newcastle Human Research Ethics Committee institutional review board (H-2018-0159).

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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244 NOVEL RESPIRABLE ANTISENSE OLIGONUCLEOTIDE (RASON) APPROACH TO PRIMARY AND METASTATIC HUMAN LUNG CANCER: PRELIMINARY RESULTS IN A MODEL SYSTEM EMPLOYING SPONTANEOUS LUNG TUMORS IN DOGS

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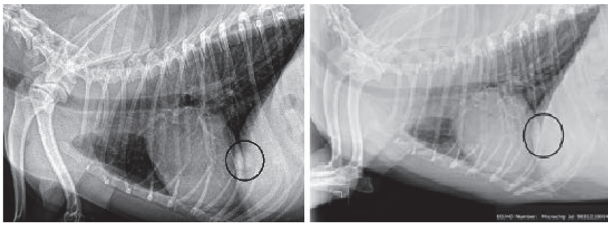
Background Antisense oligonucleotides function by targeting the messenger RNA coding for a target protein, rather than the protein itself. This laboratory previously introduced Respirable Antisense Oligonucleotides (RASONs) into human clinical

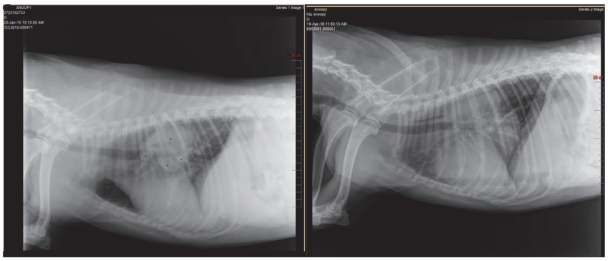
trials for asthma.[1–5] In that work we demonstrated that RASONs delivered by inhalation are absorbed into surfactant lining the surface of the lung; are distributed with high efficiency throughout the bronchial epithelium; and are taken up with therapeutic effect by both bronchial epithelial cells and immune effector cells resident throughout the bronchial epithelium, as well as in bronchial-associated lymphoid tissue (BALT). We have now re-engineered this technology to adapt it to the treatment of primary and metastatic lung tumors via immune checkpoint inhibition. While immune checkpoints expressed on lung tumors are not amenable to RASON inhibition, immune cells resident in the bronchial epithelium and BALT represent good targets for the RASON approach to checkpoint inhibition. E.g., SIRP-alpha is a receptor expressed by myeloid lineage cells such as dendritic cells (DCs), tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs). When CD47, found on the surface of tumor cells, binds to SIRP-alpha on immune effector cells, the anti-tumor action of such immune effector cells becomes significantly diminished. We hypothesized that RASONs targeting mRNA of immune checkpoint proteins found on immune effector cells would eliminate checkpoint proteins from their surface, such that when they were signaled to home in on lung tumors, they would arrive in the tumor-associated microenvironment in a state impervious to checkpoint ligands expressed on the surface of tumor cells. To test this hypothesis, we applied a RASON protocol to dogs with spontaneous lung tumors presenting to their veterinarians.

Methods In this preliminary, proof-of-principal study, two dogs with histologically confirmed metastatic lung tumors were administered RASONs targeting PD1, CTLA-4 and SIRP-alpha, by inhalation, twice weekly for eight weeks.

Results X-ray analysis performed two weeks after the conclusion of RASON treatment showed dramatic results. One dog showed complete tumor dissolution (figure 1), and the second dog showed near total tumor dissolution, with faint shadows remaining (figure 2).



Abstract 244 Figure 1 Canine 2 presented with a 3-cm spherical tumor (circled, left). After RASON treatment (right), tumor underwent complete regression



Abstract 244 Figure 2 Canine 1 presented with one 9-cm tumor and four smaller tumors ranging from 1–2 cm (tumors are marked with dot

Conclusions While these are preliminary results, and need to be dramatically expanded, they provide an initial indication that the RASON approach might prove to be an effective addition to immune checkpoint inhibition. It possesses certain advantages over small molecule or antibody approaches to checkpoint inhibition. For example, rather than being delivered systemically, RASONs are delivered by inhalation directly to the target tissues– the bronchial epithelium and BALT. Furthermore, it may be possible to reduce the toxicity of systemic treatments targeting checkpoint proteins on tumor cells, by reducing or eliminating their ligands on immune effector cells. In as much as the RASON approach to the treatment of human asthma failed in clinical trials as a result of its induction of an influx of macrophages into the lung, the ability to render TAMs impervious to the presence of tumor-associated immune checkpoints suggests that the RASON approach may hold considerable promise for the treatment of lung tumors.

Ethics Approval All research reported here involved informed consent by owners of dogs with spontaneous lung neoplasms, for which no satisfactory alternative treatment was available, and was performed in strict compliance with both the Basle Declaration, to which the laboratory is a signatory member, as well as guidelines published by the International Council for Laboratory Animal Science (ICLAS).

Consent N/A

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245 HUMAN TLR8 KNOCK-IN MICE POTENTIATE IMMUNOTHERAPY RESPONSES OF MC38 SYNGENEIC TUMORS

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Background Toll-like receptors (TLRs) serve critical roles in mediating innate immune responses against many pathogens. However, they may also bind to endogenous ligands and lead to the pathogenesis of autoimmunity. Although TLR8 belongs to the same TLR family as TLR7, its role in inflammation and tumor progression is not yet fully understood due to the lack of suitable animal models. In humans, both TLR7 and TLR8 recognize single-stranded self-RNA, viral RNA, and synthetic small molecule agonists.[1, 2] However, mouse Tlr8 is non-functional due to the absence of 5 amino acids necessary for RNA recognition. In order to create a mouse model with functional TLR8, we replaced exon 3 of mouse Tlr8 with human TLR8, therefore developing a hTLR8 knock-in (KI)