1427 EVALUATING MHC-II ANTIGEN PRESENTATION *IN VIVO* TO IDENTIFY TARGETS FOR IMMUNOTHERAPY

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Background Central to the success of immunotherapy is antigen presentation on Major Histocompatibility Class I and II (MHC-I and MHC-II).[1] While much effort has been paid to characterize MHC-I antigens recognized by $CD8^+$ T cells, our understanding of MHC-II antigens, which are recognized by $CD4^+$ T cells, is far less developed. The importance of MHC-II antigen presentation is particularly evident in lung adenocarcinoma (LUAD), where the alveolar type 2 (AT2) cell-of-origin is one of the only non-hematopoetic cell lineages known to present high levels of MHC-II.[2] These observations prompt the need to understand the contribution of direct MHC-II presentation in LUAD.[3 4] While advances in mass spectrometry have enabled direct interrogation of peptides presented by MHC-I and MHC-II, collectively known as the 'immunopeptidome', the field lacks tools to evaluate antigen presentation *in vivo*.[5–7] Here we present a novel mouse model to interrogate the MHC-II immunopeptidome *in vivo* in LUAD and set the stage for future investigations of MHC-II antigen presentation beyond lung cancer.

Methods GEMMs, including the $Kras^{Lox\text{-}stop\text{-}Lox\text{-}G12D}$; $p53^{fl/fl}$ (KP), model faithfully recapitulate histopathological features of human LUAD and represent an excellent tool to understand MHC-II in the LUAD microenvironment.[8] To profile MHC-II ligands in LUAD, we engineered a Cre-recombinase inducible affinity tagged MHC-II (H2-A^bStrep) and incorporated this allele to the KP model (KP/A^bStrep), enabling precise isolation of MHC-II peptides from malignant cells in the heterogeneous microenvironment. We thoroughly validated this novel model at the genetic, transcriptomic, and proteomic levels and isolated MHC-II peptides from LUAD tumors at various stages of tumor progression and with multiple targeted and immune therapies.

Results We demonstrate that LUAD cells present a diverse array of MHC-II peptides *in vivo* and found that source proteins that are processed and presented on MHC-II are highly divergent from those identified on LUAD MHC-I.[8] Peptides derived from secreted proteins are abundant on LUAD MHC-II complexes, including those that are lowly expressed in LUAD cells but highly expressed by other cells in the tumor microenvironment. Finally, we found that the repertoire of MHC-II peptides is highly dynamic with respect to tumor progression and treatment context.

Conclusions These results have profound implications for our understanding of the immunobiology of lung cancer in distinct tumor microenvironments. Moreover, application of the A^bStrep system to other disease and tissue contexts will pave the way forward for a deeper understanding of the MHC-II immunopeptidome *in vivo* to be exploited for next generation immunotherapies.

REFERENCES

1. Jhunjhunwala S, Hammer C, Delamarre L. Antigen presentation in cancer: insights into tumour immunogenicity and immune evasion. *Nature Reviews Cancer*, 2021:1–15. https://doi.org:10.1038/s41568–021-00339-z
2. Naranjo S, *et al.* Modeling diverse genetic subtypes of lung adenocarcinoma with a next-generation alveolar type 2 organoid platform. *Genes Dev* 2022;**36**:936–949. https://doi.org:10.1101/gad.349659.122
3. Oliveira G, *et al.* Landscape of helper and regulatory antitumour CD4(+) T cells in melanoma. *Nature* 2022;**605**:532–538. https://doi.org:10.1038/s41586–022-04682–5
4. Alspach E, *et al.* MHC-II neoantigens shape tumour immunity and response to immunotherapy. *Nature* 2019;**574**:696–701. https://doi.org:10.1038/s41586–019-1671–8
5. Sarkizova S, *et al.* A large peptidome dataset improves HLA class I epitope prediction across most of the human population. *Nature Biotechnology* 2019;**38**:199–209. https://doi.org:10.1038/s41587–019-0322–9
6. Abelin JG, *et al.* Mass Spectrometry Profiling of HLA-Associated Peptidomes in Mono-allelic Cells Enables More Accurate Epitope Prediction. *Immunity* 2017;**46**:315–326. https://doi.org:10.1016/j.immuni.2017.02.007
7. Ouspenskaia T, *et al.* Unannotated proteins expand the MHC-I-restricted immunopeptidome in cancer. *Nature Biotechnology* 2022;**40**:209–217. https://doi.org:10.1038/s41587–021-01021–3
8. Jaeger AM, *et al.* Deciphering the immunopeptidome in vivo reveals new tumour antigens. *Nature* **2022;607**:149–155. https://doi.org:10.1038/s41586–022-04839–2

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