722 EVALUATION OF THE EFFECTS OF PEMBROLIZUMAB ALONE AND IN COMBINATION(S) WITH MDSC-TARGETING AGENTS MK-0482 AND MK-4830 ON THE NATIVE CANCER PATIENT TME VIA FUNCTIONAL SPATIAL PROFILING (CIVO®)

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Background Immune checkpoint inhibitors have produced durable clinical responses in hard-to-treat cancers, but to date, such responses are limited to a minority of patients. Combination treatments of PD-1 inhibitors with drugs that target additional immune suppressive components of the tumor microenvironment (TME) have been proposed as a solution. A resource-intensive approach to investigating such combinations (> 4500 clinical trials to date) has resulted in limited success. A more efficient approach to clinical evaluation of drug combinations is needed.

CIVO® enables detailed assessment of multiple microdosed drugs and drug combinations, simultaneously introduced to discreet positions within the native microenvironment of a patient's solid tumor *in situ*.[1–3] To directly compare the effects of pembrolizumab alone and in combination with MK-0482 or MK-4830, two agents targeting myeloid-derived suppressor cells (MDSCs) via immunoglobulin-like transcript 3 (ILT3) and ILT4 respectively, we completed a trial in soft tissue sarcoma (STS) and head and neck squamous cell carcinoma (HNSCC) patients using CIVO.

Methods Thirteen patients (6 STS, 7 HNSCC) with planned surgical resection of a surface-accessible primary or metastatic tumor, enrolled in this multicenter clinical trial. The CIVO multiplexed microdose injection procedure was performed 1 to 4 days before surgery. Each injection consisted of a fluorescent marker co-formulated with vehicle, pembrolizumab, MK-0482, MK-4830, or combinations thereof. The injected portion of the surgical sample was then removed, formalin-fixed, paraffin-embedded, and evaluated for biomarkers of response via immunohistochemistry (IHC), in situ hybridization (ISH), and/or digital spatial profiling (NanoString GeoMx DSP).

Results High injection success rate was achieved with 87% resulting in visually distinct sites of drug exposure. To date, no device/microinjected drug-related adverse events have been reported.

In the STS cohort, localized TME responses to pembrolizumab were consistent with previous studies with strong interferon (IFN) response observed in tumor mutational burden (TMB)-high myxofibrosarcoma and low in TMB-low synovial sarcoma as detected by DSP. While combination injection sites containing either MK-0482 or MK-4830 resulted in decreased expression of CD163, a biomarker of M2 macrophage polarization, combining pembrolizumab with either MK-0482 or MK-4830 inhibited induction of the IFN signature.

Analysis of the HNSCC cohort is currently in progress.

Conclusions Functional spatial profiling of the cancer patient TME following multiplexed intratumoral CIVO microinjection enabled in-depth, safe, and efficient side-by-side evaluation of pembrolizumab alone and in combination with MK-0482 or MK-4830. Further study to determine whether the localized TME responses to CIVO predict the therapeutic benefit of checkpoint inhibitor combinations in different patient subsets is warranted.

Acknowledgements Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA provided drug and financial support for the study.

Trial Registration Clinical Trial Registry Number: NCT04541108

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Ethics Approval This study was approved by the Institutional Review Boards of the Oregon Health and Science University (approval #00022805), the University of Pennsylvania (approval #844966), the Louisiana State University Health Sciences Center, Shreveport (approval #00002077), and WCG (approval #20212261).

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0722