963 TME IMMUNE-PROFILING OF *EX VIVO* TUMOR TISSUES AND THEIR FUNCTIONAL RESPONSES TO IMMUNO-ONCOLOGY TREATMENTS

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Background Many clinically approved and novel preclinical (immuno)-oncology therapeutics rely on the complex interactions between various components present in the tumor microenvironment (TME). The TME is a heterogenous mixture of tumor, stromal, and immune cells that can generate pro-inflammatory and immunosuppressive milieus. Previously presented was the 3D *Ex Vivo* Patient Tissue (EVPT) Platform, which utilizes a short-term 3D *ex vivo* tumor culture system containing micro-tissues generated from human tumor material, followed by high content imaging (HCI)-based analysis. In the EVPT platform, tumor cell killing following drug therapies, including immune checkpoint inhibition (ICI), could be successfully measured in patient samples from multiple cancer indications such as lung, ovarian, breast, bladder, and skin cancer. Further sample characterization is performed using techniques such as flow cytometry, immunohistochemistry, and cytokine measurement. This study provides a summary of sample response data, biomarker characterization, and extended flow panels to provide more granular immune profiling.

Methods Patient tumor tissues and peripheral blood samples were obtained from commercial tissue providers and processed within 24 hours to preserve the TME. Fresh, minimally processed tumor material was embedded in a protein-rich hydrogel and exposed to therapies at various doses. Next, the phenotypic screening using our proprietary automated HCI analysis platform was performed. Immune profiling was done by flow cytometry. IHC biomarker and cytokine measurements were performed on fixed tissues or culture supernatants.

Results Using our functional HCI assay, ICI sensitivity was observed in about 20% of NSCLC and bladder cancer samples (n>30), aligning well with clinical response rates. In ICI-responsive samples, the presence of immune cells could be confirmed using biomarker IHC and their proliferation measured by HCI, where corresponding cytokines (IL-2, Granzyme B, IFN-y) were found to be elevated accordingly. FACS samples derived from blood and processed tumor tissue were used for detailed profiling at baseline or post-treatment. The TME composition varied between samples, displaying a diverse representation of immune cells using lymphocyte/Treg markers (CD3,CD4,CD8,CD25,FoxP3), myeloid markers (CD14,CD68, CD80,CD86,CD33), as well as NK-cells (CD16, CD56) and tumor cells (EpCAM).

Conclusions There is great potential for primary *ex vivo* assays, where all disease relevant cellular components are preserved, to provide greater translatability from preclinical studies.

In the EVPT assay, ICI sensitivity is measured, and using corresponding cross-platform biomarker analyses, further support and validation of the measured responses are provided as well as a deeper insight into patient-specific tumor immune niche composition.

Ethics Approval Protocol no FRT-19101_v1.0 submitted to CNBMDM with the no 9S/4 on the date of 12.11.2019 considers that all demanding ethics have been fulfilled and the study may take place.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0963