66 SWITCHABLE ANTIBODIES FOR INCREASED THROUGHPUT IN ITERATIVE IMMUNO-FLUORESCENT STAINING OF CANCER TISSUE USING CHIPCYTOMETRY™

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Background Understanding the spatial distribution of key immune cell populations in the tumor microenvironment is critical for our understanding of cancer and to develop novel drugs. While historically the spatial analysis of the tumor microenvironment has been limited by low plex methods like immunohistochemistry (IHC), iterative immuno-fluorescence staining is an emerging method for precise spatial multiplexing. The drawbacks of such iterative methods are often slow throughput or harsh sample treatments for the inactivation of the fluorescence dyes.

Methods Here we present the analysis of human cancer tissue with a novel class of fluorescent labeled antibodies for the ChipCytometry™ platform, which combines iterative immune-fluorescent staining with high dynamic range imaging to facilitate quantitative phenotyping with single-cell resolution. We have developed novel switchable antibodies that allow for fast and gentle signal deactivation after each staining cycle to increase throughput while preserving the integrity of the sample.

Results The results show precise expression levels for each marker in the assay, maintaining spatial information about each cell. Several immune subtypes could be identified and quantified based on protein expression profiles. Staining with the novel switchable antibodies was specific and signal deactivation was possible in five minutes for the whole tissue section while preserving the integrity of the sample. This led to an increased throughput in comparison to commercially available antibodies by reducing the overall amount of time needed for the assay. Furthermore, subsequent staining with other commercially available antibodies was not impaired, demonstrating the high flexibility of the ChipCytometry™ platform for in-depth immune profiling in clinical samples.

Conclusions ChipCytometry™ in combination with novel switchable antibodies allows for the simultaneous detection of multiple protein markers on a single tissue section for deep immune cell profiling with increased throughput in comparison to commercially available antibodies. Nonetheless, a combination of both types of antibodies is possible and expands the capabilities of ChipCytometry™, giving researchers the highest flexibility in choosing their marker panel.

Ethics Approval This study was approved by the University Leipzig Medical School ethics board under approval number 302/16-ek

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