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Imaging in cancer immunology: Phenotyping of multiple immune cell subsets *in situ* in FFPE tissue sections

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There has been a rapid grown in the field of tumor immunobiology in recent years as a result of recent successes in cancer immunotherapies and it is becoming clear that immune cells play many sometimes conflicting roles in the tumor microenvironment. However, obtaining phenotypic information about the various immune cells that play these roles in and around the tumor has been a challenge. Existing methods can either deliver phenotypic information on homogenous samples (e.g., flow cytometry or PCR) or morphologic information on single immunomarkers (standard IHC). We present here a methodology for delivering quantitative per-cell marker expression and phenotyping, analogous to that obtained from flow cytometry but from cells imaged *in situ* in FFPE tissue sections. This methodology combines: The sequential multi-marker labeling of up to 6 antigens using antibodies all of the same species in a single section; automated multispectral imaging (MSI) to remove the typically problematic FFPE tissue auto fluorescence and correct cross-talk between fluorescent channels and an automated image analysis that can quantitate the per-cell marker expression, determine the cellular phenotype, count these cells separately in the tumor compartment and in the stroma and provide high-resolution images of their distributions. We present here several examples of this new methodology in breast, lung and head and neck cancers. Each application example will show 6-plex multiplexed staining, per-cell quantitation of each marker and multi-marker cellular phenotyping from multispectral images of standard clinical biopsy sections, as well as methods to explore the spatial distributions of the phenotyped cells in and around the tumor.

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ABCB1 overexpression predicts outcome of CML patients undergoing first-line imatinib treatment: A TIDEL II sub-study

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Background & Aims: The TIDEL II trial indicates first-line imatinib treatment with selective switching to nilotinib for failure to meet specific molecular targets/intolerance, results in excellent molecular responses and overall survival. Drug transporters, particularly *OCT-1* and *ABCB1,* impact imatinib response. Here, the clinical significance of *ABCB1* overexpression in response to imatinib therapy was assessed in patients enrolled to TIDEL II.

Method: *ABCB1* mRNA expression was assessed by PCR at day 0, day 22 post imatinib therapy initiation and, where relevant, at cessation in CP-CML patients.

Results: A change in ABCB1 expression after 22 days of imatinib (compared with baseline) was observed (range 0.6-2.3 fold, median 2-fold), suggesting imatinib may alter *ABCB1* expression. This change was not related to *BCR-ABL1* expression (p=0.29 at day 0 and p=0.84 at day 28). When patients with a ≥2-fold rise in *ABCB1* expression were compared with those with a <2-fold rise significant differences in outcome were revealed. Nilotinib is also exported by *ABCB1*; accordingly, patients with ≥2-fold rise in *ABCB1* were less likely to achieve MMR when switched to nilotinib therapy (11% vs. 70% of patients with <2-fold rise).

Conclusion: This rapid PCR-based assay performed pre and 22 days-post imatinib initiation provides a potent early predictor of subsequent imatinib response in CP-CML patients; these data also suggest nilotinib is a poor therapeutic option for patients with up-regulated *ABCB1* expression in response to imatinib. Results shown here exemplify how drug transporters can influence TKI therapy and patient response and provide a new, effective and easily translatable prognostic biomarker based on *ABCB1* expression.

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