Target Exposure Scoring With Mass Spectrometry Imaging

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Intro (120)
The evaluation of drug concentration and exposure to structures, substructures or cells is a key step for setting up a new drug and to anticipate a potential lack of efficacy in tissues. These evaluations are undergone with Mass Spectrometry Imaging (MSI) but remain difficult for target exposure at high spatial resolution with high histological heterogeneity. In this study, a new workflow was set up using allowing a software development to score the accurate concentration of a drug in particular structures of tumor tissues. The objective of the workflow was to quantify a histological drug exposure level (Epacadostat, IDO1 inhibitor) through the expression of its target, IDO1 enzyme, using an automated tissue annotation and quantification approaches.

Methods (120)
In order to establish the target exposure score of the EPA into CT26 mouse tumor models, immunohistochemistry (IHC) staining was performed to target IDO1 enzyme histological localization in serial tissue sections. Then, different targeted segmentations using color-based algorithms were applied (K-Means clustering, CIELAB analysis + Nearest Neighbor Classification etc) for an automatic creation of ROIs related to IDO1 enzyme presence. After MSI acquisition and signal/pixels extraction, a correlation matrix was performed in order to assess and to score the EPA/IDO1 co-localization. Finally, an exposure score was calculated by the software percentage was assessed by dividing the EPA concentration multiplied by the pixels number of region A, B and C by EPA overall presence multiplied by the overall pixel number.

Results (300)
Target exposure analysis was carried out for the first time using a unique software that linked the topographic and molecular localization of the EPA drug (using MSI) and its IDO1 enzyme target (using immunostaining). First, an immunostaining based segmentation algorithm (based on the brown color of colorimetric reaction) was developed for IDO1 enzyme expression. It allowed identifying 3 regions based on the IDO1 staining density: A (+++), B (++) and C (+) for high, medium and low IDO1 expression levels, respectively. Then, EPA molecular signal and absolute quantities were assessed (obtained using QMSI) and automatically extracted and a ratio of MSI intensity/quantity and immunostaining density were automatically calculated for each pixel of each region (A, B and C) by the software. The results showed a quantity of 15.5 µg/g in region A vs 40 µg/g in the entire section, which means that almost 46% of EPA signal was concentrated within 20% of the entire tissue section. Thereafter, a high correlative relation was found between the IDO1 level and the EPA mean intensity/absolute quantity, where almost 52% of EPA signal was localized in region A, 32% in region B and 16% in region C. Finally, the software provided a higher histological definition capacity allowing calculating a % of exposed drug with and without the presence of its target. This result confirmed that more than just reaching the organ of interest (tumor), the drug also reached its specific target. Drug exposure specificity was therefore confirmed. As exposure at the site of action and to its specific target were identified as the most important factors for success in drug discovery and the design of chemical probes, these results showed and confirmed the high contribution of MSI for drug exposure and specificity to targets studies.

Novel aspect (20)
A new tool for drug exposure scoring in heterogeneous tissues using MSI