doi:10.1017/S1431927622005785
Microsc. Microanal. 28 (Suppl 1), 2022

Quantitative Bioimaging of Microglial Response to Brain-Targeted Treatment Using Deep Learning Based Morphometry

David Joy[1], Dan Xia[1], Kathryn Monroe[1], Pascal Sanchez[1], Meredith Calvert[1]*

[1.] Denali Therapeutics, South San Francisco, CA, USA
* Corresponding author: calvert@denali.com

Microglia are a dynamic population of brain-resident immune cells that provide immune surveillance of the tissue environment and can move between different activation states in response to changing stimuli. These activation states, associated with increases in inflammatory, phagocytotic, and pathological functions, are marked by characteristic morphological changes in cellular process length, branching complexity and soma size. Microglial cell states are traditionally viewed along a continuum between resting- with long, delicately branched processes and a small cell body, and activated- in which cells adopt an ameoboid shape with retracted, broadened processes and an enlarged soma [1,2]. This simplistic, polarized view of microglial activation has been challenged due to recent work identifying intermediate and divergent cell states associated with specific functional and transcriptional subtypes [3].

Critical changes in microglial responsiveness have been found to be associated with neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease. Additionally, genetic studies have identified variants associated with late-onset Alzheimer's disease (LOAD), including a number of genes involved in modulating microglial responsiveness. A gene encoding the microglial transmembrane immune receptor, Triggering receptor expressed on myeloid cells 2 (TREM2), was identified as a LOAD gene and individuals carrying rare heterozygous variants of TREM2 are found to have higher LOAD risk [4]. TREM2 modulates the response of microglia to the presence of amyloid-β plaques in the brain, and treatment with an agonistic anti-TREM2 antibody is found to attenuate plaque-associated pathology and neuritic dystrophy in mouse models of AD [5].

To dissect the complex interactions between microglial morphology changes, and to investigate potential therapeutic modulation of microglial response by an anti-TREM2 agonistic mAb, we developed a deep-learning enabled pipeline to 1) robustly segment microglia from 3D images of brain tissue and 2) classify microglial states from morphometric features in a mouse model of AD. These combined approaches provide an improved analytical pipeline for employing morphometric endpoints in drug development programs.

Mice were treated with an experimental agonist of TREM2 and then imaged in a time course at 1, 7, 14, and 28 days post treatment using confocal microscopy. Microglia were segmented in 3D using a convolutional deep neural network which segmented both the cell body and soma, while simultaneously separating individual touching microglia. A large feature bank of morphological descriptors were calculated for each cell, including skeletal, fractal, and Sholl-like features, across a data set comprising thousands of microglia. Dimensionality reduction and clustering techniques were used to assign phenotypic states to each cell, representing a continuum of states from resting through activated microglia.

Deep learning-based segmentation was able to segment microglia in a wide variety of configurations, densities, and shapes, and could successfully decompose microglia into soma and process sub-compartments. Morphological profiling further decomposed a wide variety of cell shapes into three phenotypic categories without any human supplied labels, suggesting those states are inherent to microglial biology. Applying this analysis to the experimental treatment revealed that the population of activated microglia doubled in response to treatment at 24 hours, an effect which persisted for 7 days, then slowly relaxed to baseline after 28 days.

Through a combination of deep-learning based segmentation, morphologic profiling, and phenotypic clustering, this pipeline improves assessment of complex morphologic endpoints in clinical drug discovery and provides a detailed time course for modeling the cellular response to treatment.

References:

[1] Deczkowska et al., Nat. Neurosci. **21** (2018), 779-786. doi: 10.1038/s41593-018-0145-x
[2] Hammond et al., Annu Rev Cell Dev Biol. **34 (**2018), 523-544. doi: 10.1146/annurev-cellbio-100616-060509
[3] Keren-Shaul et al., Cell **169 (**2017), 1276-1290. doi: 10.1016/j.cell.2017.05.018
[4] Ulland and Colonna, Nat Rev Neurosci. **14** (2018), 667-675. doi: 10.1038/s41582-018-0072-1
[5] Wang et al., J Exp Med. **217,** e20200785 (2020), https://doi.org/10.1084/jem.20200785