CASE STUDY

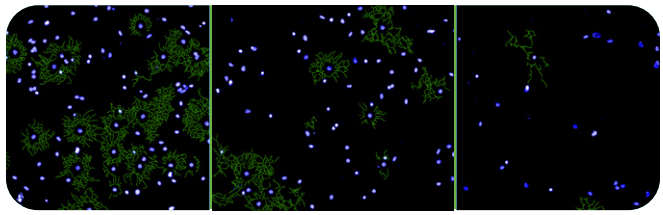
High-content analysis of drug-induced oligodendrocyte differentiation promoting remyelination in multiple sclerosis

Multiple Sclerosis (MS) is a neurological disease in young adults involving an aberrant immune-mediated response leading to progressive failure of remyelination in the central nervous system. Myelin, which provides insulation to nerve cells and enables fast communication, is lost and oligodendrocytes, which are producing the myelin-sheath in the central nervous system (Figure 1), degenerate.

Numerous scars (scleroses) develop in the nervous system which gives the disease its name. Progressive neurological symptoms range from autonomic, visual and motor to sensory problems. Currently, 2.3 million people are affected worldwide.[1] The pathology of MS is complex and its causes are largely unknown and multifactorial. There is no cure yet, and current medications to suppress the immune response show severe adverse effects.

The research group of Dr. Paul Tesar at the Case Western Reserve University School of Medicine in Cleveland is developing stem cell technologies for better understanding and treatment of nervous system disorders including multiple sclerosis. To investigate alternative therapeutic treatments, a multi-institutional team brought together by Paul Tesar performed a phenotypic high-content screen to identify bioactive small molecules enhancing oligodendrocyte maturation.[2]

There is histopathological evidence in MS patients that oligodendrocyte progenitor cells are abundant in demyelinated regions, but fail to differentiate into myelin-sheath forming oligodendrocytes. The screen



identified two existing medications currently approved for topical application that promote myelin repair and therefore might be effective as systemic treatments for multiple sclerosis.

This case study focusses on the development of the phenotypic assay on the Operetta® high content analysis system and details the image analysis strategy within Harmony® high content analysis software.

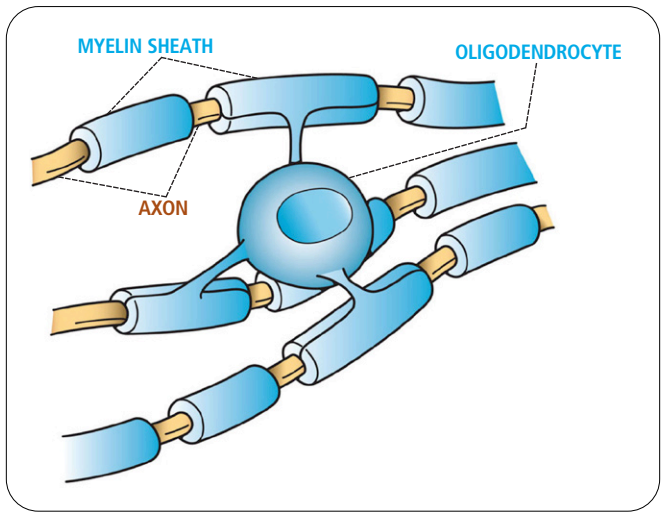


Figure 1: Central role of oligodendrocytes in the production of myelin sheaths in the central nervous system to provide insulation to up to 50 adjacent axons.[3]

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Application

Mouse epiblast stem cell-derived oligodendrocyte progenitor cells (OPCs) were seeded onto poly-D-lysine coated ViewPlate-96 plates (Revvity, #6005710) or CellCarrier-96 plates (Revvity, #6005550) coated with laminin (Sigma, #L2020; 10 mg/mL). To determine the optimal seeding density, 10,000 and 50,000 cells per well were seeded in growth medium (DMEM/F12 supplemented with N2, B-27, neurotrophin 3, cAMP, IGF-1, noggin[2]) and allowed to attach for two hours before addition of drug. Thyroid-hormone-positive controls and DMSO vehicle controls were included in the assay plate. Cells were incubated under standard conditions (37 °C, 5 % CO_2) for three days and fixed with 4 % paraformaldehyde (PFA) in phosphate buffered saline (PBS). Fixed cells were permeabilized with 0.1 % Triton X-100 and blocked with 10 % donkey serum (v/v) in PBS for two hours. Cells were labelled with an MBP (myelin basic protein) antibody (Abcam, ab7349; 1:100) for one hour at room temperature followed by detection with an Alexa Fluor 488-conjugated secondary antibody (1:500) for 45 min. Nuclei were visualized by DAPI staining. The drug of interest (denoted drug X) was tested in triplicate at eight different doses (ranging from 160 nM to 6.7 µM). Plates were imaged on the Operetta system using a 20 x long working distance (lWD) objective in widefield mode (Figure 2).

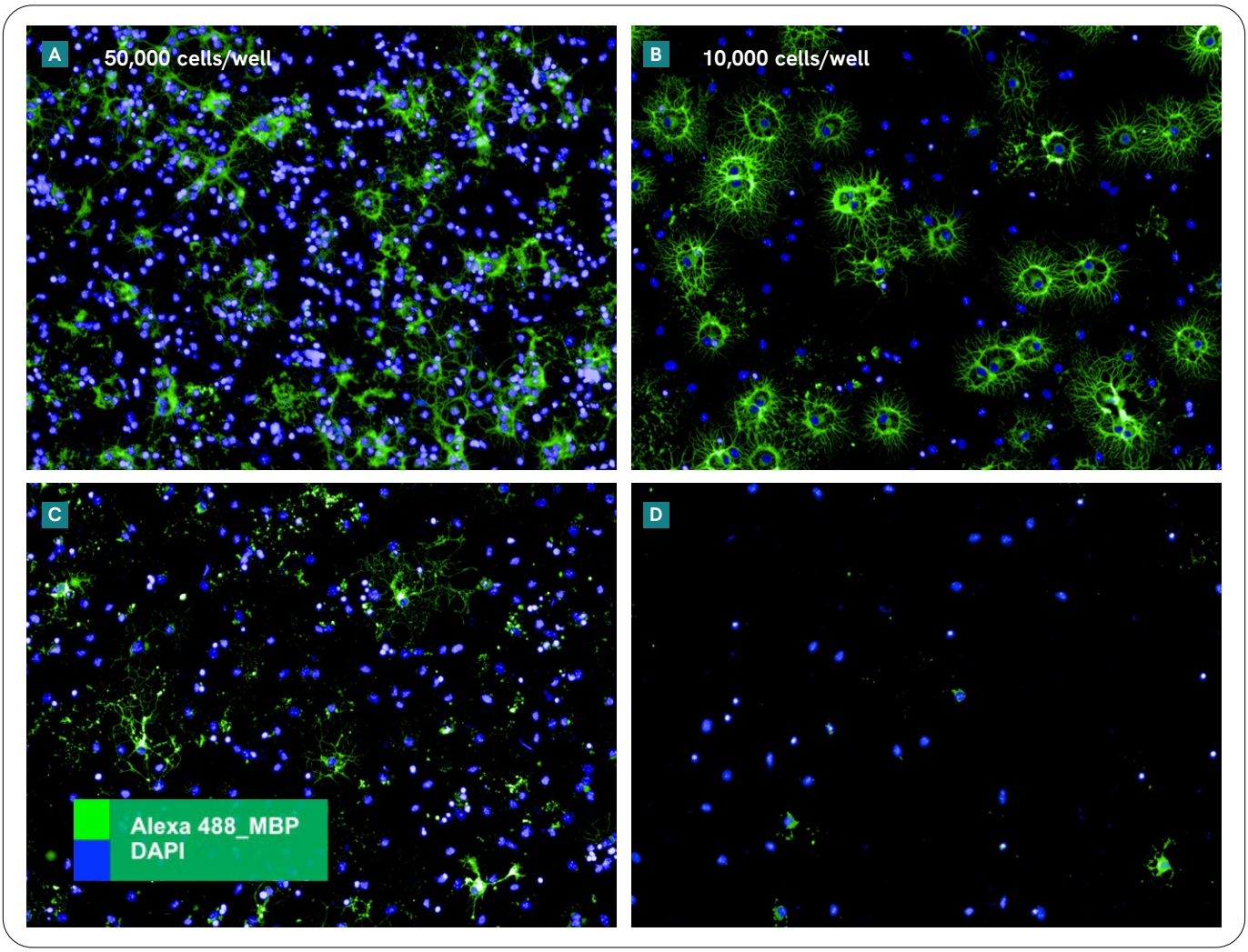

Figure 2: Drug-induced differentiation of mouse stem cell-derived oligodendrocyte progenitor cells (OPCs). Mouse epiblast stem cell-derived OPCs were seeded at two different cell densities (left: 50,000 cells/well, right: 10,000 cells/well) and treated with drug X (A + B). Cells were labeled with DAPI and an Alexa 488-labelled myelin basic protein (MBP) antibody and imaged on the Operetta system in non-confocal mode using a 20 x lWD objective. Cells in panel A and B were treated with the highest concentration of drug (6.66 µM), panel C and D show examples for 160 nM drug X- treated wells. Differentiated oligodendrocytes are characterized by their emanating MBP-positive processes.

Image analysis strategy

Automated image analysis was performed with Harmony software (Figure 3). Briefly, DAPI stained nuclei were filtered with a sliding parabola to improve contrast for segmentation. The quality of the identified nuclei population was optimized by removing small fragments (< 50 µm^2) and artifacts (based on roundness) as well as very bright, potentially pyknotic nuclei. As the processes emanating from mature oligodendrocytes resemble neurites, the CSIRO2 algorithm of the *Find Neurite* building block was used on the Alexa 488-MBP-stained images (filtered with sliding parabola) to calculate the total neurite length. Each nucleus was expanded by 50 % to provide a "cell body" for the "neurite" tree segmentation. The classification for a differentiated oligodendrocyte was then defined by setting the threshold to a minimum of 100 px total neurite length (i.e. sum of length of all segments attached to a cell, also called neurite tree). The percentage of differentiated cells in the population was calculated and normalized to thyroid hormone (positive control)-treated wells.

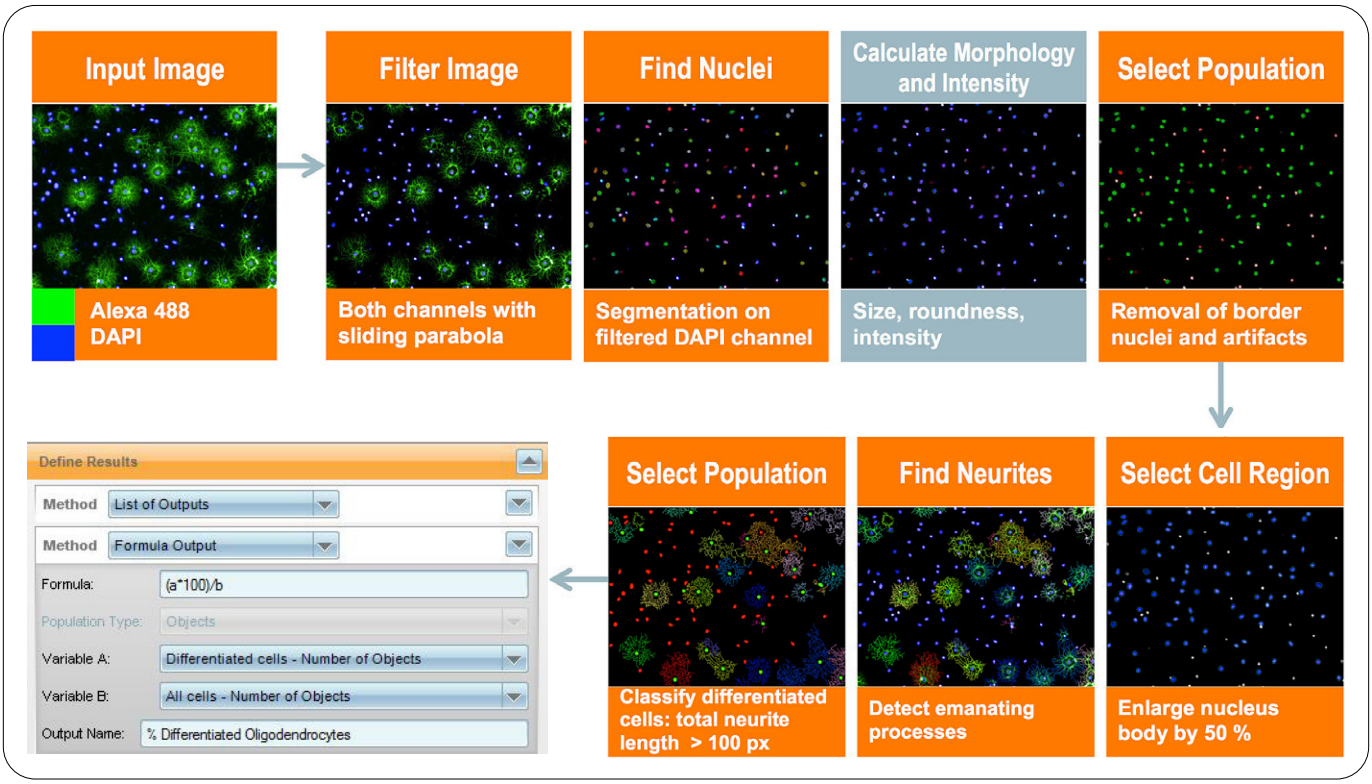

Figure 3: Image analysis workflow for oligodendrocyte classification within Harmony software: starting with a classical nuclei segmentation step on the contrast-enriched filtered DAPI-channel, nuclei are then cleared from artifacts and potentially pyknotic cells. Neurite detection is applied onto enlarged nuclei. By setting a minimum threshold of 100 px for total neurite length, cells are classified into differentiated and non-differentiated oligodendrocytes.

Dr. Paul J. Tesar

"The Operetta high content imaging system brought a pharma-like capacity to the lab and transformed our ability to rapidly identify promising molecules with therapeutic potential to enhance remyelination."

Results

During assay development, drug X was applied to two different cell densities of mouse epiblast stem cell-derived OPCs in eight different concentrations ranging from 160 nM to 6.66 µM. For both cell densities, a dose-dependent increase in differentiated mature oligodendrocytes was observed (Figure 4). The percentage of differentiated oligodendrocytes was normalized against the thyroid hormone positive control (value set to 100 %). At its highest concentration, drug X can induce OPC-differentiation up to a level of 75 % relative to the positive control. However, EC_{50} values differ substantially between the seeding densities (0.93 µM vs. 3.54 µM).

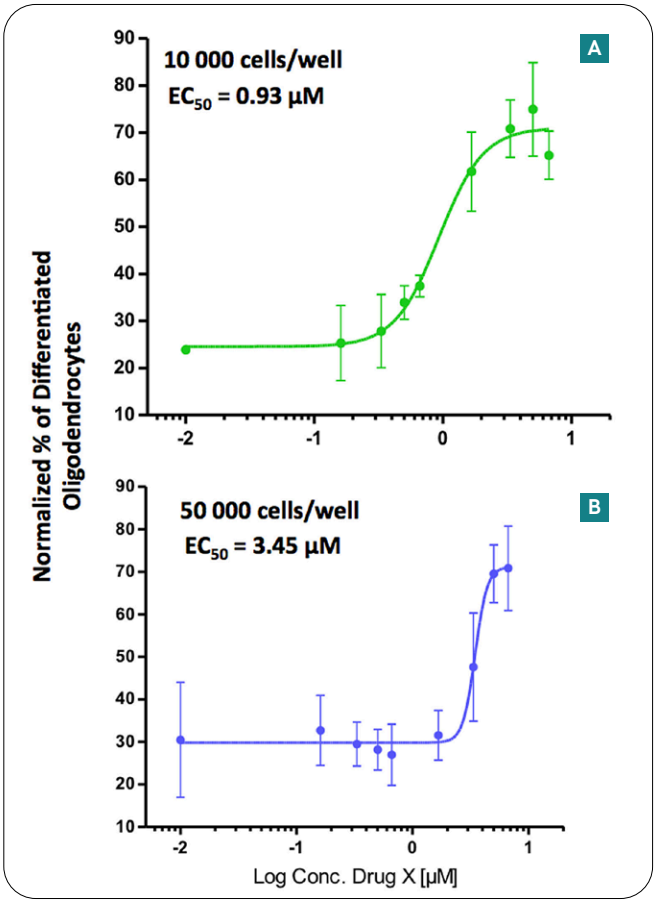

Figure 4: Drug X promotes differentiation of OPCs to oligodendrocytes in a dose-dependent manner. Eight concentrations of drug X (ranging from 160 nM to 6.67 µM) were tested with two different seeding densities (10,000 and 50,000 cells/well). Differentiated cells were classified according to a minimum of 100 px of total neurite length per cell; data were normalized against thyroid hormone positive control (set to 100 %). EC_{50} for the lower cell density was determined to be 0.93 µM drug X, while the more confluent wells led to a higher EC_{50} of 3.45 µM.

Quality parameters confirm that the assay performs better at 10,000 cells/well, as the Z` value is 0.61 compared to a Z' of 0.20 for 50,000 cells/well. As shown in Figure 2, the higher confluency leads to a less homogeneous appearance of the oligodendrocyte population and to shorter MBP-positive processes (50,000 cells/well max. total neurite length of 106 vs. 397 px at 10,000 cells/well).

Analysis of the number of oligodendrocytes per well suggests drug X to have an additional proliferative effect (Figure 5).

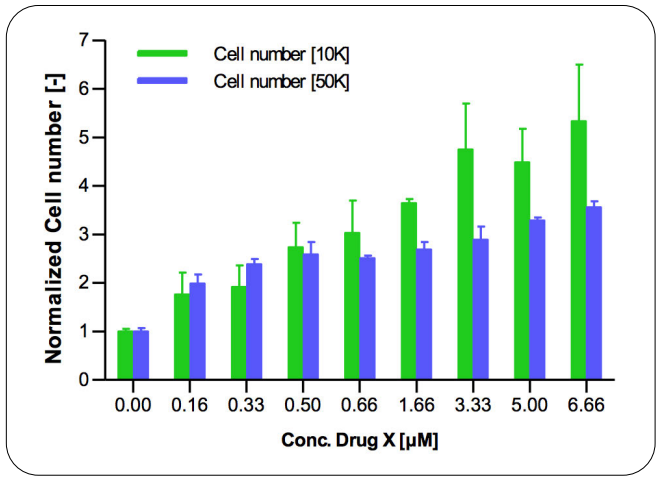

Figure 5: Drug X induces proliferation. Increasing concentrations of drug X lead to increasing normalized cell numbers (mean cell number per well relative to mean cell number of negative control wells).

Conclusion

One of the greatest challenges in multiple sclerosis therapy is the halting or reversal of the failure of remyelination in the brain in order to reverse disabilities in MS patients. The work of Paul Tesar and colleagues highlighted here, could potentially lead to such novel treatments, as it aims to control the function of stem cells in the body and thereby to help the body repair itself. High content analysis systems such as the Operetta allow the quantification of complex phenotypic changes such as changes in the length of processes emanating from oligodendrocyte brain cells. Combined with cutting-edge stem cell technologies, high-content screening is therefore a key technology enabling drug discovery within this new area of regenerative medicine.

Acknowledgement

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References

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3. https://commons.wikimedia.org/wiki/File:Oligodendrocyte_illustration.png.

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