

A Method to Classify Human Astrocytoma Cell Using Multiparametric Flow Cytometry: a Step Towards Single-cell Molecular Pathology of Solid Tumors

Rachel Grossman MD; Ilan volovitz; Netanel Shapira; Zvi Ram MD

### Introduction

Multicolor flow cytometry (FCM) has revolutionized the molecular pathology of hematological tumors enabling multiparametric classification of single tumor cells. Meanwhile, the pathological evaluation of brain tumors (BTs) and other solid tumors, relies heavily on subjective histopathological evaluation of tissue slices stained for single antigens. To date, no FCM based method has been developed to classify human astrocytoma.

### Methods

Human brain lesions were dissociated to single cells. The cells were analyzed using a five-color FCM staining panel concurrently monitoring Ki67 (a proliferation marker), GFAP (a glial marker) IL13 receptor a2 -IL13Ra2 (a glioma-associated antigen - GAA), A2B5 (a GAA and a chemoresistance marker), and cellular viability.

# Results

The Tumor-panel enabled blind classification of brain lesions to astrocytomas, to metastasis reaching the brain and to nontumorous lesions based on marker co-expression patterns consistent with the cells' biology. The panel enabled quantification of the viable tumor cell fraction in lesions following enzymatic dissociation to single-cells and following a tumorcell purification step using a density gradient.

A clinically-relevant finding that emerged from the multiparametic analysis of antigen co-expression in gliomas was the partial coexpression of IL13Ra2 and Ki67: many Ki67+ (proliferating) cells did not express the IL13Ra2 GAA (i.e., IL13Ra2-Ki67+). This may partially explain the failure of several clinical trials that had targeted toxins to gliomas via IL13Ra2.

## Conclusions

FCM can be used to classify astrocytoma cells within single-cell dissociated brain lesions. The developed method represents a first step towards a quantitative, userindependent means for multiparametric classification and study of solid tumors.

### Learning Objectives

By the conclusion of this session, participants should be able to: Describe the importance of using FCM in astrocytoma classification

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