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MULTIPARAMETRIC FLOW CYTOMETRY HIGHLIGHTS B7-H3 AS A POSSIBLE NOVEL DIAGNOSTIC/THERAPEUTIC TARGET IN GD2$^{neg/low}$ NEUROBLASTOMA VARIANTS

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High-Risk neuroblastomas (HR-NB) relapses in more than 30% of cases, despite using aggressive therapies including targeting of GD2. The presence of GD2$^{neg/low}$ NB variants and/or the surface expression of different immune checkpoints ligands including B7-H3 might contribute to therapy resistance. The main aim of this study was to set a fast, unbiased method to unequivocally identify, quantify and characterize Bone Marrow (BM) infiltrating tumor cells. In this study we used a Multiparametric Flow Cytometry (MFC) panel to analyze 41 BM aspirates from 25 NB patients, comparing results with Cytomorphological Analysis (CA) and/or immune-histochemical analysis (IHC). Spike-in experiments assessed the sensitivity of MFC. To find novel prognostic markers possibly integrating the MFC panel, Kaplan-Meier analysis on 498 primary NBs has been performed. No false-positive were detected, and MFC showed high sensitivity (0,0005%). Optimized MFC identified CD45^{neg}CD56^{pos} NB cells in 11 out of 12 (91.6%) of BM indicated as infiltrated by CA, 7 of which co-expressed high levels of GD2 and B7-H3. MFC detected CD45^{neg}CD56^{pos}GD2$^{neg/low}$ NB variants which, importantly, expressed high surface levels of B7-H3. Kaplan-Meier analysis highlighted an interesting dichotomous prognostic value of different ligands involved in NB recognition by the immune system.
To conclude this study describes a specific, sensitive and fast MFC analysis allowing a precise quantification of i) BM tumor burden; ii) surface expression of GD2; iii) surface expression of different immune checkpoint ligands. MFC might usefully support other routinely used diagnostic and prognostic tools, improving diagnosis, prognosis, and orienting novel personalized treatments in patients with GD2$^{neg/low}$ NB, who might benefit from innovative therapies combining B7-H3 targeting.