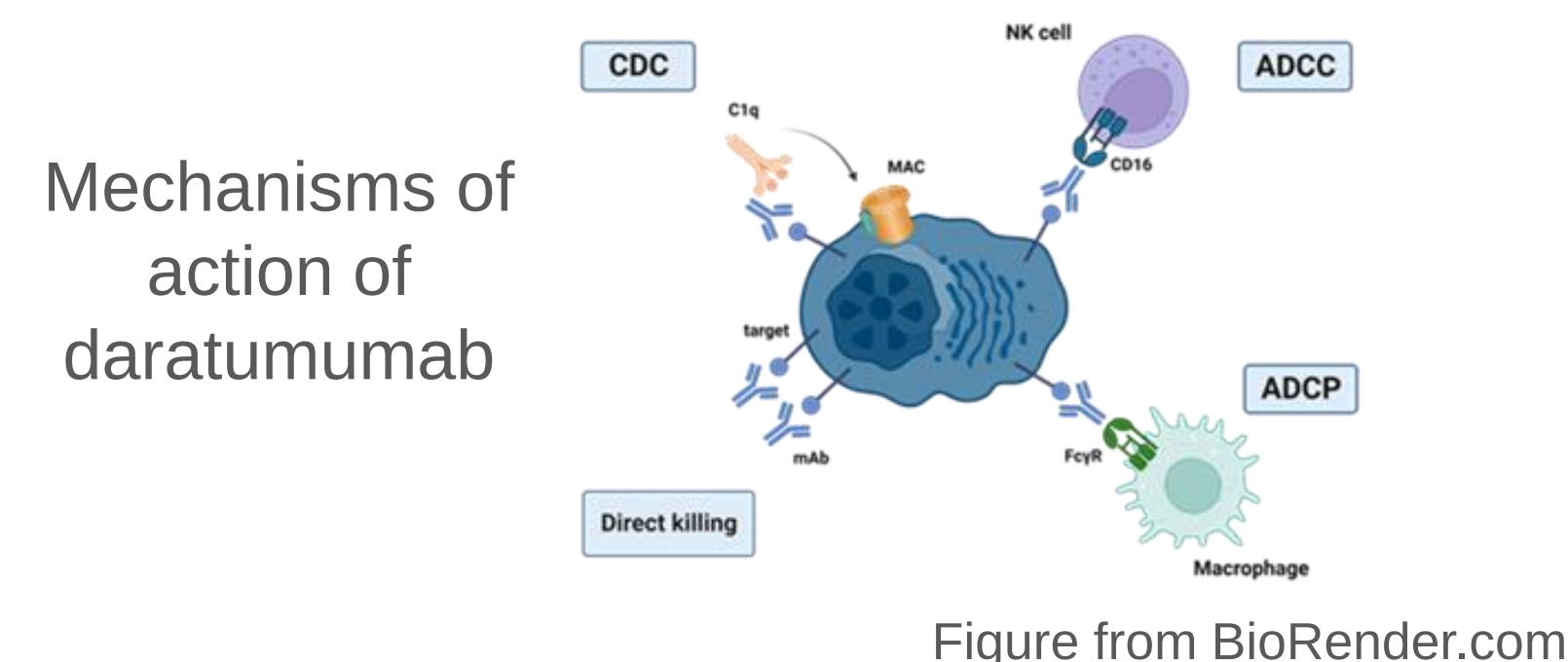


Advancing Insights into Monoclonal Antibody Mechanisms: A Novel Ex-Vivo Assay Using Whole Bone Marrow Environment to Investigate the Efficacy of Daratumumab in Multiple Myeloma

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INTRODUCTION

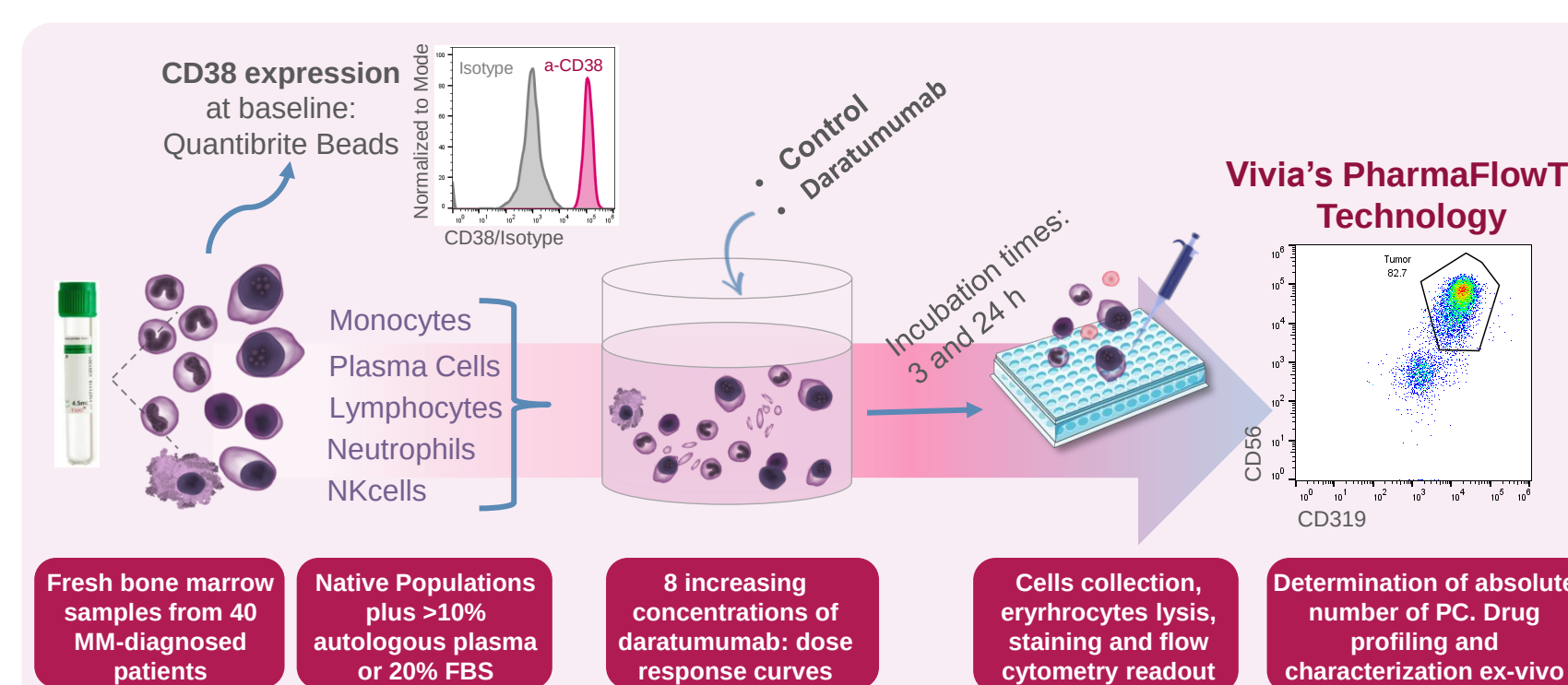
Monoclonal antibodies (mAbs) have transformed therapeutic approaches, and daratumumab has emerged as a promising option for treating multiple myeloma (MM). These mAbs exert their effects killing plasma cells (PC) through complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) involving natural killer (NK) cells, antibody-dependent cellular phagocytosis (ADCP) via macrophages, and direct induction of cell death pathways. In this context, Vivia Biotech applies a method of incubating the whole bone marrow (BM) to integrate each of those mechanisms of action by creating a biologically relevant milieu mimicking the *in vivo* microenvironment.



AIM

To develop an *ex vivo* assay that accurately evaluates the efficacy of daratumumab (DARA) in MM and allows the stratification of patients based on their response.

MATERIALS/METHODS



Vivia Biotech applies a method of incubating the BM to integrate each mechanisms of action of mAbs by creating a biologically relevant milieu mimicking the *in-vivo* microenvironment.

RESULTS

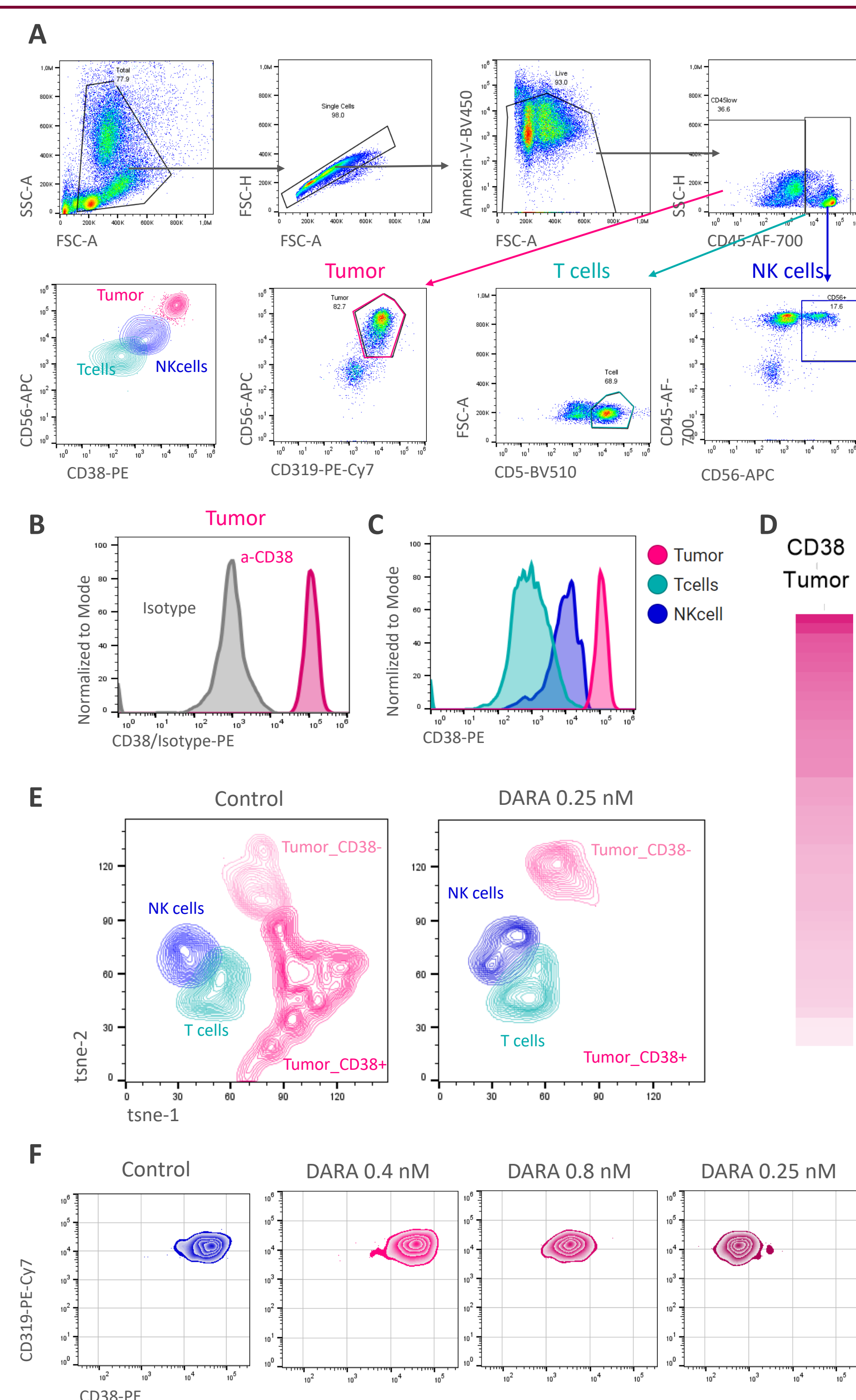


Figure 1: Flow cytometry analysis. **A.** Gating strategy followed to identify and determine absolute tumor, NK and T cells counts in the conditions assayed. After discarding debris and doublets, tumor cells were identified as live CD45^{bright}CD56⁺CD319⁺ cells. NK cells were defined as CD45⁺CD56⁺CD5⁻ cells and T cells as CD45⁺CD5⁺ cells. **B.** CD38 receptor density was quantified using Quantibrite beads following manufacturer's instructions. Isotype control was used to define the background signal. **C.** Tumor cells expressed higher levels of CD38, followed by NK and T cells. **D.** CD38 levels in tumor cells varies between samples. **E.** At the highest concentrations assayed, CD38⁺ tumor cells were not detected in DARA-treated wells. **F.** CD38 levels in tumor cells decreased after incubating with increasing concentrations of DARA due to the competition of the antibodies or internalization of the receptor.

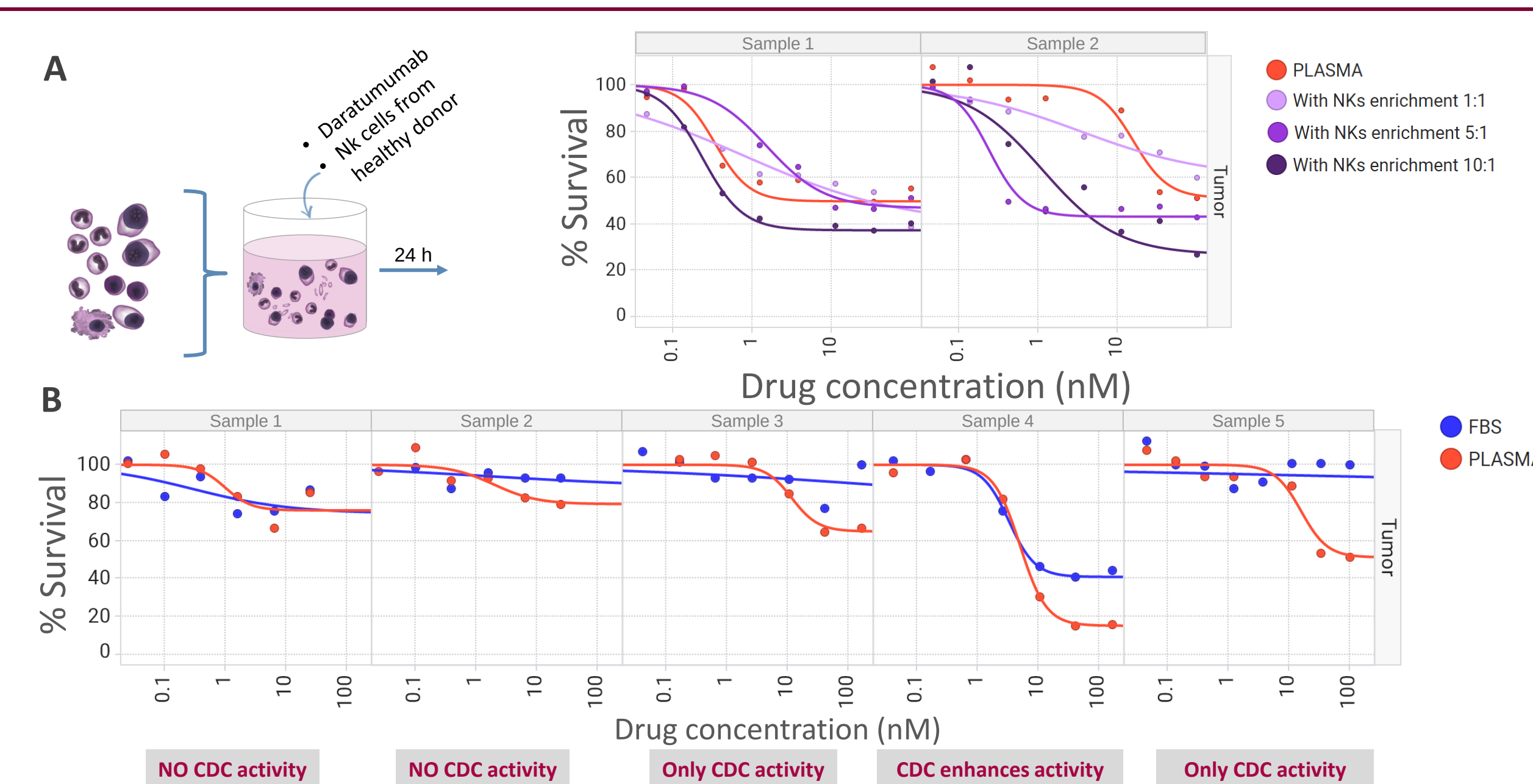


Figure 2: Vivia's methodology allows detection of ADCC and CDC-mediated tumor killing. **A.** MM samples were incubated with increasing concentrations of DARA in presence of different effector (NK cells)- target (tumor) ratios. NK cells were isolated from peripheral blood from healthy donors. The presence of higher E:T ratios increased the activity of DARA indicating ADCC-mediated activity. **B.** MM samples were incubated with increasing concentrations of DARA for 24 hours in presence of 20% autologous plasma (presence of complement) or 20% FBS. Autologous plasma triggered enhanced responses to DARA compared to the activity in absence of plasma in three out of five samples assayed.

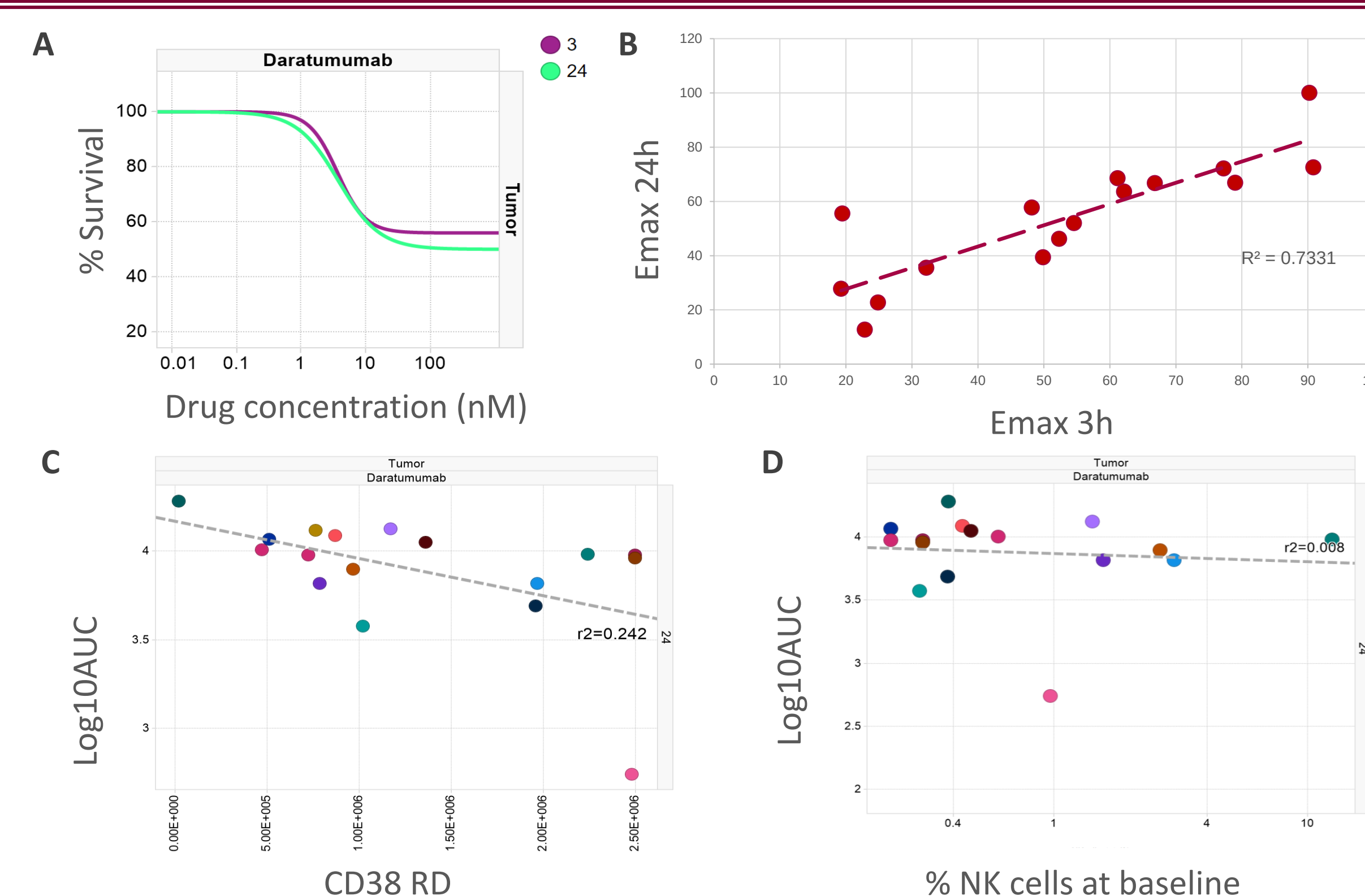


Figure 3: Correlation between activity at different incubation times and between activity and CD38 expression and NK proportion at baseline. **A.** Mean of 10 dose-response curves shows slightly better activity of DARA after 24 hours compared to 3 hours of incubation. **B.** Good correlation between Emax of the dose-response curves of DARA at 3 and 24 hours. **C.** No correlation between activity of DARA (represented as log₁₀AUC) at 24 hours and CD38 expression on tumor cells, expressed as receptor density (RD) or **D** percentage of NK cells at baseline.

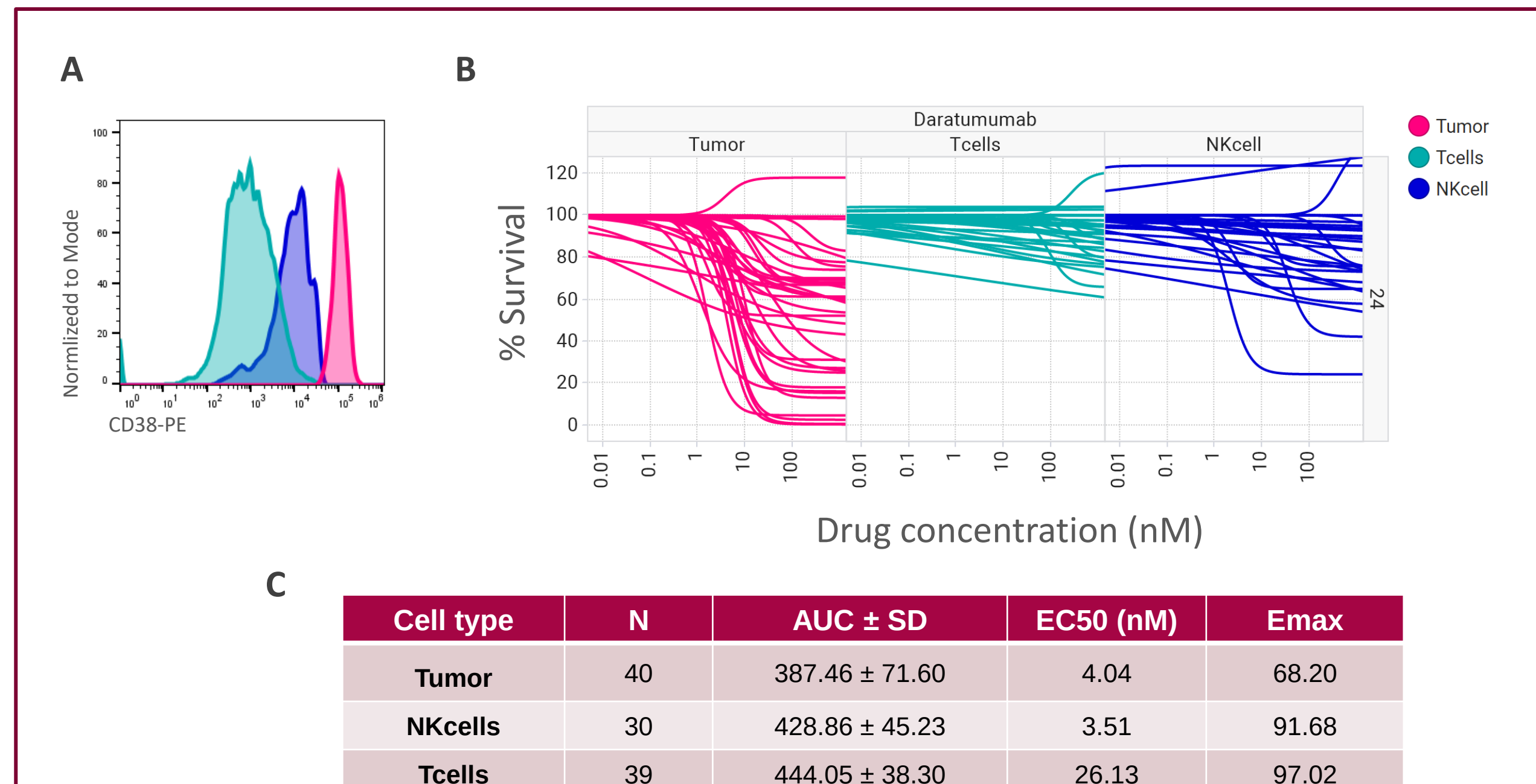


Figure 4: DARA-induced killing in different populations of cells after 24 hours of incubation. **A.** Tumor cells have higher CD38 expression levels in the cell surface than NK and T cells. **B.** DARA shows greater activity in tumor cells (pink) compared to NK cells (blue) and T cells (green) present in MM samples. **C.** Activity parameters of the calculated dose-response curves after DARA treatment in the three populations of cells identified.

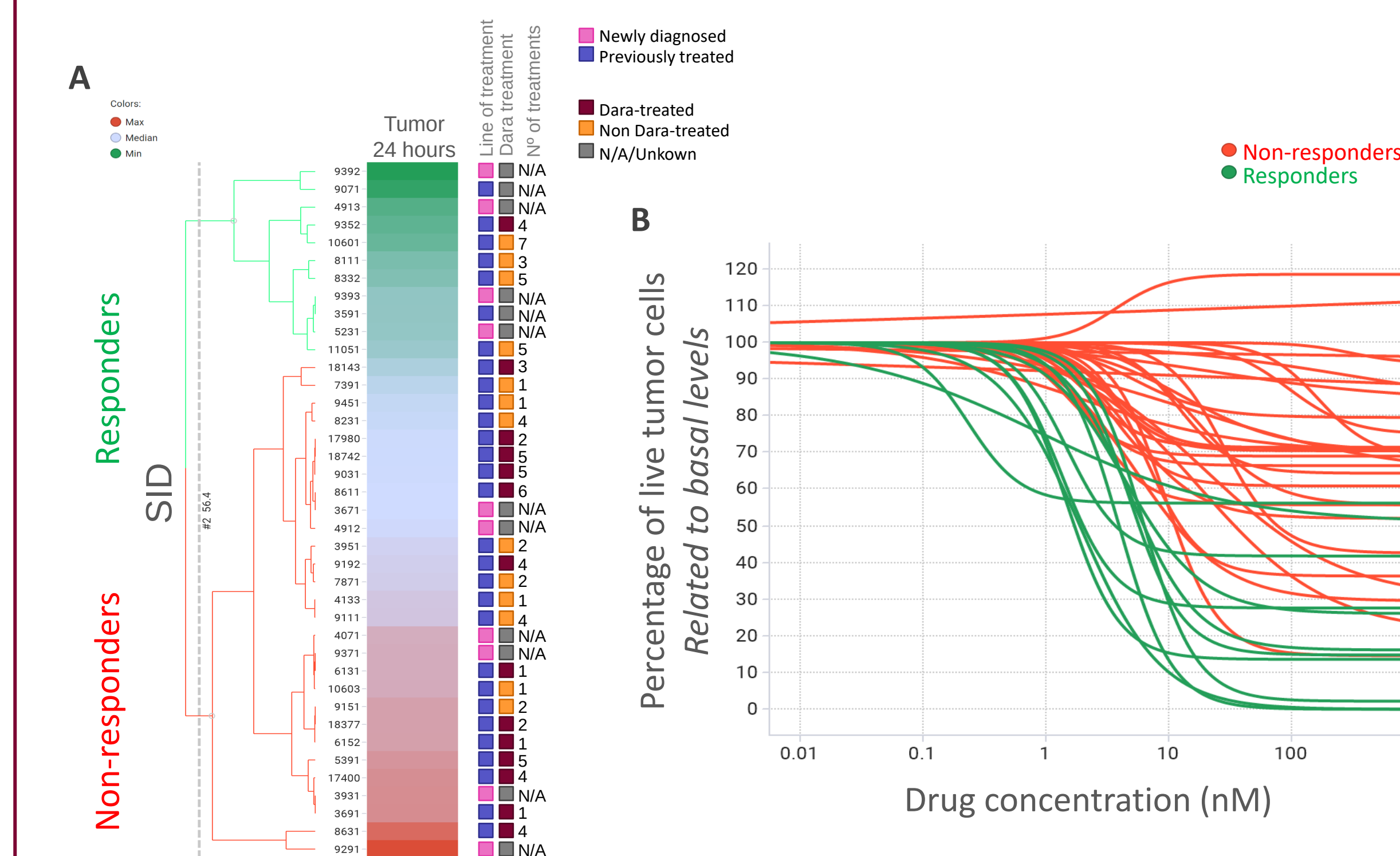


Figure 6: Classification of the samples based on their response to DARA *ex-vivo*. **A.** Cluster classification of samples in sensitive and resistant established based on the response to DARA *ex-vivo*. Line of treatment, previous treatment with DARA and number of previous treatments are indicated. Newly diagnosed and previously treated samples were evenly distributed in both groups. Samples from patients previously treated with DARA were more clustered in the resistant group. **B.** Dose response curves after treatment with increasing concentrations of daratumumab of all samples assayed, non-responders represented in red and responders in green, based in the heat map classification.

CONCLUSIONS

- Our whole BM methodology provides a comprehensive insight into the mechanism underlying the action of mAbs like DARA, capturing simultaneously activity mediated by CDC, ADCC, ADCP or direct apoptosis.
- Although in our study there was a greater effect of DARA on the immune cells that express it more, we could not find a correlation between expression levels, the number of NK cells, and Dara activity within plasma cells, suggesting multiple mechanisms involved in its efficacy.
- Although samples previously treated with DARA exhibit increased resistance in our *ex-vivo* assay, a more uniform patient cohort should be examined to establish a potential correlation between *in-vivo* DARA resistance and *ex-vivo* results. Moreover, it is essential to highlight that current MM treatments involve administration of DARA in combination with other compounds not analysed in this classification.

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