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Introduction

Antibody-drug conjugates (ADCs) are a promising therapy using monoclonal antibodies to deliver potent drugs to tumor sites. Clinical development requires a comprehensive methodological approach thorough evaluation of ADCs, considering factors like ex vivo activity or hematotoxicity.



Materials & Methods

Fresh Bone Marrow (BM) samples from over 40 patients with hematological malignancies or Normal BM (NBM) were tested with increasing ADC concentrations. Target expression, receptor internalization and hematotoxicity were evaluated by flow cytometry. Next-generation (NGS) and RNA sequencing (RNAseq) data were evaluated by bioinformatic to identify differential gene expression patterns and pathways involved in resistance.

Results

Assay Selection

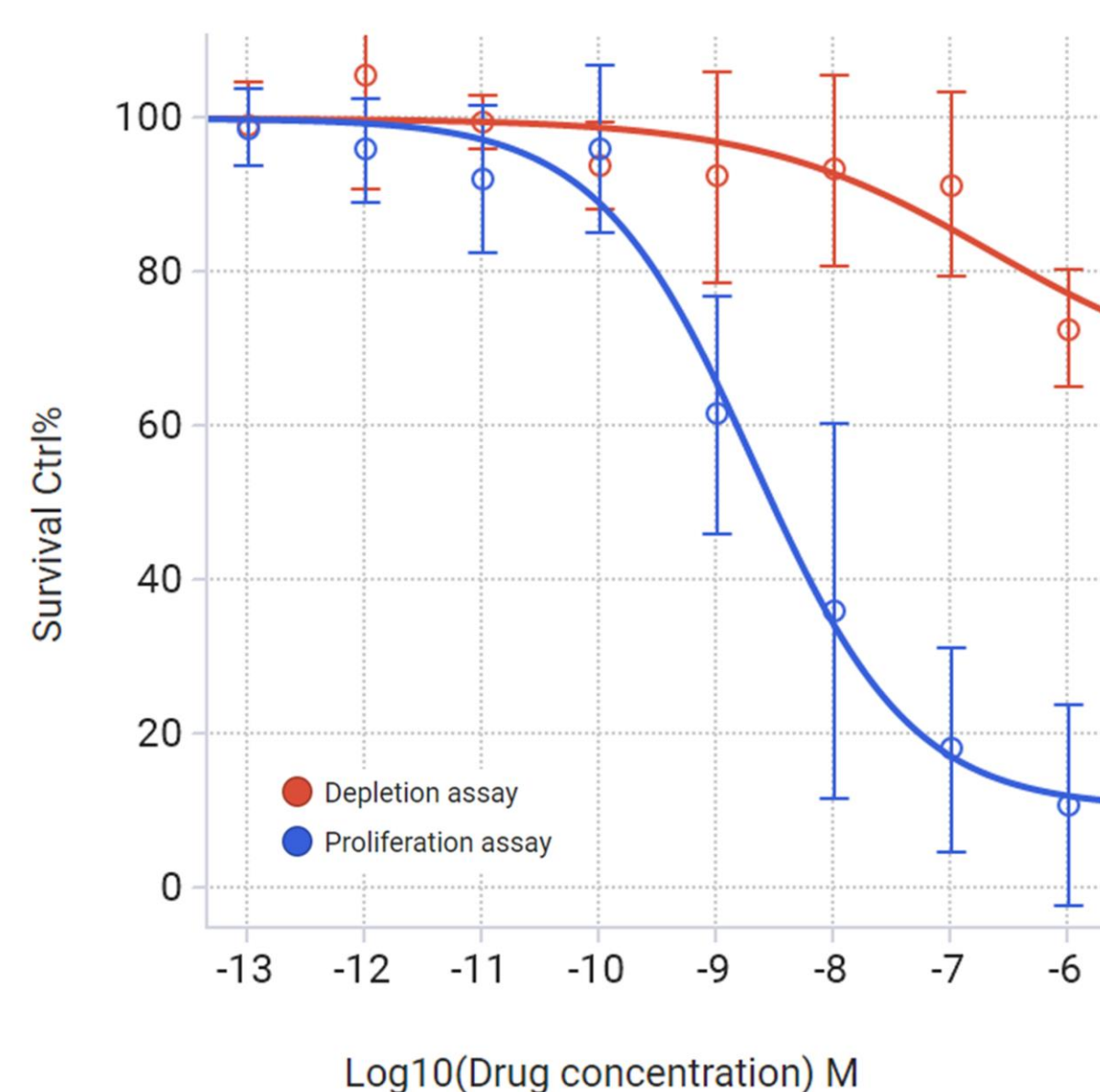


Figure 1: 9 AML patient-derived samples were incubated with one ADC compound linked with an anti-tubulin payload on two different assays showing that cells need proliferation to capture ADC activity.

Candidates Selection

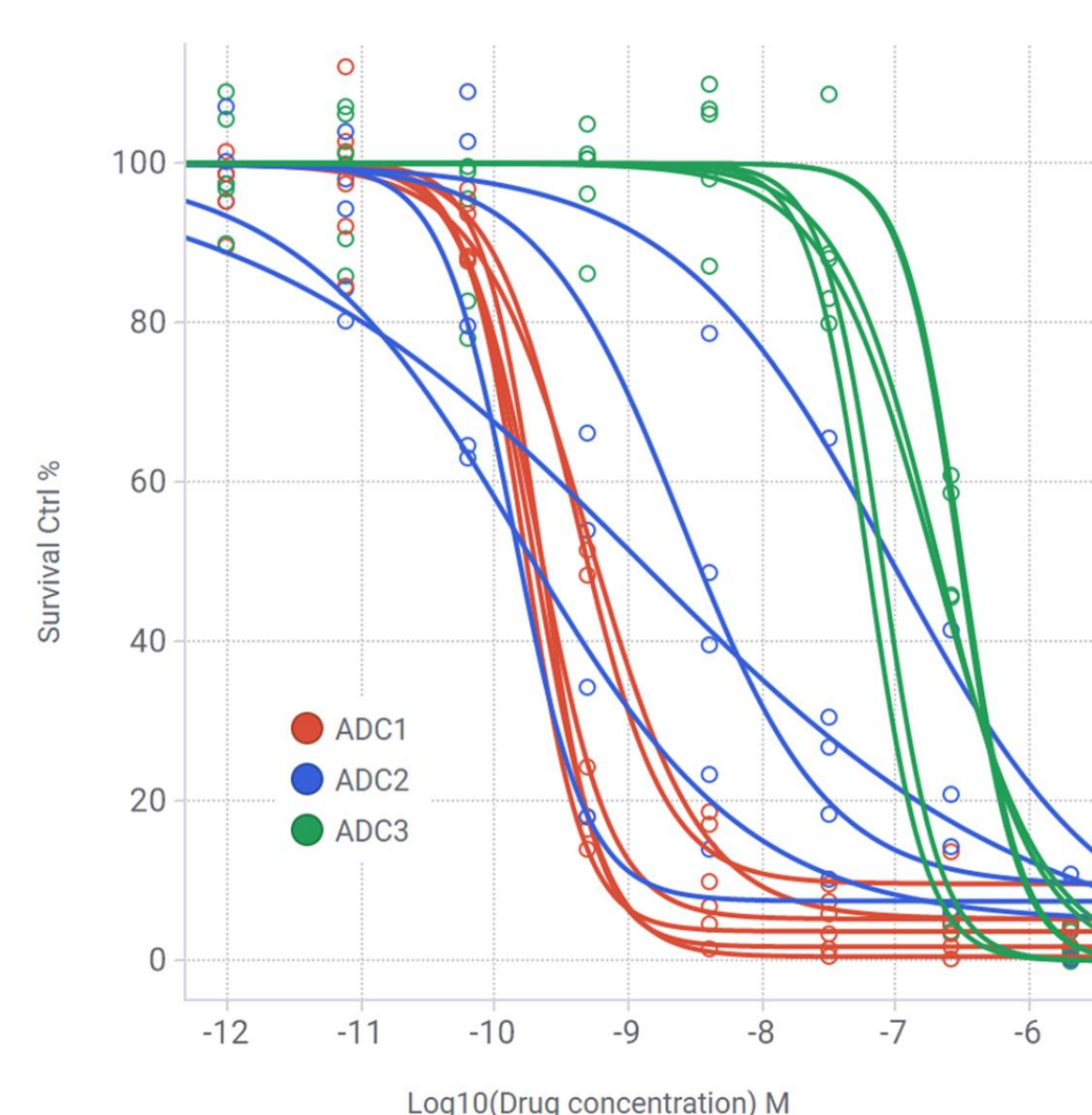


Figure 2: Comparative Hit-to-Lead in real time results of 3 ADCs (color-coded) tested on 6 AML patient-derived samples. ADC#1 demonstrated superior efficacy in similar conditions (72h).

Inter-patient variability

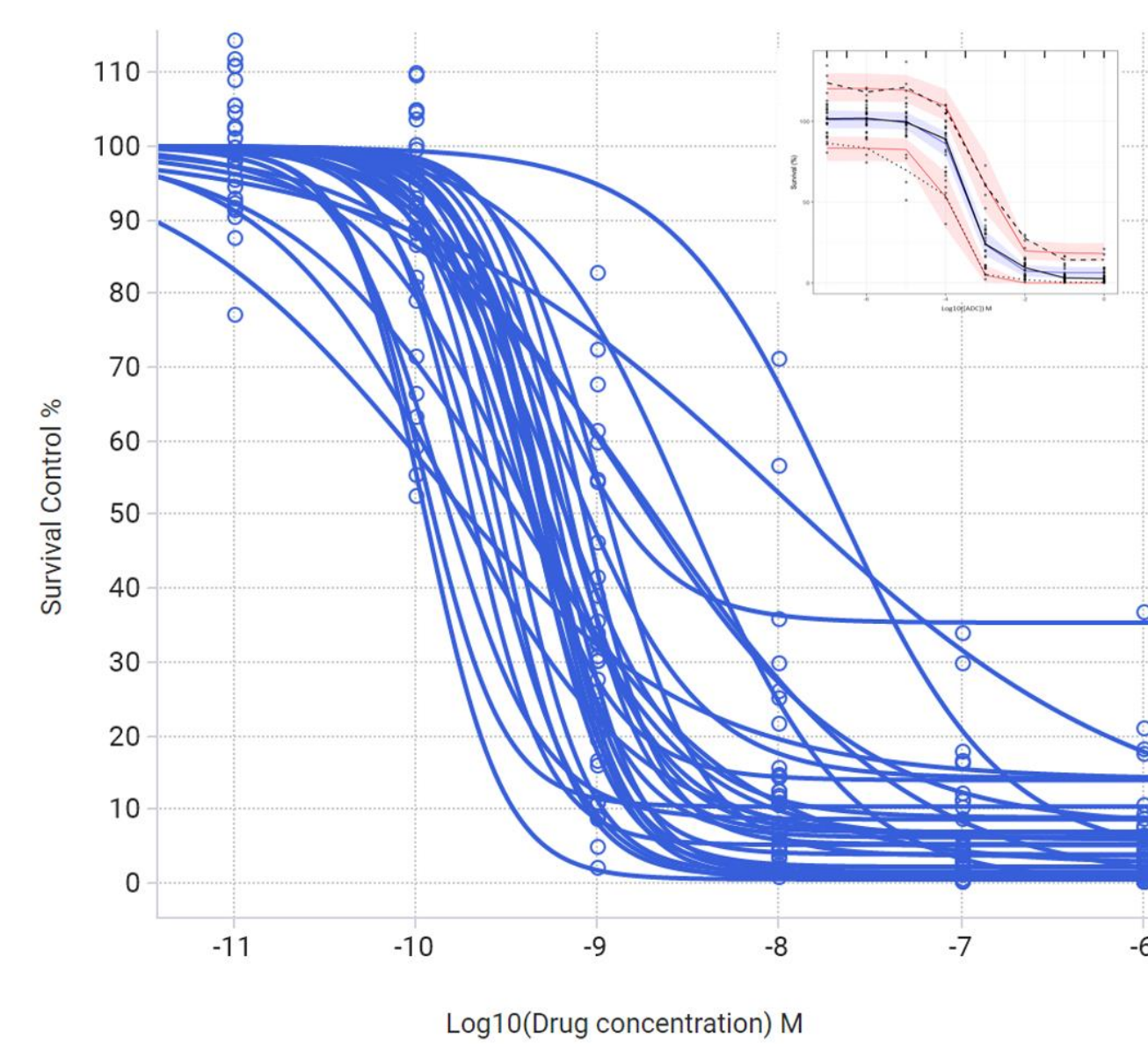


Figure 3: Inter-patient variability shown in the ex vivo assay from a cohort of 30 AML patients. On top-right the VPC (Visual Predictive Check) plot from a mixed-effect population model analysis showing statistical boundaries and confidence intervals.

Target Expression

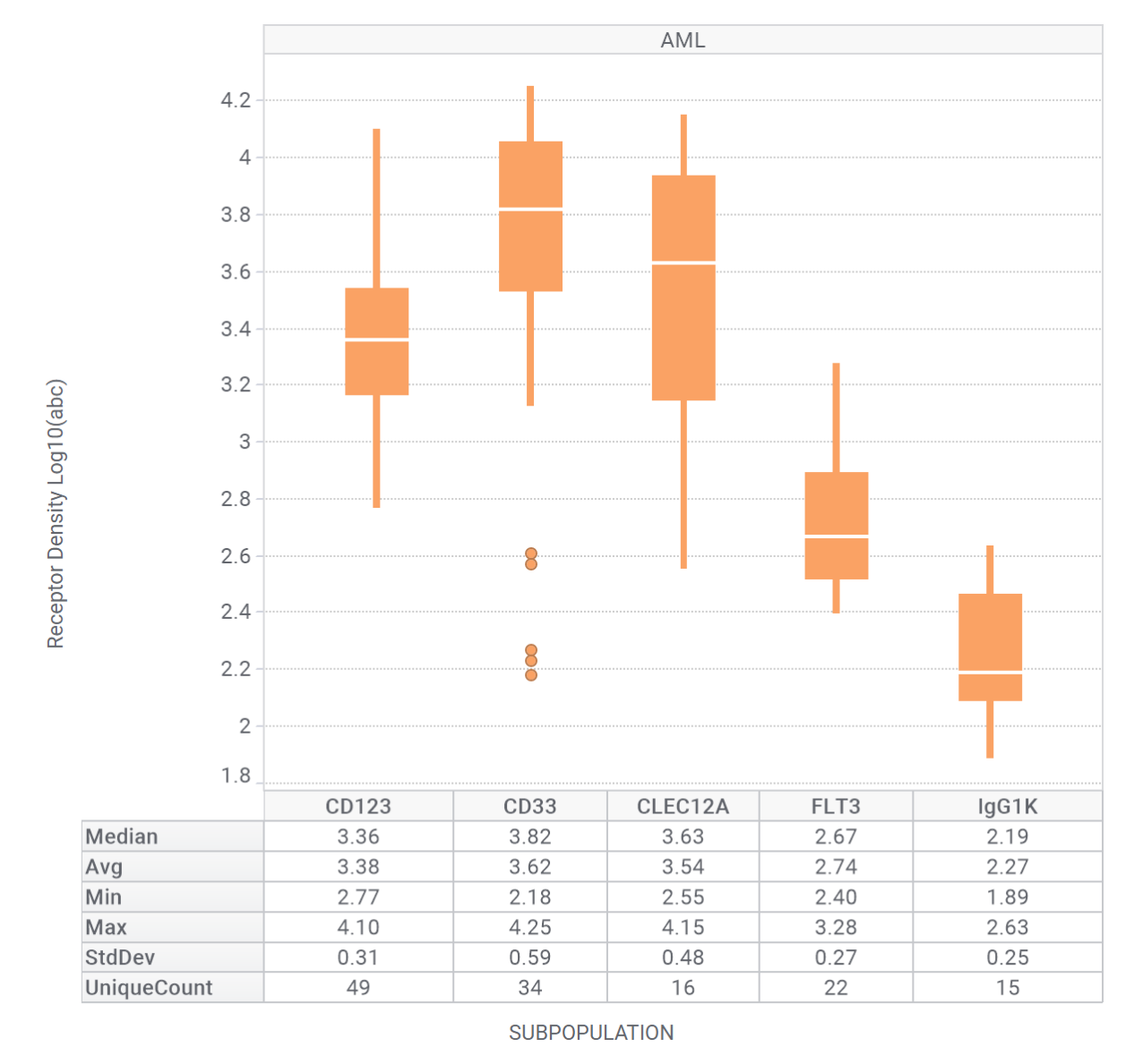


Figure 4: Distribution of 4 target expression (Receptor Density) and isotype across AML patient's samples. A marked variation is observed across different targets, as well as among patients within the same target.

Expression & Activity Correlation

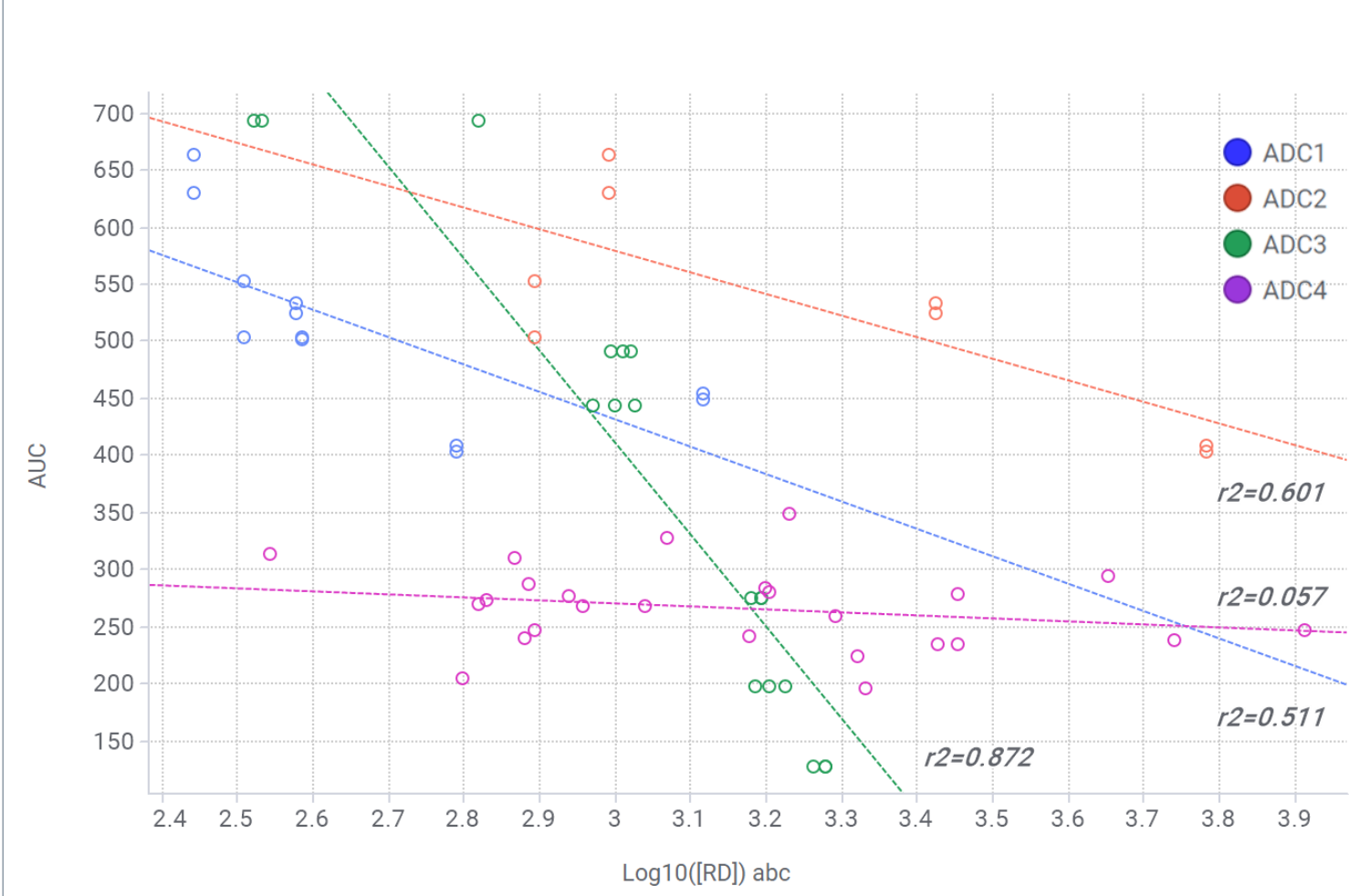


Figure 5: Examples of correlation between receptor density (RD) antibody binding capacity (abc) values with ex vivo activity of 4 different ADCs (color-coded), calculated as area under the curve (AUC).

Target Internalization & Activity Correlation

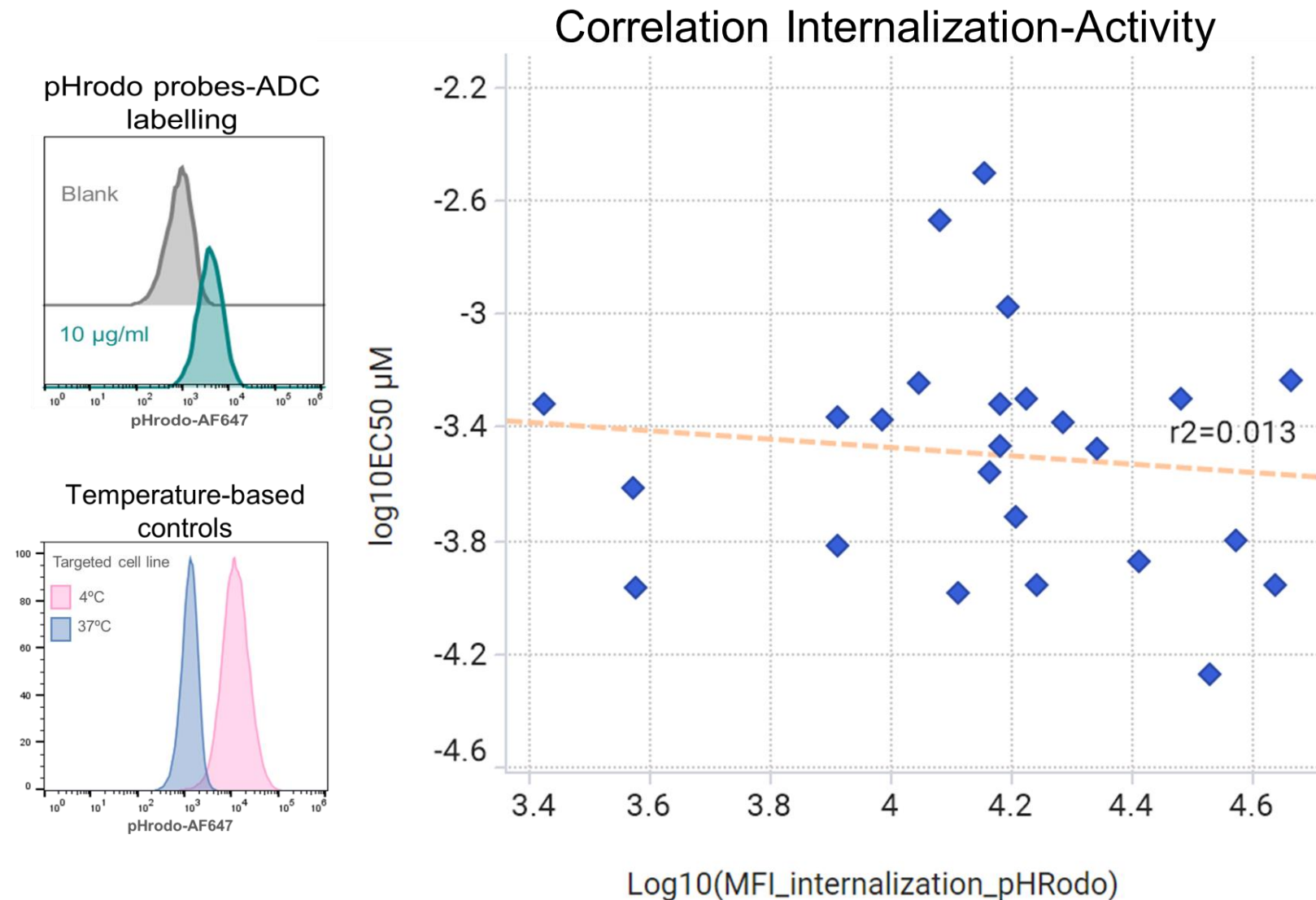


Figure 6: Left panels show ADC internalization using a pH-sensitive probe and appropriate temperature-based controls. Right panel demonstrates absence of correlation with activity (EC_{50}) for a highly internalized target.

ADC Selectivity

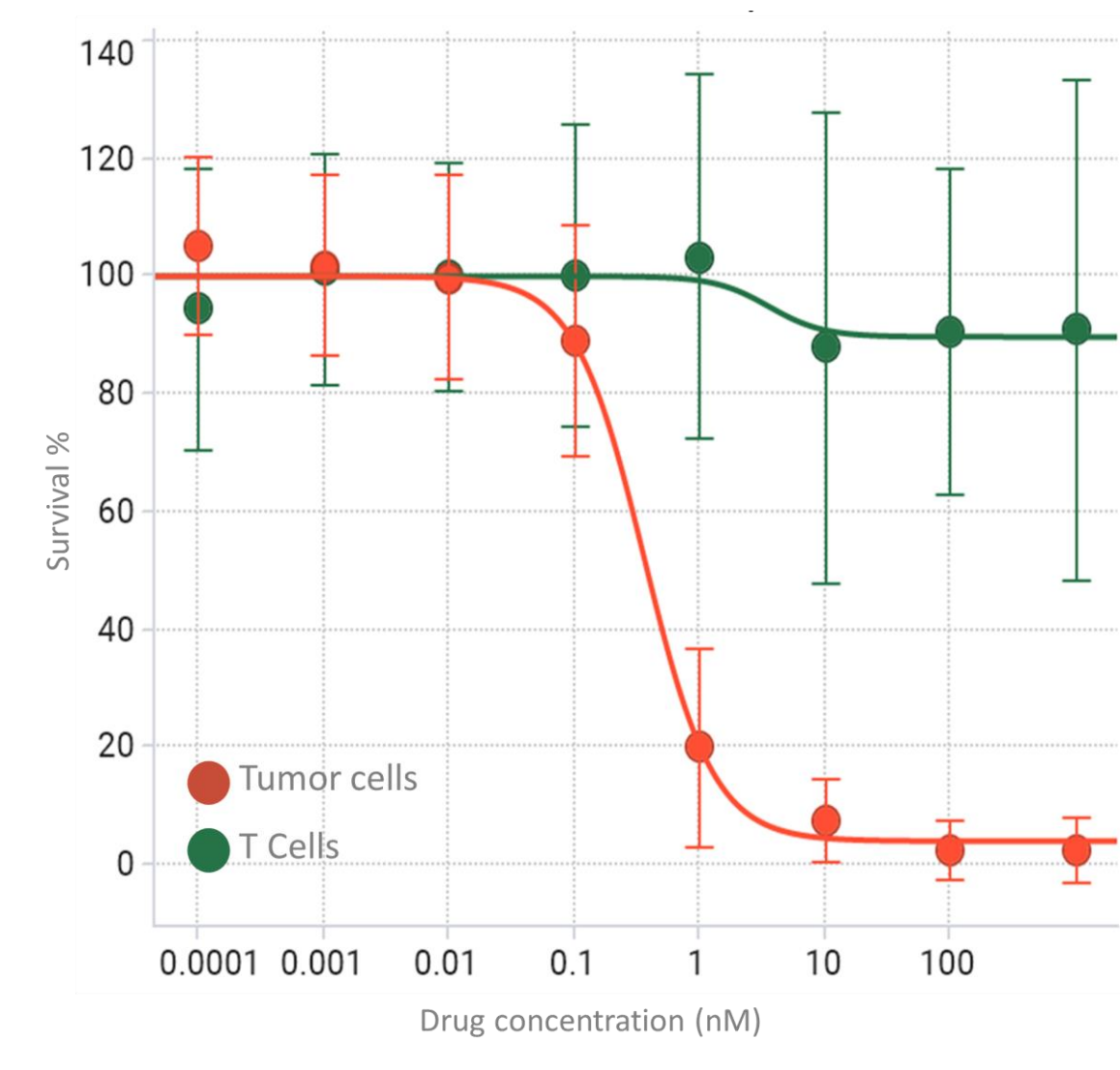


Figure 7: ADC shows high selectivity for target populations (tumor) with no activity on off-target cells (T-Lymphocytes).

ADC Hematotoxicity

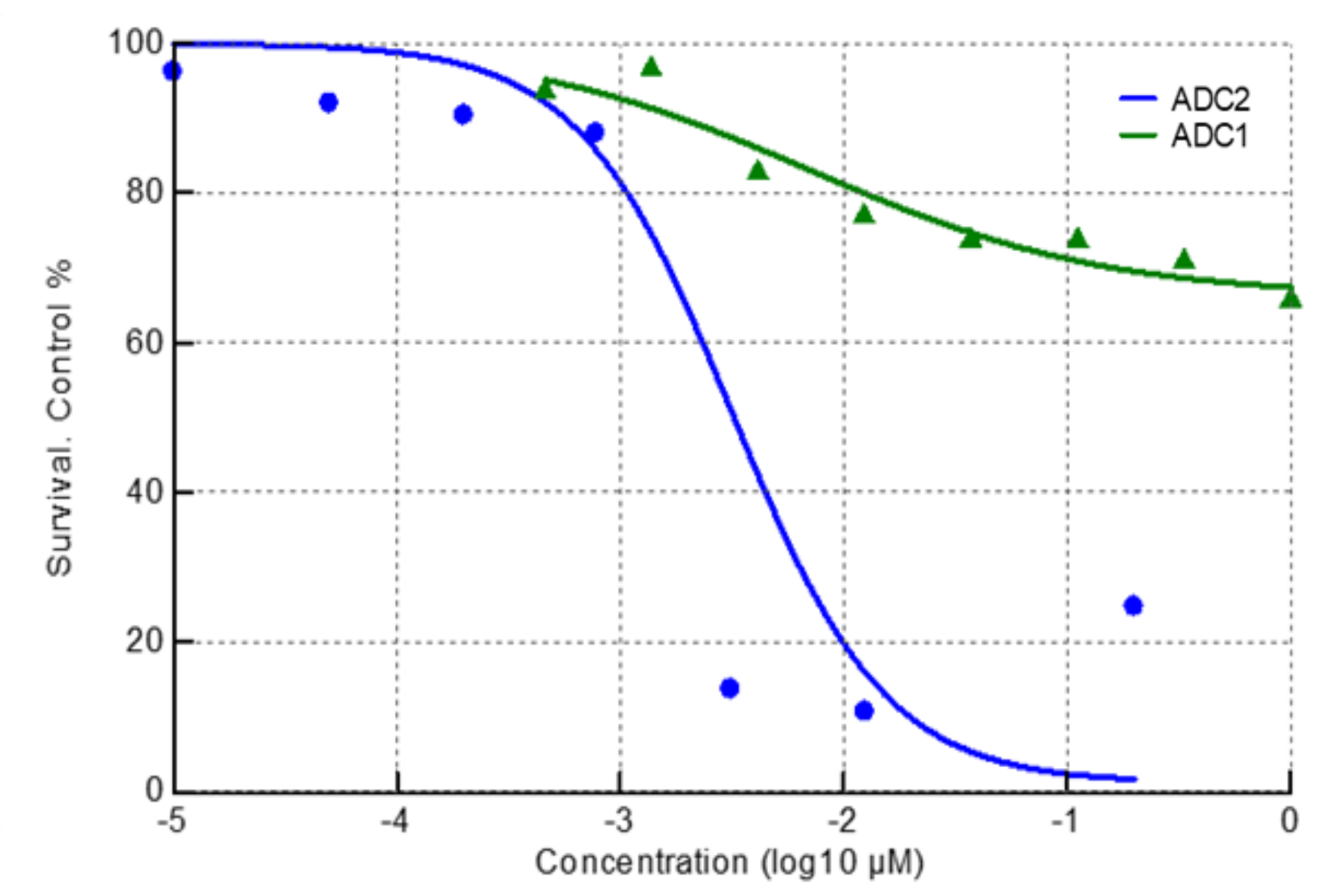


Figure 8: Representative analysis of two different ADCs with same target on CD34+ NBM cells, showing that ADC2 (Blue line) exhibits significantly higher hematotoxicity vs ADC1 (Green Line)

Combinatory Therapies

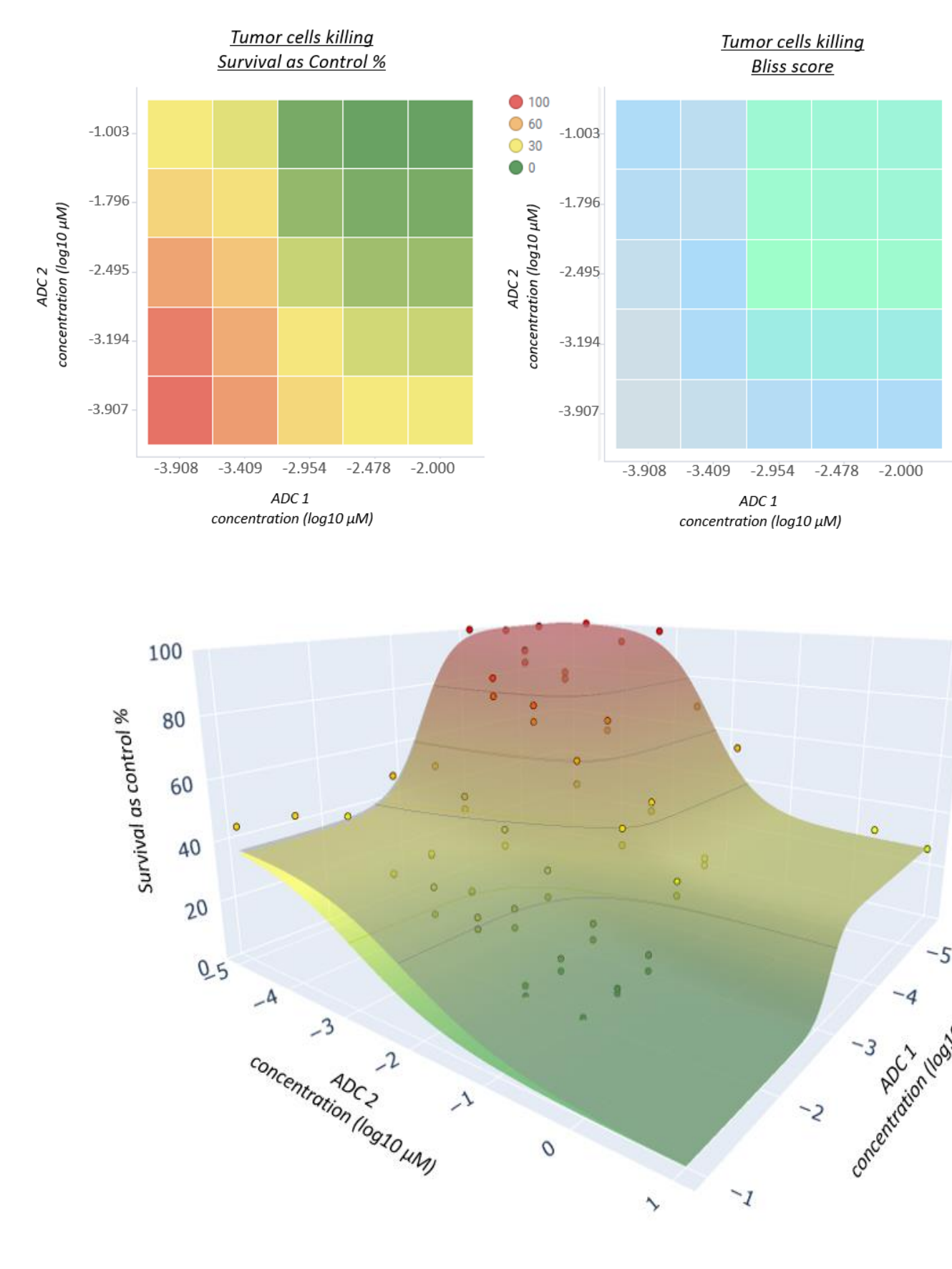


Figure 9: Combination matrix showing ex vivo effect (left) and synergy score (right) on top as color scale heatmaps. Below an interaction surface model showing synergy with respect to an additive surface model (gray).

Molecular-based Mutational profile

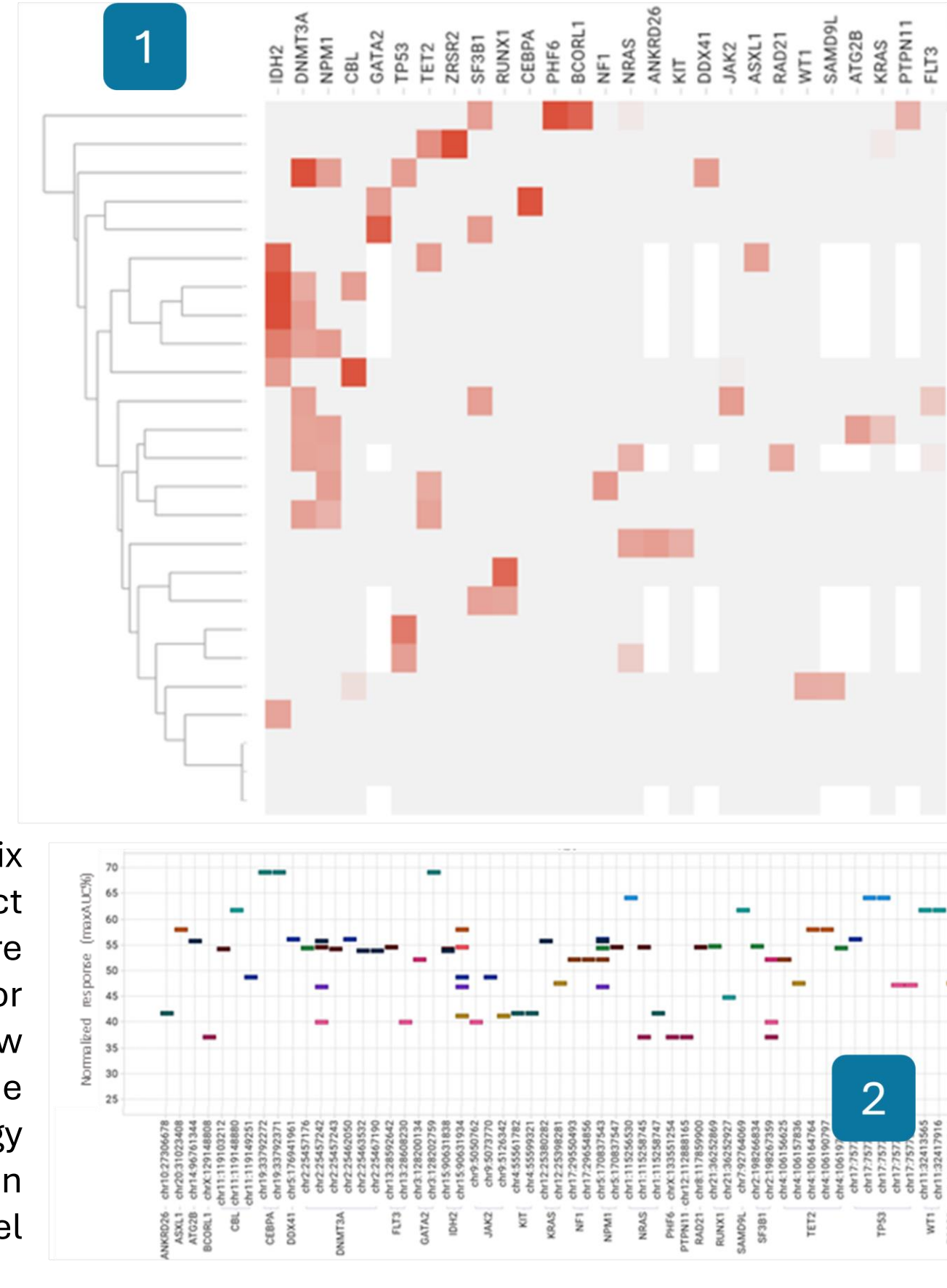


Figure 10a: 1) Patient's sample classification (rows) based on a clusters analysis of the mutational profile (cols) from genomic sequencing data. 2) Correlation with ex vivo functional assay results.

Transcriptional analysis

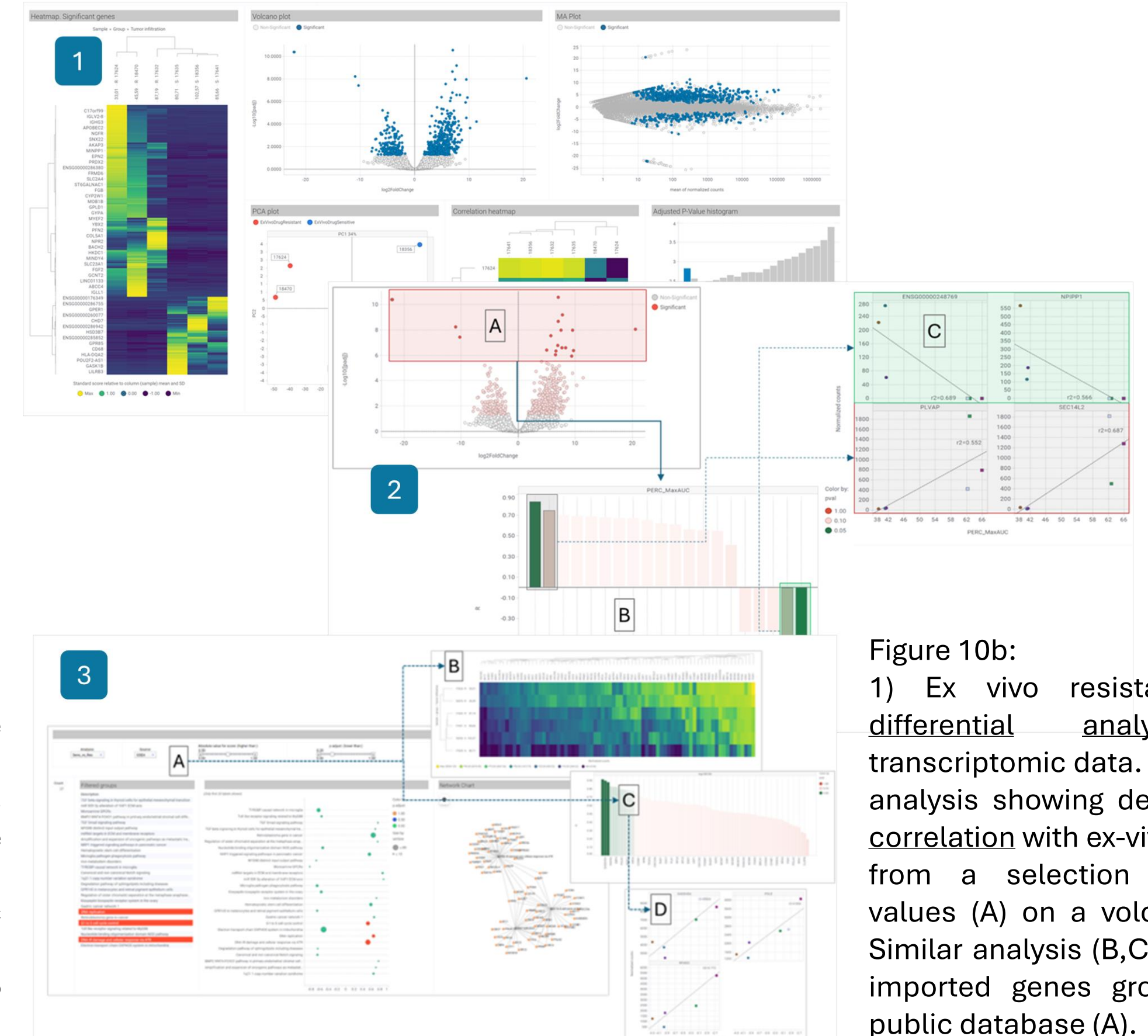


Figure 10b: 1) Ex vivo resistance based differential analysis from transcriptomic data. 2) Interactive analysis showing details (B) and correlation with ex-vivo results (C) from a selection of extreme values (A) on a volcano plot. 3) Similar analysis (B,C,D) based on imported genes groups from a public database (A).

Conclusions

- We provide a comprehensive methodological approach for a thorough understanding of ADC activity alone or in combinations for clinical development.
- This approach integrates and correlates ex vivo data for activity, target expression, occupancy, internalization and molecular data.
- We found that ADC activity often depends on the type of assay or candidates that facilitates their selection for further preclinical evaluation.
- Target expression or internalization alone didn't always correlate with effectiveness indicating that additional sample-specific factors must be related to the sensitivity to ADCs.
- Hematotoxicity varied among ADCs, influenced by the ADC or linker stability reflecting how this assay is important for determining the toxicity related to ADCs.
- By using NGS and/or RNAseq, we identified mutations and gene expressions that could be related to resistance mechanisms.