Background: Bispecific immunotherapy against T cells elicit complex cellular responses involving T cells activation, proliferation, generation of memory clones, resulting in the depletion of tumor cells by cell lysis rather than apoptosis. Multiparametric Flow cytometry techniques can capture these multiple changes in different cell subsets in a mixed population such as, whole bone marrow or blood patient samples. We pioneered automated flow cytometry to evaluate the pharmacological activity of drug candidates in patient samples, preserving the whole sample (Native Environment). This approach has been validated with 80% clinical correlation in 2nd Line Acute Myeloid Leukemia. Vivia commercializes a Precision Medicine such as whole bone marrow or blood patient samples. We pioneered automated flow cytometry integrated model.

Methods: Bone marrow and peripheral blood samples from adult patients diagnosed with AML, MM, NHL or CLL in Spanish centers from the PETHEMA group were included. Whole marrow samples maintaining their Native Environment were incubated for 48h in 96 well plates containing compounds and their combination. Pharmacological responses were calculated using population models. Induction response in AML was assessed according to the Chevallier criteria (2003). Patients obtaining an CR/CRi were classified as responders and the remaining as non-responders.

Results: Our ex vivo automated flow cytometry platform was able to capture the distinct behavior of multiple cell subpopulations occurring in the same well. Although the number of T cells was low, with 0.7 ratios of 1.10 to 1.50, T cells proliferated to large numbers concordant with tumor cells being depleted, and not by apoptosis consistent with T cell loss. T cells activation and exhaustion markers (CD25, CD69, ICOS, PD1, CD62L) showed potent T cell activation prior of robust proliferation. Thus, when adding the bispecific antibody in some samples T cell number even decreases, but a small new activated subset emerges, concordant with effective albeit not complete cell depletion. Kinetics were mainly driven by EC50 ratios and levels of target surface expression. Overlapping the dose responses of a bispecific antibody on tumor cell depletion across multiple samples showed a good patient stratification, from most sensitive to most resistant samples. These curves are equivalent to the curves used daily to guide therapy in AML patients for approved drugs, show here CFI-IDA for reference. If validated with clinical correlation studies, these curves may enable to guide patient immunotherapy.

Conclusion: These Native Environment Precision Medicine ex vivo assays may capture the pharmacological activity of immunotherapy bispecific antibodies. This would enable its application in different phases of drug discovery and development, from screening 100s Ab Hits, to hit-to-lead selection, or screening 10s of different combinations to identify the best combination partners, or even in the future Companion Diagnostics and associated molecular biomarkers. However, these applications would greatly benefit from new PKPD Population Models that integrate these biomarkers. However, these applications would greatly benefit from new PKPD Population Models that integrate these biomarkers. However, these applications would greatly benefit from new PKPD Population Models that integrate these biomarkers. However, these applications would greatly benefit from new PKPD Population Models that integrate these biomarkers. However, these applications would greatly benefit from new PKPD Population Models that integrate these biomarkers.