Novel bioassay predicts cancer patients' response to immunotherapy

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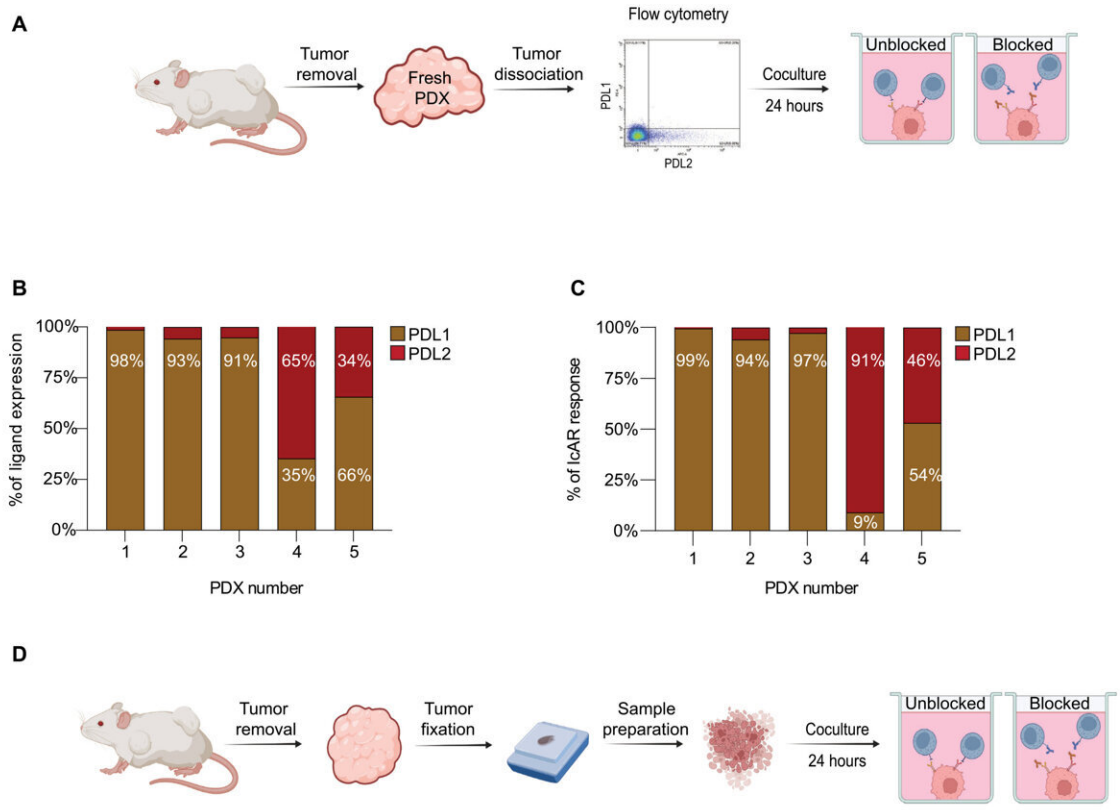

IcAR response to fresh and fixed PDXs and generation of an IcAR score.(**A**) Schematic representation of the IcAR assay protocol. Following flow cytometry determination of surface expression levels of PDL1 and PDL2 and coexpression of PDL1 and PDL2 (double) in fresh PDXs (extracted from mice), the IcAR functional assay was performed on both unblocked ligands and ligands blocked using anti-PDL1, anti-PDL2, and anti-PD1 antibodies. (**B**) PDL1 and PDL2 levels were determined in five PDXs, and the assay results are presented as percent of surface expression of each ligand and of coexpression (double). (**C**)

Coculture with IcAR-PD1 cells was performed for each PDX using anti-PD1 (pembrolizumab), anti-PDL1 (durvalumab), and anti-PDL2 antibodies; results are displayed as percent of response to each ligand out of the total IcAR response (unblocked). (**D**) Coculture with IcAR-PD1 cells was performed for each FFPE tissue sample using anti-PD1, anti-PDL1, and anti-PDL2 antibodies; results are displayed as percent of response to each ligand out of the total IcAR response (unblocked). (**E**) Correlation between IcAR response (namely, IL-2 levels) from fresh and fixed PDXs ($n = 5$). (**F**) Example of surface coverage of a well, measured with a JuLI Stage cell recorder, to produce normalized response per surface area. (**G**) Different PDXs give different scores, depending on the functionality of binding of each ligand (**P