# Automated 42 PDX 3D *In Vitro* Tumor Models of the TME Screen Immuno-Oncology and Targeted Compounds and Biologics for Antitumor Effects and MOA

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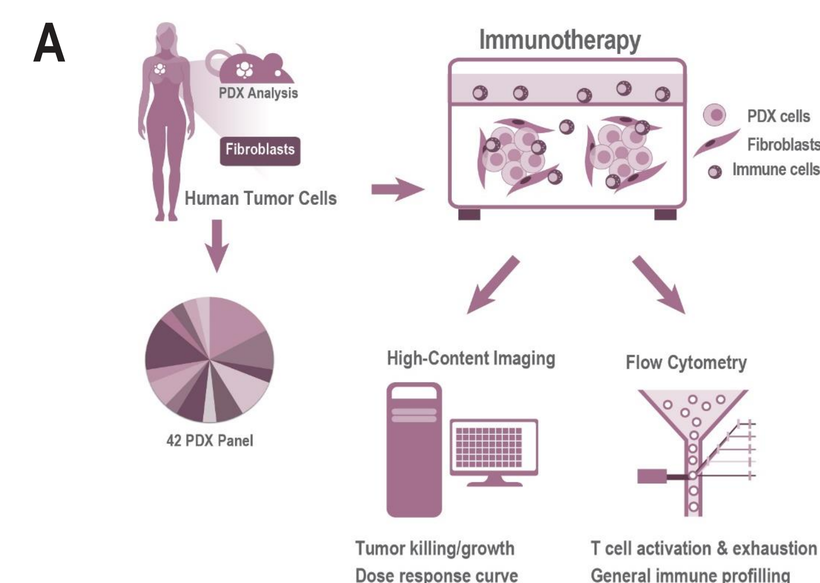
## 1 ABSTRACT

High throughput screening has been widely utilized for its efficiency in testing novel therapeutic agents. However, the conventional screening using 2D culture of cancer cell lines lacks the biological complexity of the tumor microenvironment or the immune compartment which limits their application in testing immune-oncology therapies. Particularly with the passing of the FDA Modernization Act 2.0, it is critical to create more biologically relevant 3D *in vitro* models that can be used to reduce, refine and ultimately replace preclinical animal models. Here we have developed a highly automated 3D high throughput screening platform comprised of 42 patient-derived xenograft models in coculture with fibroblasts in engineered extracellular matrix hydrogels that resemble tumor stromal microenvironment. The PDX models cover a diverse range of tumor and histological types including NSCLC, CRC, gastric, TNBC, RCC, bladder, ovarian, melanoma, pancreatic, and others. Moreover, PBMCs or other immune cells can be incorporated into the system for evaluating immuno-oncology drugs. The panel has been tested against chemotherapy drug cisplatin and the immunotherapy biologic, Solitomab, an EpCAM/CD3 bispecific antibody. The endpoint analyses for antitumor effects include the dose response of tumor size and tumor cell death percentage based on high content imaging analysis. In addition, immune cells and supernatant in the IO assay can be recovered from the wells and used for flow cytometry profiling and cytokine assays. In summary, the novel 42-PDX panel described here offers a unique and powerful tool for rapidly generating preclinical data and a better understanding the drug activity at the pharmacology stages, opening the door for faster and more human relevant drug discovery for cancer patients.

## 2 PLATFORM

A




B

| Cancer Type | Abbr. | Models |
|---|---|---|
| Bladder | BXF (2) | 1036, 1352 |
| Colon | CXF (4) | 94, 243, 269, 1103 |
| Gastric | GXA or GXF (4) | 251, 3023, 3067, 3080 |
| Head neck | HNXF (1) | 1853 |
| Liver | LIXAH (1) | 575 |
| Lung (NSCLC, adeno) | LXFA (7) | 289, 526, 586, 629, 923, 1647, 2184 |
| Lung (NSCLC, epidermoid) | LXFE (1) | 66 |
| Lung (NSCLC, large cell) | LXFL (3) | 430, 529, 1674 |
| Breast | MAXF (1) | 401 |
| Melanoma | MEXF (5) | 622, 1539, 1792, 1829, 2106, |
| Ovarian | OVXF (2) | 899, 1023 |
| Pancreatic | PAXF (4) | 546, 1997, 2005, 2035 |
| Mesothelioma | PXF (1) | 698 |
| RCC | RXF (4) | 486, 1781, 2282, 2516 |
| Sarcoma | SXFO (1) | 678 |
| Uterus | UXF (1) | 1138 |

**A)** A seamless workflow for growing 3D patient-derived tumors models and assaying immunotherapy drugs. Assay readouts include tumor growth/killing assay using high-content imaging analysis and T cell activation & exhaustion and general immune profiling using flow cytometry. **B)** A fixed panel of 42-3D tumor models using low passage, PDX-derived cell lines across major histotypes. * Visit https://compendium.criver.com/ to learn the PDX lines in the panel.

## 5 SUMMARY

- Cypre's 3D tumor model platform is automated for generating high-throughput screening data in a 96-well format. The present study showcases a panel of 42 independent solid tumor PDX models for efficacy and MOA readouts via high content imaging and flow cytometry.
- The models mimic the human tumor microenvironment by coculturing patient-derived (PDX) tumor cells, human fibroblasts, and human PBMCs in an engineered extracellular matrix hydrogel.
- Interestingly, NK, macrophages and Tregs are preserved in the models.
- Solitomab screening revealed a dose-dependent increase in CD25+, CD69+, PD1+ and HLA-DR+ T cells. A range of responders / partial / non-responders were enumerated.
- The combination of Nivolumab and Ipilimumab displayed a synergistic tumor killing effect *in vitro* that strongly correlated with the *in vivo* PDX model, and further revealed an expansion of CD8+ T cells and secretion of proinflammatory cytokines like IFNg and TNFa.
- These data showcase the application of Cypre 3D tumor platform in predictive immuno-oncology drug screening for efficacy and MOA readouts.

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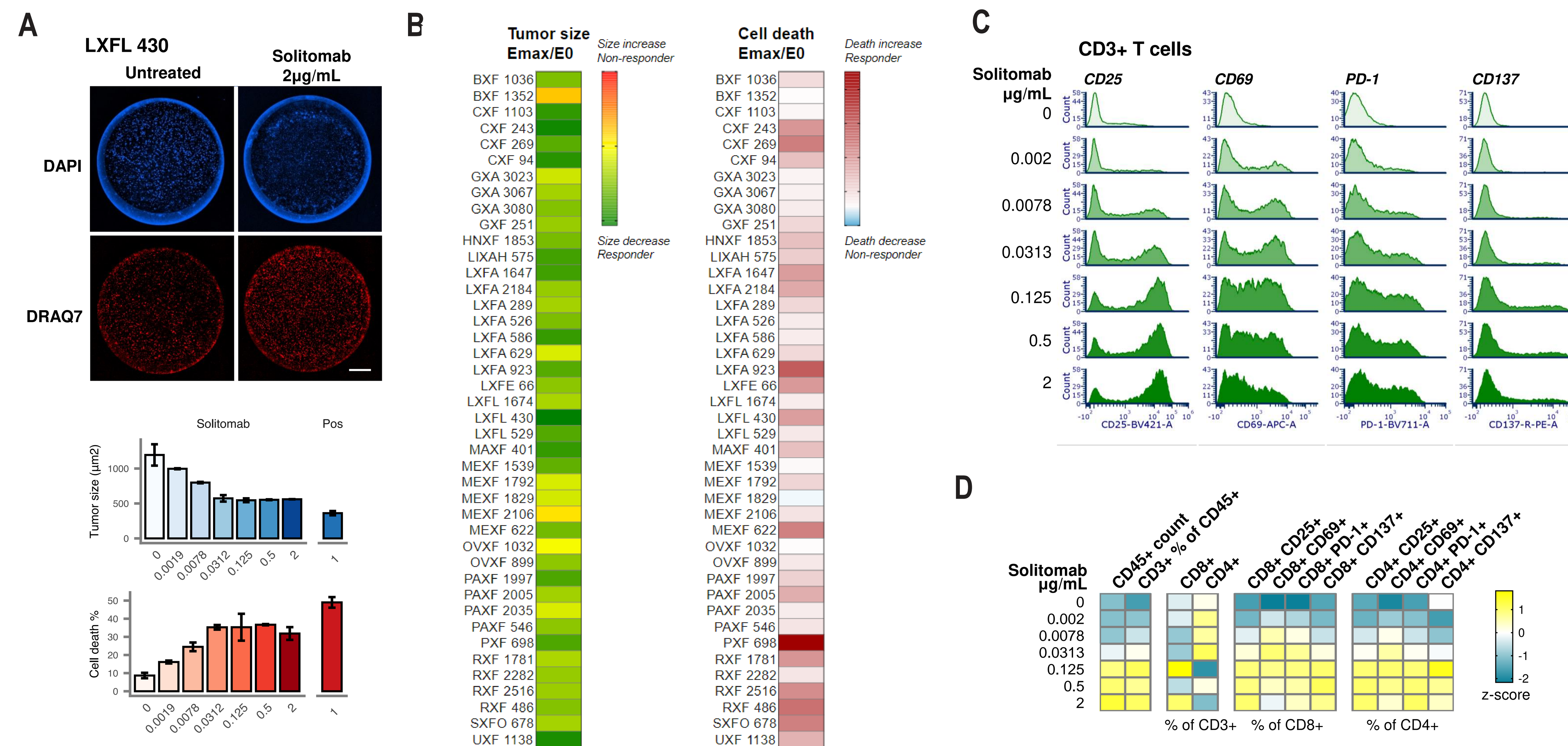
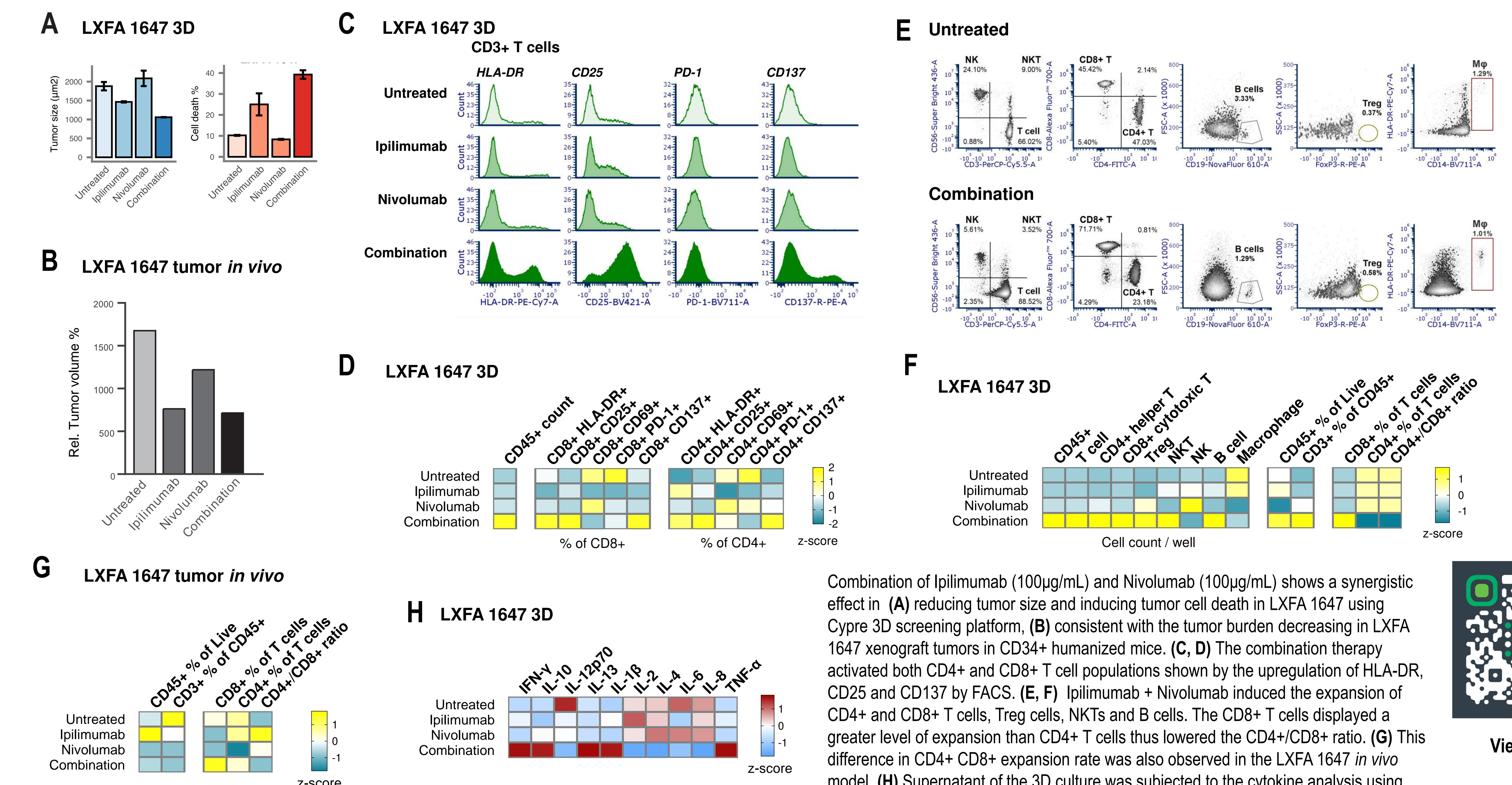
## 3 Dose response analysis of tumor killing and T cell activation & exhaustion by solitomab using the Cypre 42 PDX Panel of 3D tumor models




The Cypre 42 PDX panel were treated with allogenic PBMCs and 6 doses of solitomab and staurosporine 1μM as positive control. **(A)** The 3D culture were stained with Hoechst and DRAQ7 and subjected to high throughput image analysis. The representative 2x images of responder line LXFL430. scale bar = 500μm **(B)** Dose response profile of tumorsphere size and percentage of DRAQ7+ dead cells in LXFL 430 model treated with solitomab. **(C)** The efficacy of solitomab in all 42 PDX models was visualized by the heatmaps of tumor size and cell death Emax/E0 values. **(D)** Solitomab induced T cell activation and exhaustion in LXFL430 cells was shown by increase in CD25, CD69, CD137 and PD-1 expression by FACS. **(E)** Quantitation of T cell activation and exhaustion in CD4+ and CD8+ T cells by solitomab in a dose-dependent manner.

## 4 3D *in vitro* efficacy evaluation of Nivolumab-Ipilimumab combination in LXFA 1647 correlates with *in vivo* outcome; flow cytometry and cytokine analysis reveal mechanism of action.




Combination of Ipilimumab (100μg/mL) and Nivolumab (100μg/mL) shows a synergistic effect in **(A)** reducing tumor size and inducing tumor cell death in LXFA 1647 using Cypre 3D screening platform, **(B)** consistent with the tumor burden decreasing in LXFA 1647 xenograft tumors in CD34+ humanized mice. **(C, D)** The combination therapy activated both CD4+ and CD8+ T cell populations shown by the upregulation of HLA-DR, CD25 and CD137 by FACS. **(E, F)** Ipilimumab + Nivolumab induced the expansion of CD4+ and CD8+ T cells, Treg cells, NKTs and B cells. The CD8+ T cells displayed a greater level of expansion than CD4+ T cells thus lowered the CD4+/CD8+ ratio. **(G)** This difference in CD4+ CD8+ expansion rate was also observed in the LXFA 1647 *in vivo* model. **(H)** Supernatant of the 3D culture was subjected to the cytokine analysis using MSD proinflammatory panel. Secretion of IFN-γ, IL-10, IL-13, IL-1β and TNF-α was upregulated in the combination therapy.

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