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Application of MiniPDX® in clinical indication identification and anti-tumor drug development

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Abstract

MiniPDX® (Mini-patient-derived xenograft) is a novel, rapid (7~10days), and accurate method for drug sensitivity validation in-vivo. It can also be characterized as an in vivo version of an organoid assay. The testing material can be fresh patient tumor samples or tissues from established PDX models.

We have systematically evaluated and compared the response rates of MiniPDX® assays and PDX assays pairwise in 26 PDX models across 3 types of cancers to 12 clinically relevant regimens for chemical and targeting drugs. The results demonstrate a high correlation between drug responses of the two assays, with sensitivity of 80% and specificity of 93%. More and more studies have shown that MiniPDX® drug sensitivity test results are consistent with clinical responses in most patients, which indicates MiniPDX® models have great potential in guiding personalized medicine.

LIDE has successfully conducted over 3,000 MiniPDX® tests for clinical precision medicine, covering more than 50 indications. The application of MiniPDX® extends beyond therapeutic selection, also helping facilitate decision-making and prioritization of in the discovery pipeline for novel agents.

Importantly, the MiniPDX® Mouse Trial using fresh tumor samples generated from the clinic is beneficial for determination of potential clinical indications. Only thousands of fresh cells leftover from MiniPDX® preparation are sufficient to get genomic and transcription data by OncoVee ® K-cell technology. The combination of MiniPDX® assay and omics data would be very useful for determination of potential biomarkers to distinguish responders and non-responders in population with certain indication(s) and leveraged for patient stratification and selection criteria in clinical trial design.

In addition, we have developed a new version of MiniPDX® assay for immunotherapy called IO-FIVE (Immuno-Oncology Fast In Vivo Efficacy test). This assay uses fresh patient cancer cells with autologous immune cells to maximally mimic human tumor microenvironment before seeding in immune-deficient mice. IO-FIVE allows for the tracking of cellular viability and phenotype pre- and post-treatment, thus providing a robust metric of in vivo immuno-therapeutic efficacy within two weeks.

Materials and Methods

Tumor tissue acquisition: Fresh surgical tumor specimens were acquired from patients at participating hospitals. Tumor tissue acquisition was approved by the ethics committees of each participating hospital and agreed to by each patient via written informed consent and was carried out according to state and institutional regulations on experimental use of human tissues.

Establishing the PDX model: Fresh surgically removed gastric cancer, lung cancer and pancreatic cancer tissues were used for establishing PDX models. Tumor cells were subcutaneously implanted into immune-deficient mice as previously described and stably propagated for three passages.

Establishing the MiniPDX® model: We developed an in vivo drug sensitivity MiniPDX® assay by using a modified microencapsulation and hollow fiber culture system (Oncovee® MiniPDX® LIDE Biotech) according to the manufacturer's instruction. Tumors≥500 mm^3 in size with a necrotic area <30% were used. Briefly, tumor tissues were washed with Hank's balanced salt solution (HBSS) to remove non-tumor tissues and necrotic tumor tissue in a biosafety cabinet. After the tumor tissues were morselized, they were digested with collagenase at 37℃ for 1-4 h. Cells were pelleted by centrifugation at 600g for 5 min followed by removal of blood cells and fibroblasts with magnetic beads. Cells were then washed with HBSS and filled into OncoVee capsules. Capsules were implanted subcutaneously via a small skin incision with 3 capsules per mouse (5-week-old nu/nu mouse).

MiniPDX® drug sensitivity assays: Mice bearing MiniPDX® capsules were treated with appropriate drugs or their combinations for 7 days. Thereafter, the implanted capsules were removed and tumor cell proliferation was evaluated using the CellTiter Glo Luminescent Cell Viability Assay kit (G7571, Promega, Madison, WI, US), as instructed by the manufacturer.

Establishing the IO-FIVE model: a new version of MiniPDX® assay for immunotherapy (IO-FIVE, Immuno-Oncology Fast In Vivo Efficacy test). Briefly, patient derived tumor cells with autologous TILs (tumor infiltrated lymphocytes) or PBMCs were co-transferred into mini-capsules before embedding subcutaneously in immunodeficient mice. Immunophenotyping was carried out by Flow cytometry analysis before and after treatment (immunotherapy, target therapy or chemical therapy). A few thousand of cells in the mini-capsule samples were processed and analyzed by RNA-seq.

Results

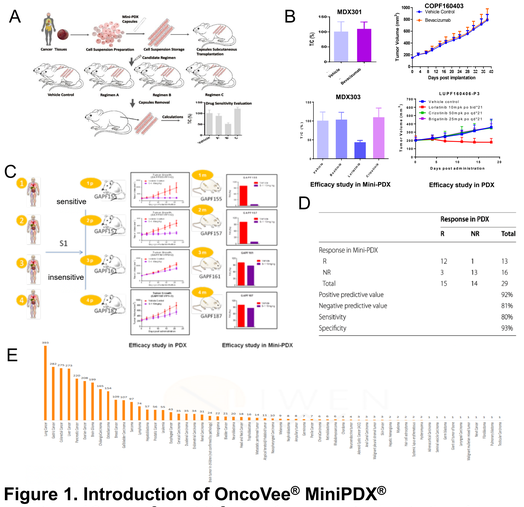

Figure 1. Introduction of OncoVee® MiniPDX®

A. Method of OncoVee® MiniPDX® Assay for rapid systemic detection of drug sensitivity in *vivo*.
B. MiniPDX® has a high degree of correlation with PDX efficacy
C. Representative results of pairwise efficacy tests in 4 PDX xenograft models against S-1 regimens. After the treatment, GAPF155 and GAPF1577 showed a marked decrease in tumor volume or cell viability but GAPF161 and GAPF187 did not.
D. Correlation response of MiniPDX® versus PDX assays in PDX models
E. LIDE has completed over 3,000 MiniPDX® tests for clinical precision medicine, covering more than 50 indications.

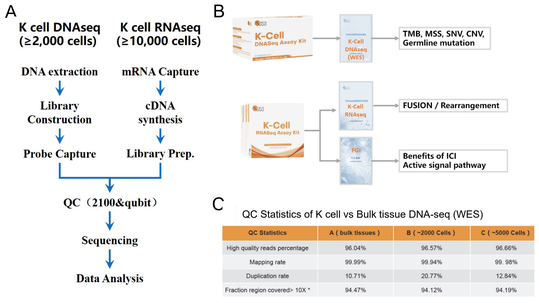

Figure 2. K-cell Assay Kit：Novel solution for RNA/DNA preparation in limited samples.

A. K-Cell Omics analysis refers to the application of WES (Whole Exome Sequencing) and RNAseq on small amount of sample (as few as thousands of cells) to obtain information related to clinical medication.
B. K-cell DNAseq is used to find driver gene variations and TMB/MSI status; K-CELL RNA-seq can detect the Fusion/Rearrangement of samples; Functional Genomic Imaging (FGI) helps judge patient benefit from immune checkpoint inhibitors (ICI) and detect abnormal gene expression or activation of signal pathways using gene signatures.
C. When compared with bulk tissue sample, inputs of 2K cells and 5K cells can achieve equivalent WES QC parameters (high quality reads percentage, mapping rate, and fraction region covered>10X).

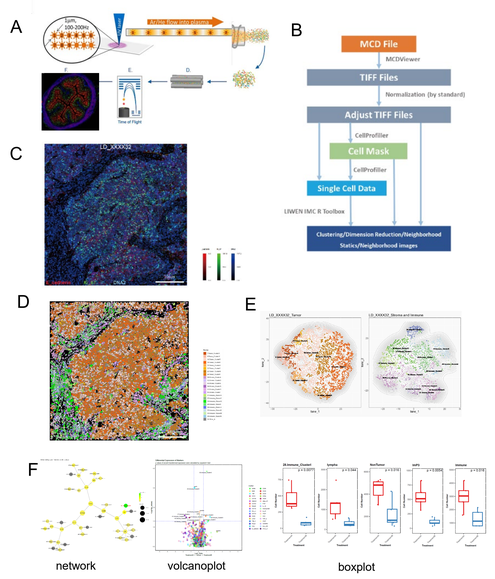

Figure 3. IMC: Simultaneously Detect over 30 markers on FFPE Samples

A. The Principle of Imaging Mass Cytometry(IMC).
B. LIDE's IMC data analysis pipeline: From raw images to expression, phenotype and spatial data at single cell level.
C. Morphological analysis: Show the expression of different markers in the sample, to explore the co-location and co-expression relationship between markers.
D. Subgroup tissue localization in situ: Reveal the spatial relationship between tumor, stroma and each immune subgroup.
E. Phenotypic analysis: Obtained subgroup composition of tissue samples.
F. Find subgroups and markers with significant differences (network, volcano plot, boxplot).

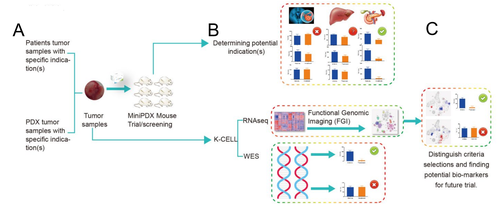

Figure 4. MiniPDX® Mouse Trial

A. Potential drug indications can be assessed using fresh patient tumors or established PDX models through MiniPDX® Mouse Trials.
B. RNA or DNA can be extracted and enriched from only thousands of cells using K-Cell technology from MiniPDX® preparations, enabling further RNAseq analysis via FGI.
C. Omics data help identify bio-markers to differentiate responders from non-responders in certain populations, aiding in patient stratification for clinical trial criteria confirmation of inclusive/exclusive criteria in clinical trials.

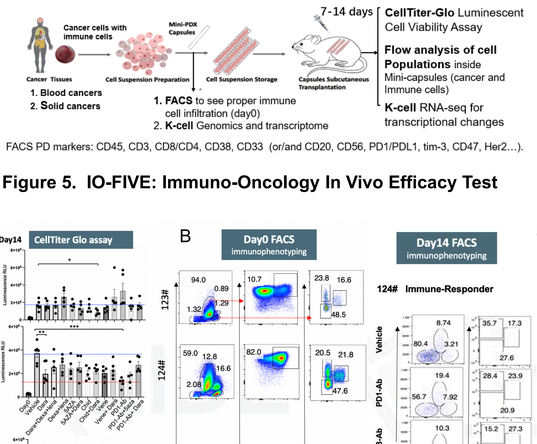

Figure 5. IO-FIVE: Immuno-Oncology In Vivo Efficacy Test

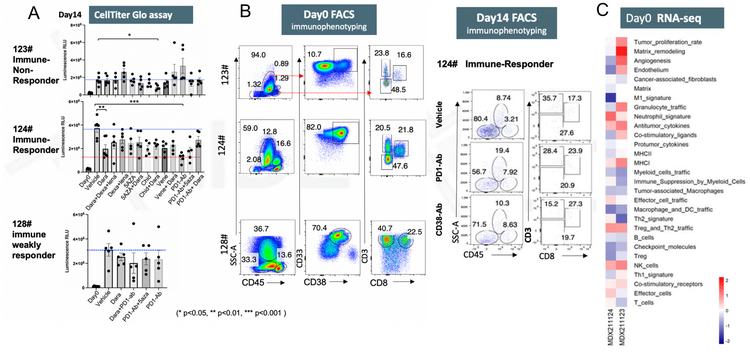

Figure 6. IO-FIVE for AML Case study of IO-FIVE assay in three AML (Acute myeloid leukemia) patients. A. CTG result of day0 and day14 post drug(s) treatment, including CD38(daratumumab), PD1 antibody(Sintilimab). B. Flow cytometry analysis on day0 and day14 for immunophenotyping. C. RNA-seq for the two AML samples of day0.

Summary and Conclusion

MiniPDX® is a proven technology with 30+ published supporting papers, demonstrating real world evidence of its benefit at clinic. After years of refinement and validation in China, LIDE is excited to bring the technology to the North American and European markets

Additionally, IO-FIVE is mainly used to test the function of immune-regulatory drugs as well as well as target drugs in tumor microenvironment. At present, several Investigator Initiated Trials (II-T) are on-going to further validate the correlation of IO-FIVE results to clinic endpoints.

References

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