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

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Abstract

MiniPDX® represents a novel in vivo drug sensitivity test with fast turnaround (7-10 days), using either fresh patient tumor samples or tissues from established PDX models. We systematically evaluated and compared the response rates of MiniPDX® assays and PDX assays pair-wise in 26 PDX models across 3 types of cancers to 12 clinically relevant regimens for chemical and targeting drugs.

The results showed a high correlation between drug responses of the two assays, with sensitivity and specificity of 80% and 93%, respectively. More and more studies showed that MiniPDX® drug sensitivity test results were consistent with clinical responses in most patients, which indicated MiniPDX® models have great potential in guiding personalized medicine.

LIDE has completed over 3,000 MiniPDX® tests for clinical precision medicine, covering more than 50 indications. MiniPDX® was used to rank clinically approved drugs or drug regimens to guide the selection of the best individualized treatment strategy. At the same time, screening other compounds facilitates selection of the best drug candidates for further R&D.

Importantly, performing MiniPDX® Mouse Trial using fresh tumor samples generated from 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 bio-markers in order to distinguish responders and non-responders in population with certain indication(s) and leveraged for patient stratification and selection criteria in clinical trial design.

Currently, we are developing a new version of MiniPDX® assay for immunotherapy (IO-FIVE, Immuno-Oncology Fast In Vivo Efficacy test) by using fresh patient cancer cells with autologous immune cells to maximally mimic human tumor microenvironment before seeding in immune-deficient mice. Total cell viability and cell phenotyping are tracked before and after drug treatment to evaluate its in vivo 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 (n=14), lung cancer (n=10) and pancreatic cancer tissues (n=2) 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°C 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

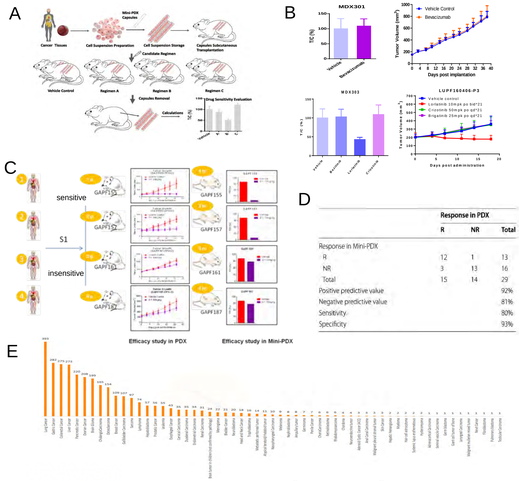

Figure 1. Introduction of OncoVee® MiniPDX®
A. Development of OncoVee® MiniPDX® Assay for rapid systemic detection of drug sensitivity in vivo.
B. MiniPDX® has a high degree of correlation with PDX efficacy.
C. Representative results of pairwise efficacy tests in 4 PDX xenograft models against S-1 regimens. 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.

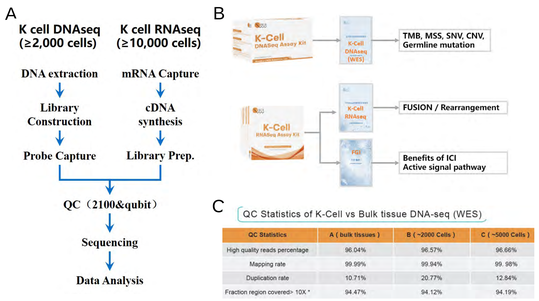

Figure 2. K-cell Assay Kit：Novel solution for RNA/DNA preparation in limited samples.
A. K-Cell Omics analysis refers to the application of WES (Whole Exome Sequencing) and RNAseq on small amount of sample (as few as thousands of cells) to obtain information related to clinical medication.
B. K-cell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

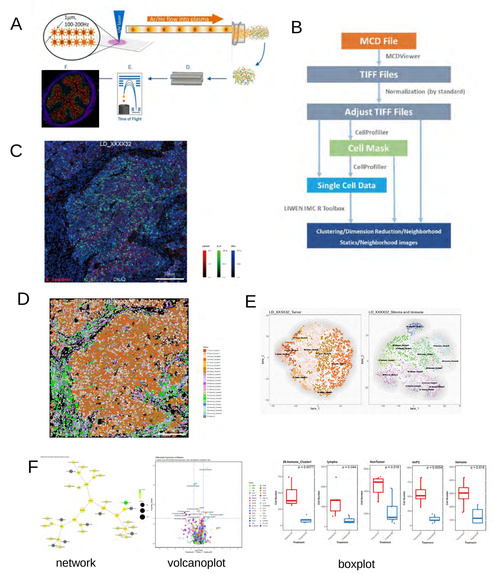

Figure 3. Imaging Mass Cytometry (IMC) analysis: FFPE detection in a high-throughput manner.
A. Workflow of Imaging Mass Cytometry.
B. IMC data analysis process.
C. Morphological analysis: Show the expression of different markers in the sample, to explore the co-location and co-expression relationship between markers.
D. Subgroup tissue localization in situ: Reveal the location relationship between tumor, stroma and each immune subgroup.
E. Phenotypic analysis: Obtained subgroup composition of tissue samples.
F. Find subgroups with significant differences (network, volcano plot, boxplot).

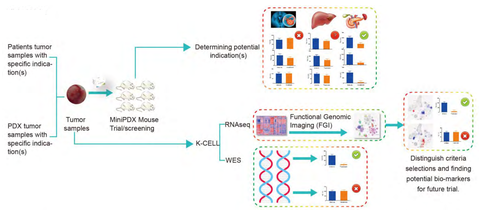

Figure 4. MiniPDX® Mouse Trial: Using either fresh tumor samples directly from patients or tumor from established PDX models, potential clinical indications of candidate drugs can be determined via MiniPDX® Mouse Trial. RNA or DNA can be extracted and enriched from only thousands of cells left from MiniPDX® preparation by K-Cell technology, while RNAseq can be further analyzed via FGI. The Omics date would be useful for determination of potential bio-markers in order to distinguish responders vs. non-responders in population with certain indications and further applied in patient stratification to confirm inclusive/exclusive criteria in clinical trials.

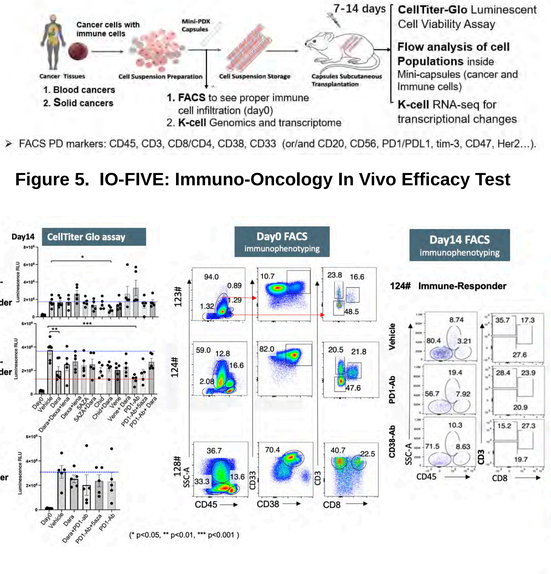

Figure 5. IO-FIVE: Immuno-Oncology In Vivo Efficacy Test

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

Summary and Conclusion

MiniPDX® is a proven technology with 20+ 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 result to clinic endpoint.

References

1. Feifei Zhang, et al. Characterization of drug responses of mini patient derived xenografts in mice for predicting cancer patient clinical therapeutic response. Cancer Communication.2018 Sep 26;38(1):60
2. Ming Zhan, et al. Guided chemotherapy based on patient-derived mini-xenograft models improves survival of gallbladder carcinoma patients. 2018 Jul 17;38(1):48.
3. Yunke Huang, et al. A Novel, Personalized Drug-Screening System for Platinum-Resistant Ovarian Cancer Patients: A Preliminary Clinical Report. 2021 Mar 29:13:2849-2867.

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