GenenDesign

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Accelerating biomarker discovery for companion diagnostics through mouse trials with PDX models in clinical settings

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Background

Current issues in precision medicine and companion diagnostics development

- Difficulties in predictive biomarker discovery
  - Lack of high-quality and well controlled tumor samples
  - Lack of sufficient drug response and genomic information of clinical samples
- Difficulties in biomarker validation
  - Difficulties to repeat experiments and test hypothesis in clinical settings
  - Long and costly process for validating biomarkers in clinical trials

Biomarker discovery with PDX models as support

- PDX models better resemble human cancer tissues than cancer cell line xenograft models in tumor heterogeneity and hierarchy
- Large number of PDX models provide better coverage of cancer complexity
- Simultaneous testing of multiple drugs and combinations
- Reproducibility and flexibility
- Convenient tissue collection for high sample quality and reliable analysis
- Quick data acquisition and significant cost reduction

GenenDesign PDX Platform

GenenDesign PDX tumor models have been established by serial passage of surgically removed or biopsy human tumors in immunodeficient mice and display a diverse range of inter- and intra-tumor complexity and heterogeneity in histopathology. The majority of GenenDesign PDX tumor models represent cancer types that are prevalent in Asian patients, including gastric cancers, lung cancers, liver cancers, esophageal cancers, and colorectal cancers (Table 1). Efforts are also made to derive acquired resistance PDX models under continuous drug treatment (Table 2).

Table 1 PDX model summary

Tumor Origin	Established (>P3)	Early passage (P1-P2)	Total
Stomach	219	1	220
Liver	59	9	68
Pancreas	68	0	68
Esophagus	209	19	228
Colon	97	6	103
Lung	152	38	190
Breast	3	15	18
Ovarian	14	5	19
Gall Bladder	8	0	8
GIST	10	0	10
Others	24	9	33
TOTAL	863	102	965

Table 2 Acquired resistance PDX model summary

Drug	Target	Model Type	Acquired Resistance
Herceptin	Her2 amplification	Stomach	6
Lapatinib		Stomach	6
AZD4547	FGFR2 amplification	Stomach	5
		Liver	3
BGJ398		Stomach	6
Crizotinib	c-Met amplification/ ALK fusion	Stomach	7
		Lung	1
		Liver	1
XL184	c-Met amplification	Liver	2
		Lung	1
Iressa/Tarceva	EGFR mutation	Lung	4
Selumetinib	MEK (K-RAS mutation)	Lung	2
Paclitaxel	N/A	Stomach	6
		Lung	22
		Esophagus	3
Docetaxel	N/A	Stomach	1
		Lung	7
FOLFOX	N/A	Stomach	13
Cisplatin	N/A	Lung	7
Carboplatin	N/A	Lung	1
Total			104

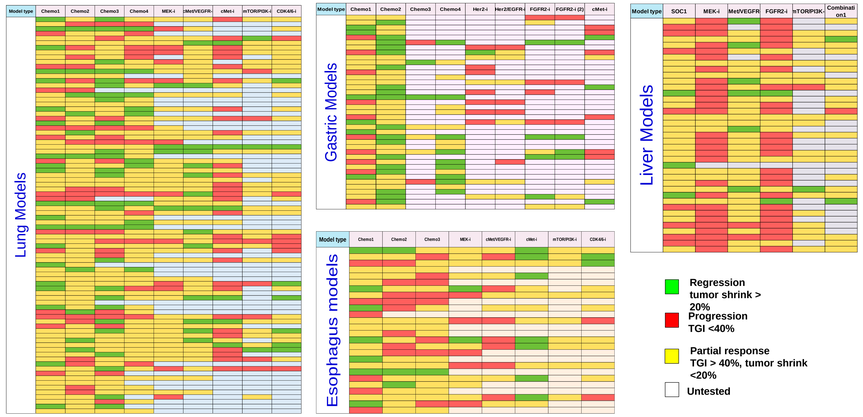
Molecular Characterization of PDX Tumor Models

Table 3 Molecular Profiling Platform at GenenDesign

Methods	Profiling Targets
Exome-Seq (Illumina Hi-Seq, Agilent 51 Mb, 120x)	DNA mutations and insertion/deletions
RNA-Seq (Illumina Hi-Seq, PE 90bp, 50 million reads)	RNA expression, fusion, and alternative splicing
SNP microarray (Affy SNP 6.0)	DNA copy number variations (CNV)
Gene expression microarray (Affy U133 plus 2.0)	mRNA expression levels
Advanced Cell Diagnosis RNAscope *in situ* assay	mRNA expression in individual cells
Others (PCR sequencing, western, qPCR, FISH, IHC)	Hot spot mutations, gene expression and amplification

Functional Characterization of PDX Tumor Models

Figure 1 Representative Functional Data Panels for Biomarker Discovery



Model Type	Drug	Model selection	Complete and ongoing studies
PDX gastric	Vehicle	all gastric cancer models	58
	Herceptin	Her2 amplification models	29
	Lapatinib	Her2 amplification models	27
	AZD4547	FGFR amplified or highly expressed	20
	BGJ398	FGFR amplified or highly expressed	20
	Crizotinib	c-Met, ALK amplified or highly expressed	27
	Palbociclib	all gastric cancer models	22
	PF04691502	all gastric cancer models	5
	FOLFOX	all gastric cancer models	58
	Paclitaxel	all gastric cancer models	54
	Combination	potential resistance models for single arm therapy	28
PDX lung	Vehicle	all lung cancer models	109
	Iressa / Tarceva	EGFR mutant	9
	Afatinib	EGFR mutant	1
	Erbitux	EGFR overexpression	24
	AZD4547	FGFR amplified or highly expressed	10
	BGJ398	FGFR amplified or highly expressed	11
	Selumetinib	all lung cancer models	87
	Crizotinib	all lung cancer models	83
	Cabozantinib	all lung cancer models	79
	Palbociclib	all lung cancer models	79
	PF04691502	all lung cancer models	62
	Paclitaxel	all lung cancer models	105
	Docetaxel	all lung cancer models	91
	Cisplatin	all lung cancer models	96
	Carboplatin	all lung cancer models	83
	Combination	potential resistance models for single arm therapy	69

Model Type	Drug	Model selection	Complete and ongoing studies
PDX liver	Vehicle	all liver models	42
	Sorafenib	all liver models	40
	Crizotinib	all liver models	40
	XL184	all liver models	40
	AZD4547	all liver models	42
	PF04691502	all liver models	20
	Combination	potential resistance models for single arm therapy	32
PDX esophageal	Vehicle	all esophageal models	28
	Crizotinib	c-Met amplified or highly expressed	24
	XL184	c-Met amplified or highly expressed	24
	AZD4547	FGFR amplified or highly expressed	5
	Selumetinib	all esophageal models	24
	Palbociclib	all esophageal models	13
	PF04691502	all esophageal models	11
	Paclitaxel	all esophageal models	28
	Cisplatin	all esophageal models	25
	FOLFOX	all esophageal models	26
PDX CRC	Vehicle	all CRC models	15
	Selumetinib	all CRC models	14
	Erbitux	EGFR overexpression	11
	Palbociclib	all CRC models	14
	Irinotecan	all CRC models	14
	Oxaliplatin	all CRC models	13
	FOLFORI	all CRC models	14

Through our in-house efforts, PDX models of different tumor types were tested with relevant SoC and clinical candidates in a **"biomarker-driven"**, **multi-drug/multi-arm** clinical trial setting. So far, more than 1500 data sets have been generated.

Organization of Drug Tests into Mouse Trials

Figure 2 Master Mouse Trial Scheme
- One cancer type tested by multiple drugs

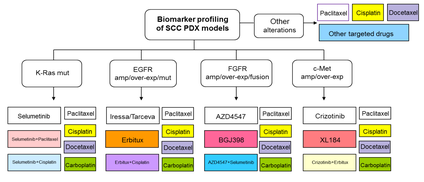


Figure 3 Basket Mouse Trial Scheme
- Multiple cancer types tested by one drug

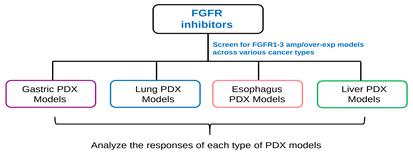


Table 5 Functional Data Panels by Cancer Types

PDX Model Type	Content	# of Data Sets
Lung	Drug response data sets from treatment with chemotherapies and targeted drugs	750
Liver		250
Gastric		250
CRC		150
Esophagus		100

Table 6 Functional Data Panels by Oncology Targets

Oncogene Target	Cancer Type	Data Sets
c-Met	Lung, Liver, Gastric, Esophagus	250
K-Ras	Lung, Esophagus, CRC	150
FGFR	Lung, Liver, Gastric, Esophagus	100
MAPK pathway	Gastric	80
EGFR	Lung, CRC	60
HER2	Lung, Liver, Esophagus, CRC	60
Cell cycle regulators	Lung, Liver, Esophagus, CRC	60

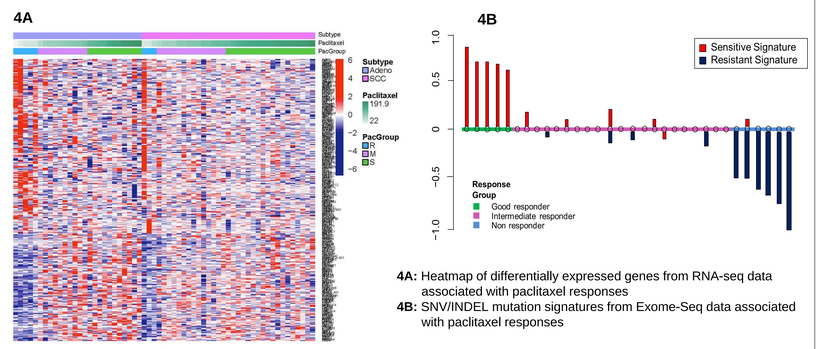
All data presented here were generated from GenenDesign internal studies.
The number of data sets were calculated as of Oct. 2015.

Analysis of Combined Genomic Profiles and Drug Response Data for Biomarker Discovery

Table 7 Selected biomarkers associated with targeted drug treatments

Targeted Therapy	Cancer Type	Identified Senitive Biomarkers in PDX models	Identified Resistant Biomarkers in PDX models
Herceptin	Gastric	Her2 amp/over-exp	c-Met amp/over-exp; PTEN deletion; FGFR2 amp; CCNE1 amp; EGFR over-exp
Crizotinib	NSCLC	ALK fusion; c-Met amp	ALK mut; EGFR over-exp; K-Ras mut
AZD4547 / BGJ398	Liver, Gastric, Esophagus	FGFR2 amp/over-exp; FGFR1/3 highly over-exp	FGFR2 gatekeeper mut; certain miRNAs; IGF1R amp; HER2 amp
Iressa / Tarceva	Lung	EGFR exon 19 deletions; EGFR L858R substitution mutations	EGFR T790M; K-Ras mut; c-Met amp; HGF over-exp
Erbitux	Lung, CRC	EGFR over-exp	K-Ras mut; c-Met amp; HGF over-exp
Selumetinib	Lung, Esophagus, CRC	K-Ras mut; PI3KCA mut;	PKA over-exp; TAF3 over-exp; RXRA over-exp
Palbociclib	Lung, Gastric, Esophagus, CRC	CDKN2A deletion; CCND1 amplification; Over-expression of RB	RB deletion; Over-expression of p16; CCNE1 amp
PF04691502	Lung, Liver, Gastric, Esophagus	ongoing	ongoing

Figure 4 Genomic and Drug Response Analysis of Chemotherapy Treatment NSCLC Tumors



4A: Heatmap of differentially expressed genes from RNA-seq data associated with paclitaxel responses
4B: SNV/INDEL mutation signatures from Exome-Seq data associated with paclitaxel responses

Table 8 Selected Biomarkers Associated with Paclitaxel and Cisplatin Treatments in NSCLC

Chemo-therapy	Cancer Type	Top Pathways Associated with Chemo-Response	Selected Biomarkers Associated with Chemo-Resistance
Paclitaxel	NSCLC	Microtubule formation, Mitotic checkpoints, PI3K/AKT, Apoptosis, Membrane transportation, DNA repair	KRT18(CK18), AKT3, TUBB3(beta3-tubulin), CXCL8(IL-8), LDHA
Cisplatin	NSCLC	Membrane transportation, PI3K/AKT, Cell Death, Cell cycle arrest, DNA repair, EMT and Epigenetic pathways	ERCC1, RRM1, CCL26(Chemokine ligand 26), SIRT1(Sirtuin 1), ATF2

Summary

1. PDX models can be characterized and classified at pathological, molecular and functional levels.
2. Paired genomic profiles and functional data from mouse trials provide rich information for accelerated biomarker discovery.
3. Predictive biomarkers associated with targeted drug responses previously identified from clinical studies were also found in our PDX model studies.
4. Predictive biomarkers associated with chemotherapy responses were also identified from bioinformatics analysis and can be potentially used for chemotherapeutic treatment selection.

References

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