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Characterization of a PDX panel covering molecular diversity of non-small cell lung cancer to accelerate the development of precision therapy

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Introduction

Lung cancer remains the first cause of cancer-related deaths worldwide, of which Non-Small Cell Lung Cancer (NSCLC) represents more than 80% of patients with advanced disease at the time of diagnosis. NSCLC is a highly heterogenous disease, and the identification of its main actionable oncogenic drivers (i.e. EGFR, ALK, PI3K/AKT/mTOR, RET, MET, BRAF and NTRK/ROS1) and the development of specific inhibitors against these targets has transformed therapeutic care.

Despite these new therapeutic options, NSCLC remains a lethal disease in the majority of patients due to tumor plasticity and selection leading to frequent resistance development and disease progression. Efforts are therefore needed to identify drugs and drug combinations that can prevent or overcome these resistance pathways.

Patient-Derived Xenografts (PDX) models developed in immune-compromised mice recapitulate the disease more faithfully than any other *in vivo* model in terms of histopathologic and genomic features. They have proven their relevance in the study of pathways leading to the development and progression of cancer, to the mechanisms linked to tumor resistance and to the identification of novel effective therapies, facilitating the translation of preclinical results in the clinical setting.

We describe a platform of over 38 NSCLC PDX models covering most of the molecular diversity of the disease, that have been fully characterized at the molecular level and for their response to a panel of cytotoxic chemotherapies and targeted therapies.

Materials & Methods

PDX model establishment and *in vivo* assays:
NSCLC PDX models have been established in immunodeficient mice from tumor biopsies collected in treatment-naïve patients or in patients having acquired resistance following an initial objective response to a variety of targeted inhibitors (EGFRi, ALKi, ROSi, BRAFi,…) in the MATCH-R clinical trial. In addition, 4 PDX models were established from circulating tumor cells (CTC) isolated from the blood of advanced NSCLC patients.
Molecular analyses were done included gene expression (RNASeq), copy number variation and whole exome sequencing. *In vivo* drug efficacy assays were performed with standards of care as single agent. Cetuximab; EGFRi (Afatinib, Erlotinib; Osimertinib); Selumetinib; Lorlatinib; Cisplatin; Docetaxel.
LCX-001-BAH and LC-F-26 models were established in highly immunodeficient mice humanized with human PBMCs and CD34+ hematopoietic stem cells.

Cellular model derivation and *in vitro* assay:
PDX-derived cell cultures were obtained from tumors explanted from mice and isolated by mechanical and enzymatic dissociation and maintained in culture at 37°C in a humidified atmosphere with 5% CO2 in complete growth medium.
Cells are maintained in culture in our XT Advanced medium (DMEM/F12 with 1% Penicillin-Streptomycin (10,000 U/mL), 2mM L-Glutamine, Insuline-Transferrin-Selenium 1X, Albumax II 0.4g/L and 8% FCS with or without Rho-associated kinase (ROCK) inhibitor, Y-27632 supplementation.
For ATP content measurement (CellTiter Glo® assay kit), cells were seeded in 96-well plates at a density of $1.25.10^3$ to 5.10^3 cells/well. Cells are incubated 48h at 37°C prior to the addition of Osimertinib. Cell viability is assessed before drugs' addition (T0) and 5 days after test molecules addition.

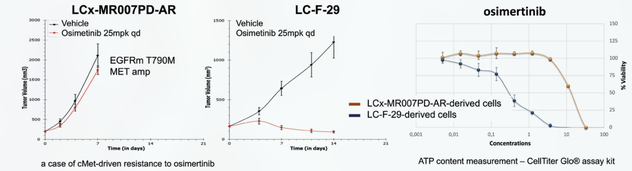
PDX engraftment on humanized mice:
Some of our NSCLC PDX were engrafted on PBMC or CD34+ stem cells humanized mice. For PBMC humanized mice, PDX were engrafted before injection of PBMC from 2 different donors (2.10^6 IP). For CD34+ humanized mice, 75.10^3 CD34+ cells were injected IV in NXG mice and 15 to 18 weeks later, PDX were engrafted only on mice presenting at least 25% of human cells in blood.
Tumor-Infiltrating Lymphocytes were purified from tumors using Tumor Dissociation Kit and CD45 (TIL) MicroBeads from Miltenyi. After antibodies staining, cells were acquired on a MACSQuant® Analyzer 10 Flow Cytometer and analyses were performed using FloLogic™ software.

NSCL cancer-derived xenograft panel and derived cellular models

	Origin	Histological sub type	Smoker status	Patient's Tumor marker status	PDX Key mutations	Cetuximab	EGFRi [2]	Selumetinib	Lorlatinib	Cisplatin	Docetaxel	PDX-DC 3 available
LC-F-12	Primary	Adenocarcinoma	No Smoker	EGFR (L858R)	EGFR; PIK3CA	NR	R	NR	/	/	/	Yes
SC131	Metastasis	Adenocarcinoma		KRAS (G12V)	EGFR; KRAS (G12V); ATM; STK11	NR	NR	/	/	NR	R	Yes
LC-F-04	Primary	Adenocarcinoma			TP53	NR	NR	NR	/	/	/	No
LC-F-09	Primary	Adenocarcinoma and Adenosquamous carcinoma	Smoker		TP53; PIK3CA	NR	NR	NR	/	/	/	No
LC-F-25	Primary	Adenocarcinoma			KRAS (G12C); TP53; ATM	/	NR	/	/	/	/	No
Lcx-MR007PD-AR	Primary	Adenocarcinoma	No Smoker	EGFR (L858R); MET amplification	EGFR; MET amplification	NR	NR		/	/	/	Yes
LC-F-29	Primary	Adenocarcinoma	Smoker	EGFR (G719A)	EGFR; TP53	NR	HR	NR	/	/	/	Yes
Lcx-MR131PD	Primary	Adenocarcinoma		EGFR (L858R and T790M); PTEN; TP53	EGFR; PTEN; TP53	/	HR	/	/	/	/	No
Lcx-MR228PD-AR	Metastasis		Smoker	EGFR (del ex19); TP53	EGFR; TP53	/	/	/	NR	/	/	No
Lcx-MR135PD2-AR	Metastasis			TP53; BRCA1; ATM	TP53	/	/	/	NR	/	/	No
Lcx-MR135PD	Primary	Adenocarcinoma		TP53, NF2	TP53	/	/	/	HR	/	/	No
Lcx-MR057PD	Primary	Adenocarcinoma	Smoker	ALK (C1156Y); TSC2		/	/	/	HR	/	/	No
Lcx-MR210PD	Primary			ALK (C1156Y)		/	/	/	HR	/	/	No
CTCx-LC-39	CTC isolated from patient blood	CTC 1			TP53	/	/	/	/	R	/	No
CTCx-LC-61	CTC isolated from patient blood	CTC 1			TP53; PTEN; RB1	/	/	/	/	R	/	Yes
CTCx-LC-58	CTC isolated from patient blood	CTC 1			TP53; RB1	/	/	/	/	NR	/	Yes
CTCx-LC-8	CTC isolated from patient blood	CTC 1			TP53; PTEN; BRCA2	/	/	/	/	NR	/	Yes
IC9LC11	Primary	Squamous Cell Carcinoma			TP53	R	/	/	/	NR	R	No
IC11LC13	Primary	Squamous Cell Carcinoma			CDKN2A; TP53	NR	/	/	/	NR	NR	No
IC1-TEP	Metastasis	Squamous Cell Carcinoma			BRCA2; CDKN2A; MLL2; TP53	HR	/	/	/	NR	NR	No
ML8LC9	Primary	Large Cell Carcinoma			MLH1; STK11	NR	/	/	/	NR	NR	No
IC20-DAN	Primary	Squamous Cell Carcinoma			TP53; PTEN; RB1; ALK; CDKN2A	R	/	/	/	NR	HR	Yes
IC8LC10	Metastasis	Adenocarcinoma			TP53; CDKN2A; MLL3;MSH2	/	/	/	/	NR	/	Yes
ML1LC2-MAR	Primary	Large Cell Carcinoma		PI3K (E545K)	NF1; PIK3CA; RB1; TP53	NR	/	/	/	HR	R	No
IC14LC18	Metastasis	Cystic serous papillary adenocarcinoma			PI3KCA; RB1; TP53;	/	/	/	/	/	R	No
ML5LC66	Primary	Adenocarcinoma		PI3K (E545K)	TP53; RB1; PIK3CA	/	/	/	/	/	NR	No
LC-F-01	Primary	Squamous Cell Carcinoma				/	/	/	/	/	/	No
LC-F-10	Primary	Large-cell neuroendocrin carcinoma	Smoker		TP53; PIK3CA	/	/	/	/	/	/	No
LC-F-11	Primary	Large cell carcinoma	Smoker			/	/	/	/	/	/	No
LC-F-13	Primary	Large cell carcinoma			TP53; BRCA2	/	/	/	/	/	/	No
LC-F-15	Primary	Adenocarcinoma	Smoker		KRAS (G12C); ATM	/	/	/	/	/	/	No
LC-F-17	Primary	Squamous Cell Carcinoma			TP53	/	/	/	/	/	/	No
LC-F-23	Primary	Squamous Cell Carcinoma			TP53	/	/	/	/	/	/	No
LC-F-26	Primary	Adenocarcinoma			PTEN; TP53	/	/	/	/	/	/	Yes
LC-F-31	Primary	Squamous Cell Carcinoma			PTEN	/	/	/	/	/	/	No
LCX-001-BAH	Primary	Adenocarcinoma			MSH6; TP53	/	/	/	/	/	/	Yes
LCx-MR320PD-AR	Metastasis		Smoker	TP53; BRAF (V600E)	TP53; BRAF; DNA-PK	/	/	/	/	/	/	No

1. Circulating Tumor Cells · 2. Afatinib or Erlotinib or Osimertinib · 3. PDX-derived cells
NR Non Responder (TGI < 58%) R Responder (90% > TGI > 58%) HR High Responder (TGI > 90%)

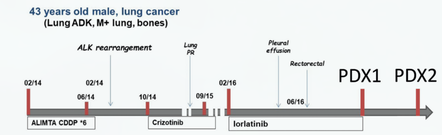
I Osimertinib responses in NSCLC PDX and derived cellular model



a case of cMet-driven resistance to osimertinib

ATP content measurement – CellTiter Glo® assay kit

II Different responses to Lorlatinib of PDX generated from the same patient



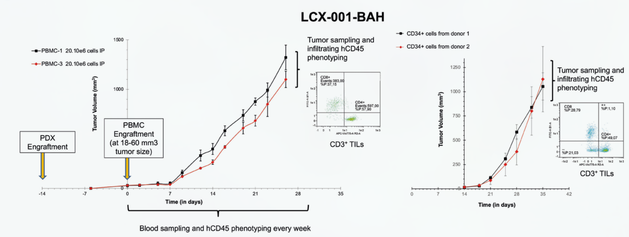
LCx-MR135PD

October 2016
Site : left lower lung lobe

LCx-MR135PD2-AR

June 2017
Site : right adrenal gland

III Engraftment of a NSCLC PDX on humanized mice and TILs phenotyping



Results

1. Two cellular models were derived from NSCLC PDX. LCx-MR007PD-AR was obtained from a MET-amplified and EGFR mutated (T790M) tumor which was non-responder to osimertinib. LC-F-29 was obtained from an EGFR mutated (G719A) tumor sensitive to Osimertinib. The *in vitro* assay is very well correlated with this *in vivo* sensitivity, since LC-F-29 cellular model was at least 20-fold more sensitive than LCx-MR007PD-AR (Fig. I).
2. Different sensitivities can be observed in PDX models developed from multiple metastases from the same patient. The use of matched models like these could facilitate the identification of relevant mechanisms of drug resistance (Fig. II).
3. The LCX-001-BAH NSCLC PDX model is able to grow on humanized mice. The growing tumors are infiltrated by human cells, assessed by TILs purification and phenotyping (Fig. III).

Conclusion & Perspectives

This panel of NSCLC PDX models provides a powerful preclinical platform to improve our knowledge on the mechanisms underlying resistance to treatment and to rapidly evaluate response to new treatments and translate this knowledge to the clinic. In addition to this PDX panel, we derived cellular models (PDX-DC) to off er a time- and cost-effective preclinical screening tool with good correlation with in vivo responses.
Engrafted on highly immunodeficient mice humanized with human PBMCs or CD34+ cells, these PDX models should facilitate bispecific T-Cell engager antibody testing or immune-checkpoint inhibitors evaluation.