Characterization of a panel of 79 PDX-derived cell lines with a focus on the EGFR exon 20 insertion mutation-driven NSCLC model LXFE 2478

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LB-B05

AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics, Oct. 26-30, 2019, in Boston, Massachusetts

1 INTRODUCTION

Patient-derived tumor xenograft (PDX) models have become indispensable for the preclinical profiling of novel anti-cancer agents as they retain histological, molecular and pharmacological characteristics of the parental patient tumors and for many tumor types collectively replicate the diversity of patient tumors. To make PDX models available for *in vitro* assays, we have established a panel of 79 low passage cell lines derived from PDXs representing 18 different tumor histologies. Using, among others, the non-small cell lung cancer model LXFE 2478 which is driven by the exon 20 insertion EGFR mutant M766_A767insASV as an example, we demonstrate similarities of PDX models and the corresponding PDX-derived cell lines.

Cancer type	Abbreviation (no. of models)	Models
Bladder	BXF (3)	1036, 1218, 1352
Central nervous system	CNXF (3)	498, 2599, 2611
Colon	CXF (5)	94*, 243, 269*, 280, 1103
Gastric (Asian)	GXA (5)	3011, 3013, 3023, 3067, 3080
Gastric (Caucasian)	GXF (2)	251, 1172
Head & Neck	HNXF (2)	1853, 1859
Liver (cholangiocarcinoma)	LIXFC (2)	575, 2050
Lung (NSCLC, adeno)	LXFA (11)	289, 526, 586, 623, 629, 677, 737, 923, 983, 1647, 2184
Lung (NSCLC, epidermoid)	LXFE (2)	66*, 2478
Lung (NSCLC, large cell)	LXFL (5)	430, 529, 1072, 1121, 1674
Mammary (triple negative)	MAXFTN (2)	401, MX1
Melanoma	MEXF (14)	274, 276, 394*, 462, 520, 535, 622, 1341, 1539*, 1737, 1792, 1829, 2090, 2106
Ovary	OVXF (2)	899, 1023
Pancreas	PAXF (8)	546, 1657, 1986, 1997, 1998, 2005, 2035, 2059
Pleuramesothelioma	PXF (3)	698, 1118, 1752
Renal Cell	RXF (7)	393, 486, 1183, 1220, 1781, 2282, 2516* **
Sarcoma (osteo)	SXFO (1)	678
Sarcoma (soft tissue)	SXFS (1)	1301
Uterus	UXF (1)	1138*

* corresponding PDX no longer available, ** established directly from patient tumor

Access to biological and clinical data for PDX-derived cell lines: https://compendium.criver.com/

Table 1. The Charles River Collection of PDX-derived Cell Lines

- Currently 79 PDX-derived cell lines (72 of them also available as PDX) have been established from tumors grown subcutaneously in nude mice.
- Authenticity was confirmed by unique STR profiles matching between PDX and the corresponding cell line.
- Cell lines were not subcloned, absence of mouse fibroblasts was confirmed by qRT-PCR.
- Characterization: molecularly (gene expression, gene copy no. alteration, WES mutation profile), immunohistochemically for selected proteins, histologically as subcutaneous xenografts in mice
- Starting from a master stock, cell lines are used for maximally 20 passages.

Reference for all discussed EGFR-targeting agents: Simon Vyse and Paul H. Huang, https://www.nature.com/articles/s41392-019-0038-9

2 RESULTS

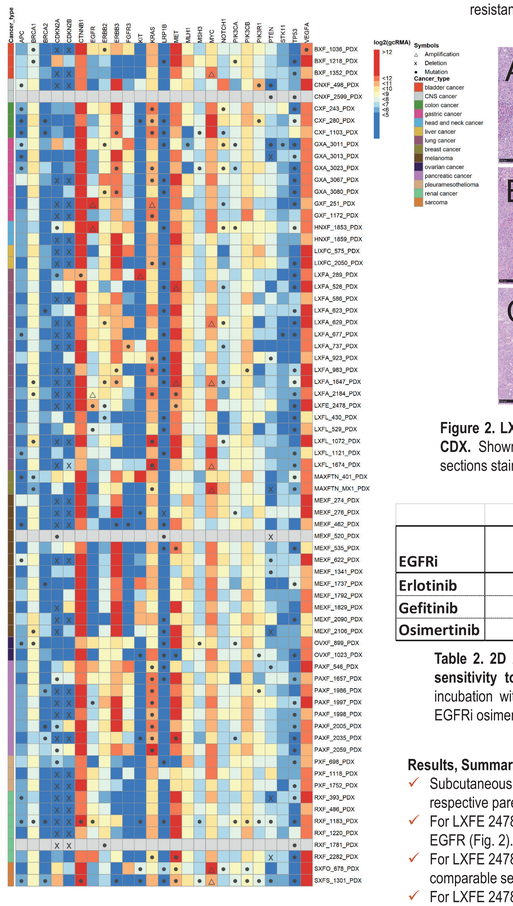

Figure 1. Status of 25 cancer-related genes in 71 PDXs Information on gene expression, gene copy number alterations and mutation status is given.

Non-small cell lung cancer model LXFE 2478 (available as PDX, CDX and cell line), profile

- Established from the brain metastasis of an adeno-squamous lung cancer of a 44-year-old female patient
- Carries a heterozygous EGFR exon 20 insertion mutation (M766_A767insASV)
- Sensitivity of the patient tumor to paclitaxel/cisplatin/cetuximab; resistance to radiotherapy, cisplatin/erlotinib/pemetrexed, PDL-1 antibody

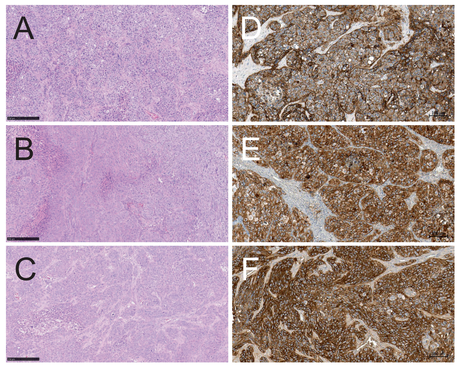

Figure 2. LXFE 2478: matching histology and EGFR expression of patient tumor, PDX and CDX. Shown are H&E stained sections of the patient tumor (A), the PDX (B) and the CDX (C) and sections stained with an anti-EGFR antibody (D, E, F).

		IC_{50} [µM]		
EGFRi	intended EGFR target (EGFRi generation)	LXFA 2478 (EGFR exon 20 ins. mut.)	GXF 251 (wt EGFR amp.)	LXFA 526 (wt EGFR)
Erlotinib	L858R, exon 19 del (1.)	3.70	0.280	30.0
Gefitinib	L858R, exon 19 del (1.)	3.83	0.469	13.5
Osimertinib	T790M (3.)	0.261	0.629	2.42

Table 2. 2D *in vitro* assay with PDX-derived cell lines: correlation of EGFR status and sensitivity to different EGFR inhibitors. Shown are the IC_{50} values after three days of incubation with the "first generation" EGFRi erlotinib and gefitinib and the "third generation" EGFRi osimertinib. *In vitro* and *in vivo* (Fig. 3 and data not shown) sensitivity profiles are similar.

Results, Summary

- ✓ Subcutaneous tumor xenografts established from PDX-derived cell lines mirror the histology of the respective parental PDX models and usually also of the patient tumors (Fig. 2).
- ✓ For LXFE 2478 both the PDX model and the corresponding PDX-derived cell line express high levels of EGFR (Fig. 2).
- ✓ For LXFE 2478 the PDX and the PDX-derived cell line subcutaneously implanted in mice display comparable sensitivity to a variety of EGFR inhibitors (Fig. 3) and cytotoxics (data not shown).
- ✓ For LXFE 2478 the sensitivity of the PDX-derived cell line to a variety of EGFR inhibitors in an *in vitro* 2D cell survival and proliferation assay in general corresponds to the EGFRi sensitivity of the corresponding PDX model *in vivo* (Table 2).
- ✓ For several additional PDX models carrying a wt EGFR gene and representing various histotypes, the sensitivity to "first generation" EGFR inhibitors of the PDX-derived cell lines in the 2D *in vitro* assay is in line with the *in vivo* sensitivity of the corresponding PDXs to EGFR-targeting agents (Fig. 4).

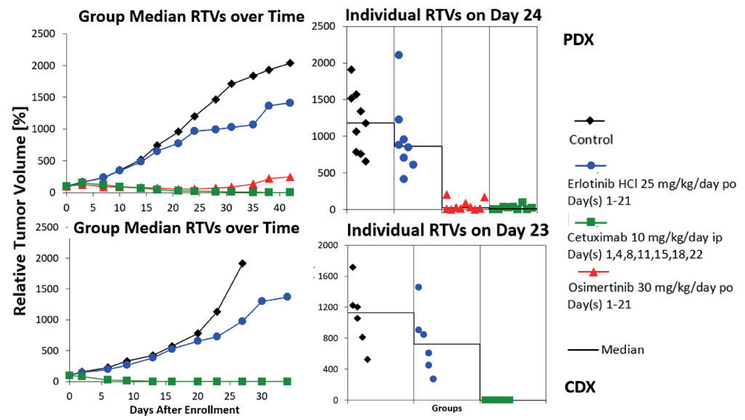

Figure 3. Matching *in vivo* sensitivities of LXFE 2478 PDX and CDX to EGFR-targeting agents. Plots of group median relative tumor volumes over time and of individual relative tumor volumes on days 23 or 24 are shown. Day 0 is the day of enrollment (tumor volume: 50-250 mm^3), day 1 is the first day of dosing.

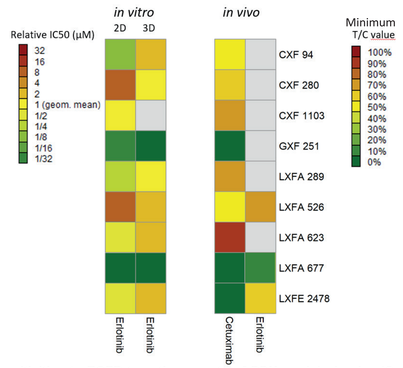

Figure 4. Matching sensitivities to EGFR-targeting agents of PDX models *in vivo* (Cetuximab, EGFRi) and *ex vivo* (3D, EGFRi) and of the corresponding PDX-derived cell lines *in vitro* (2D, EGFRi). Responses to cetuximab and "first generation" EGFRi erlotinib are tumor model-specific and typically match, irrespective of the assay format (data for 8 PDX models exhibiting wt EGFR with or without gene amplification). Notably, LXFE 2478, due to an EGFR exon 20 insertion mutation, displays differential sensitivities to cetuximab and erlotinib (see also Fig. 3).

3 CONCLUSION

As shown here for EGFR-targeting drugs, *in vitro* response data obtained with PDX-derived cell lines can predict the *in vivo* behavior of the corresponding PDXs and can thereby accelerate, and reduce the costs of, the discovery of novel anti-cancer agents.