Target Expression and Target Modulation Studies in Patient-Derived Tumor Xenografts Using a Bank of Protein Lysates

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Introduction

In this tech note, we present an efficient method that facilitates the preclinical analysis of antitumor compounds. By using specialized human tumor models and multiplex bead suspension assays, we are able to decide which tumor models to use for preclinical profiling of anticancer agents as well as identify biomarkers for specific drug response or resistance.

Methods

Protein Tumor Lysate Bank

By directly transplanting patient tumors subcutaneously into immune-compromised nude mice, more than 400 solid tumors, including all major human tumor types, have been established in serial passage (Fiebig et al. 1992, Fiebig and Burger 2001). At Oncotest, we have generated a protein tumor lysate bank consisting of 150 tumor models (Table 1).

Table 1. Xenografts selected for the protein tumor lysate bank (n = 150).

Bladder	4	Lymphoma	4	Pancreas	4
Colon	19	Mammary	14	Pleuramesothelioma	2
Gastric	4	Melanoma	12	Prostate	5
Head and neck	4	Non-small cell lung cancer (NSCLC)	33	Sarcomas	4
Kidney	6			Uterus	5
Leukemias	5	Small cell lung	6	Others	8
Liver	3	Ovarian	8		

Multiplex Bead Suspension Assays

Bead suspension assays are flexible bioassay systems that allow the parallel detection and quantitation of many targets in a single sample. For example, we can measure up to 28 cytokines and chemokines or 7 phosphorylated signal transduction proteins simultaneously. In a typical multiplex bead suspension assay, a target protein is captured from a crude cell lysate, tissue lysate, or serum with bead-bound antibodies. The total target protein amount and/or its phosphorylation status is quantitated with a secondary biotinylated antibody followed by an incubation step with streptavidin-phycoerythrin to complete a sandwich immunoassay. In this study, we used Bio-Rad's Bio-Plex multiplex suspension array system and Oncotest xenograft lysates (Figure 1).

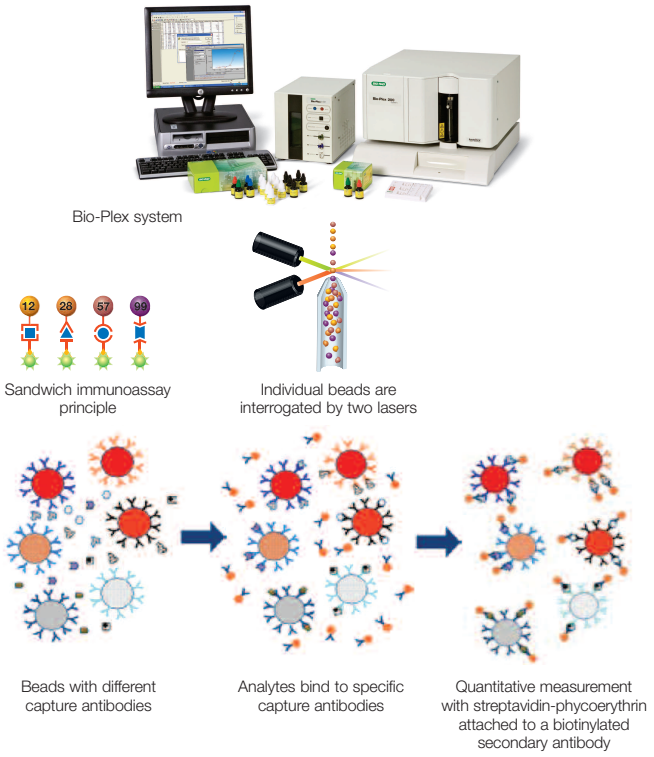

Fig. 1. Multiplex bead suspension assay test principle. The Bio-Plex multiplex system and Oncotest xenograft lysates (n = 150) were used to determine total target amount and phosphorylation status.

Results and Discussion

Expression Profiling

Multiplex bead suspension assays (Table 2) were used to determine the expression and phosphorylation state of a multitude of molecular markers involved in signal transduction, cell cycle, apoptosis, and so on. The expression profiles allowed the selection of optimal tumor models for the in vivo testing of novel anticancer agents (Figure 2). Furthermore, we used the complete molecular profiles to identify biomarkers predictive for drug response or resistance.

Target Monitoring

Another application of the multiplex bead suspension assays was the monitoring of target expression and activity (e.g., phospho-EGFR) during therapy experiments in tumor-bearing nude mice (Figure 3).

Table 2. Multiplex bead assays available at Oncotest as of February 2006. Highlighted assays are developed and manufactured at Bio-Rad.

Phosphorylated Signal Transduction Proteins
Phospho-Akt/PKB (Ser^{473})
Phospho-ATF-2 (Thr^{71})
Phospho-c-Jun (Ser^{63})
Phospho-c-Kit
Phospho-c-Met
Phospho-CREB (Ser^{133})
Phospho-EGF receptor
Phospho-ERK/MAP kinase 1/2 (Thr^{185}/Tyr^{187})
Phospho-GSK-3α/β (Ser^{21}/Ser^{9})
Phospho-HSP27 (Ser^{78})
Phospho-IκB-α (Ser^{32})
Phospho-IRS-1
Phospho-JNK/SAPK1 (Thr^{183}/Tyr^{185})
Phospho-Jun (Ser^{73})
Phospho-Lck
Phospho-NF-κB (Ser^{536})
Phospho-p38 MAPK (Thr^{180}/Tyr^{182})
Phospho-p53 (Ser^{15})
Phospho-p70 S6 kinase (Thr^{421}/Ser^{424})
Phospho-p90RSK (Thr^{359}/Ser^{363})
Phospho-PDGF receptor a
Phospho-PRAS40 (Thr^{245})
Phospho-Rb (Ser^{249}/Thr^{252})
Phospho-Rb (Thr^{821})
Phospho-RSK1/MAPKAP kinase 1a (Ser^{380})
Phospho-STAT1 (Tyr^{701})
Phospho-STAT2
Phospho-STAT3 (Tyr^{705})
Phospho-STAT3 (Ser^{727})
Phospho-STAT5A/B (Tyr$^{694/699}$)
Phospho-Tau (Ser^{199})
Phospho-Tau (Thr^{181})
Phospho-TrkA (Tyr^{490})

Total Signal Transduction Proteins
Total Akt/PKB
Total ATF-2
Total active β-catenin
Total c-Kit
Total c-Jun
Total CREB
Total EGF receptor
Total ERK/MAP kinase 1/2
Total ERK2
Total HSP27
Total IκB-α
Total IRS-1
Total JNK/SAPK1
Total Lck
Total p38 MAPK
Total p53
Total p70 S6 kinase
Total p90RSK
Total Rb
Total STAT1
Total STAT3
Total Tau

Apoptosis-Associated Proteins
Active caspase 3
Bcl-2
Cleaved PARP
Single-stranded DNA

Transcription Factors
AP-2
CREB
EGR
HIF
NF1
NF-κB
NFAT
PPAR
SRE
YY1

Matrix Metalloproteins
MMP-1
MMP-2
MMP-3
MMP-7
MMP-8
MMP-9
MMP-12
MMP-13

Death Receptors
DR5
TNF-RI
TNF-RII

Cytokines and Chemokines
Eotaxin
GM-CSF
G-CSF
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-8
IL-10
IL-12 (p40)
IL-12 (p70)
IL-13
IL-15
IL-17
Interferon-α2
Interferon-γ
IP-10
MCP-1
MIP-1α
MIP-1β
RANTES
TNF-α
TNF-β

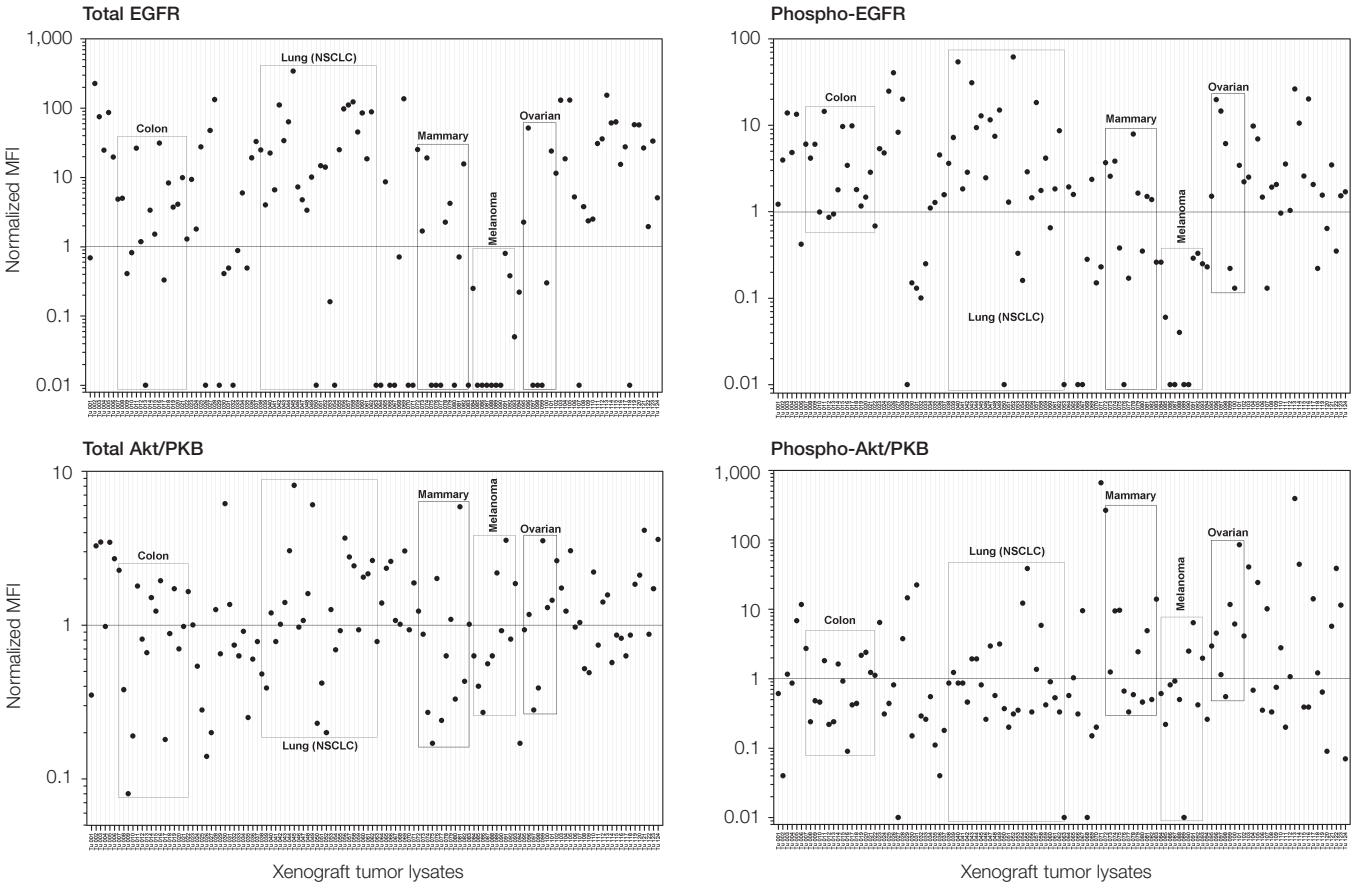

Fig. 2. Expression profiling of total proteins and phosphoproteins in 124 human tumor models. Upper panels, total and phospho-EGFR amounts; lower panels, Akt/PKB amounts. Each parameter was measured with the Bio-Plex system, and the black line shows the geometric mean value over all tumors. Tumors above the line exhibit enhanced protein expression or phosphorylation, respectively. These xenografts are suitable tumor models for target-directed in vivo studies.

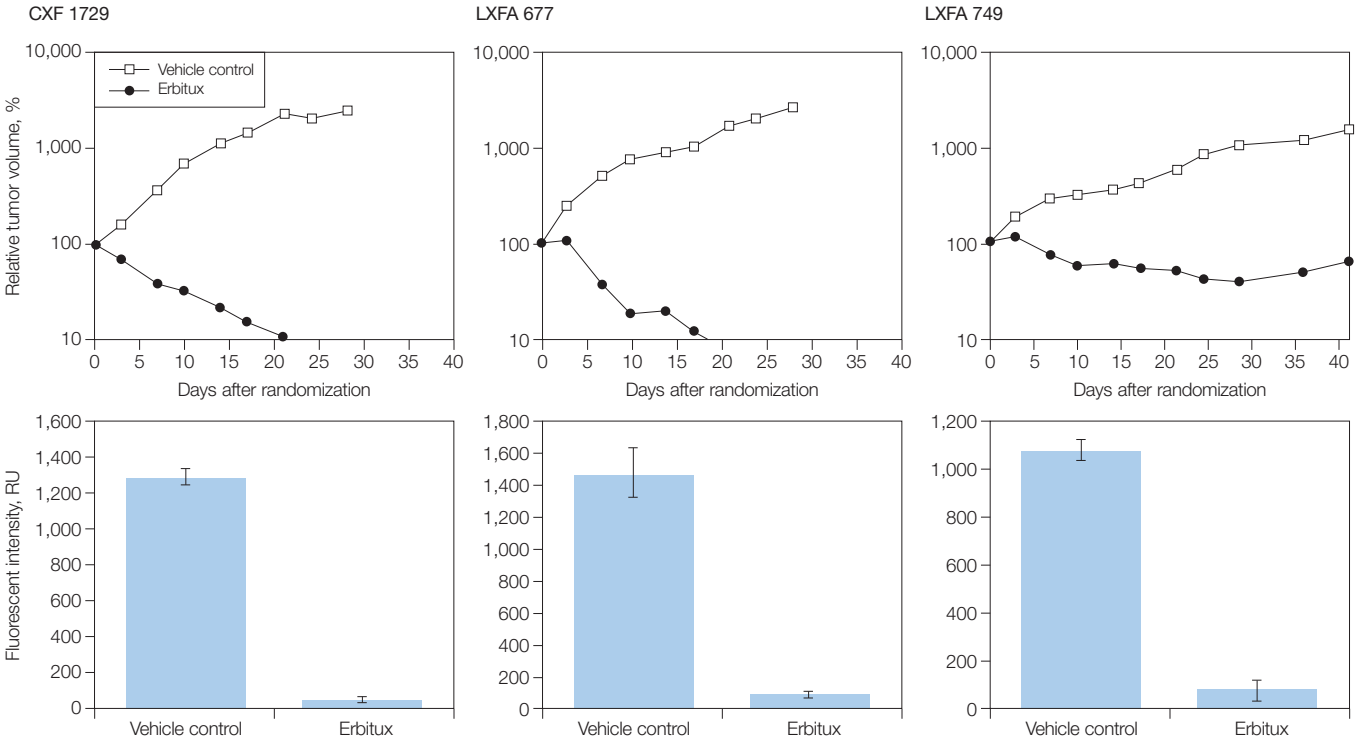

Fig. 3. Target monitoring for EGFR-inhibitory monoclonal antibody Erbitux in three sensitive tumor xenografts. Upper panels show tumor volume inhibition over time after intraperitoneal treatment with Erbitux on days 1, 8, and 15 at 30 mg/kg. Lower panels show the drop of EGFR phosphorylation 24 hr after administration of a single dose of Erbitux. CXF = colon carcinoma xenograft Freiburg; LXFA = lung adenocarcinoma xenograft Freiburg.

Conclusions

The detailed molecular characterization of Oncotest's tumor collection allows an optimal choice of tumor models for the preclinical profiling of promising new anticancer agents. This approach accelerates and improves the preclinical evaluation of novel target-directed compounds. In addition, the identification of biomarkers specific for drug response or resistance can be used as a diagnostic tool to determine the most promising individual therapy for cancer patients.

Acknowledgement

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