A new and fast *in vitro* screen to predict *in vivo* drug efficacy using primary cultures from patient-derived tumorgrafts

XenTech

Booth # C19

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INTRODUCTION

XenTech offers a large collection (over 100) of patient-derived tumorgrafts for in vivo preclinical evaluation of drug efficacy. These models reproduce accurately the behavior of the original tumors, including responses to chemotherapy (Marangoni et al. Clin Cancer Res 2007; 13: 3989-3998). As *in vivo* assays are time, money and animal consuming, we describe here a convenient *in vitro* pre-screening step to profile compound activity in panels of tumorgraft-derived primary cell cultures. We have tested more than 60 different tumorgrafts including breast, lung, colon, melanoma and glioblastoma for their capacity to generate primary cell cultures usable for *in vitro* cytotoxicity assays. Tumor cells isolated from xenografts are cultured for a few days and used at very low passage (less than 5). Today, we succeeded with a series of 20 tumorgraft models. Standard chemotherapies were tested *in vitro* to generate drug-associated IC50. Tested drugs were: adriamycin, 5-fluorouracil, irinotecan, topotecan, oxaliplatin, cisplatin and etoposide. A profile of drug sensitivity according to IC50 was defined for each model and compared to *in vivo* drug responses based on inhibition of tumor growth. Starting from *in vivo* growing tumorgrafts, this assay system allows measurement of drug-induced antiproliferative and cytotoxic effects in less than 2 weeks.

This *ex vivo* model system offers a useful platform for drug activity profiling, complementary to classical screening on tumor cell lines. Moreover, this primary tumor cell culture system will be useful for rapid screening of tumor drug response levels and selection of specific tumorgraft models for in vivo assays.

MATERIALS AND METHODS

In vitro viability assay

Primary cell cultures were obtained from tumors freshly explanted from mice in sterile condition and isolated by mechanical and enzymatic dissociation. Tissue was minced with scalpels and dissociated with a tissue shredder (gentleMACS™ ,Miltenyi Biotech) in standard culture medium supplemented with dispase, collagenase IV, hyaluronidase and DNAse. The cell suspension was filtered through a 100-µm nylon mesh. Cells were adapted for 2-5 days in standard culture conditions in Ham's F12/DMEM medium supplemented with 10% fetal bovine serum. Adherent cells were harvested by trypsinization. For the assay, cells were seeded in 96-well plates at a density of $2.5{\times}10^3$ to $10{\times}10^3$ cells/well, and incubated overnight at 37°C prior to addition of test compounds. Each drug was tested at 10 concentrations covering five log. After 3 days, cell viability and doubling time were assessed by measuring ATP cell content (CellTiter-Glo® Promega). IC50 were calculated using GraphPad Prism5 software as the concentration of drug that inhibits cell viability by 50%. Growth inhibition is calculated as a percentage of ATP value compared to vehicle-treated controls.

Quality control

-The doubling time specific for each model was used as an internal quality parameter.
- Non mouse origin of cells was assessed by :
  - Immunohistochemistry, with an anti-MHC class I H-2Kd (Acris Antibodies GmbH).
  - Flow cytometry, with anti-mouse CD90.2 (R&D systems) for fibroblasts staining.
  - RT-PCR, with murine B2Microglobulin specific primers(MWG operon) as described below :
    Total RNA from cell pellets were reverse transcripted using First strand synthesis kit (Roche) and the murine B2Microglobulin amplification was performed. cDNA from successive dilutions of murine RNA in human RNA from liver tissues were used as standard.
    Using a linear regression curve of Cq plotted against murine tissue percentage, the murine contamination was determined in our primary cells.

In vivo antitumor activity of drugs

All experiments were performed in accordance with French legislation concerning the protection of laboratory animals and with a currently valid license for experiments on vertebrate animals. In vivo studies are conducted in conformity with national veterinarian regulations as defined by the French Ministry in charge and the Ile de France Ethics Committee. Technicians are fully trained and validated on all techniques described in this experimental protocol.

Tumor fragments were implanted subcutaneously in the interscapular region of immunocompromised mice. Solid tumor xenografts grown in female nude mice were allowed to reach a volume of ~75-150 mm3 before randomizing the mice into groups of 8 to 10 based on tumor size. Tumors were measured two or three times a week after initiation of treatment, and volumes were determined using the formula : volume = (width2 x length)/2. Drug antitumor activity was evaluated with the National Cancer Institute T/C parameter: T/C = 100 x (T/C) = the percentage ratio between the mean tumor volume of a treated group (T) and the mean tumor volume of the control group at a defined day. A T/C value of 42% is considered significant and a value<10% is considered excellent antitumor activity by the NCI.

CONCLUSIONS

A mid-throughput in vitro assay system for antitumor drug activity profiling has been developed from a large panel of patient-derived tumorgrafts growing onto nude mice.

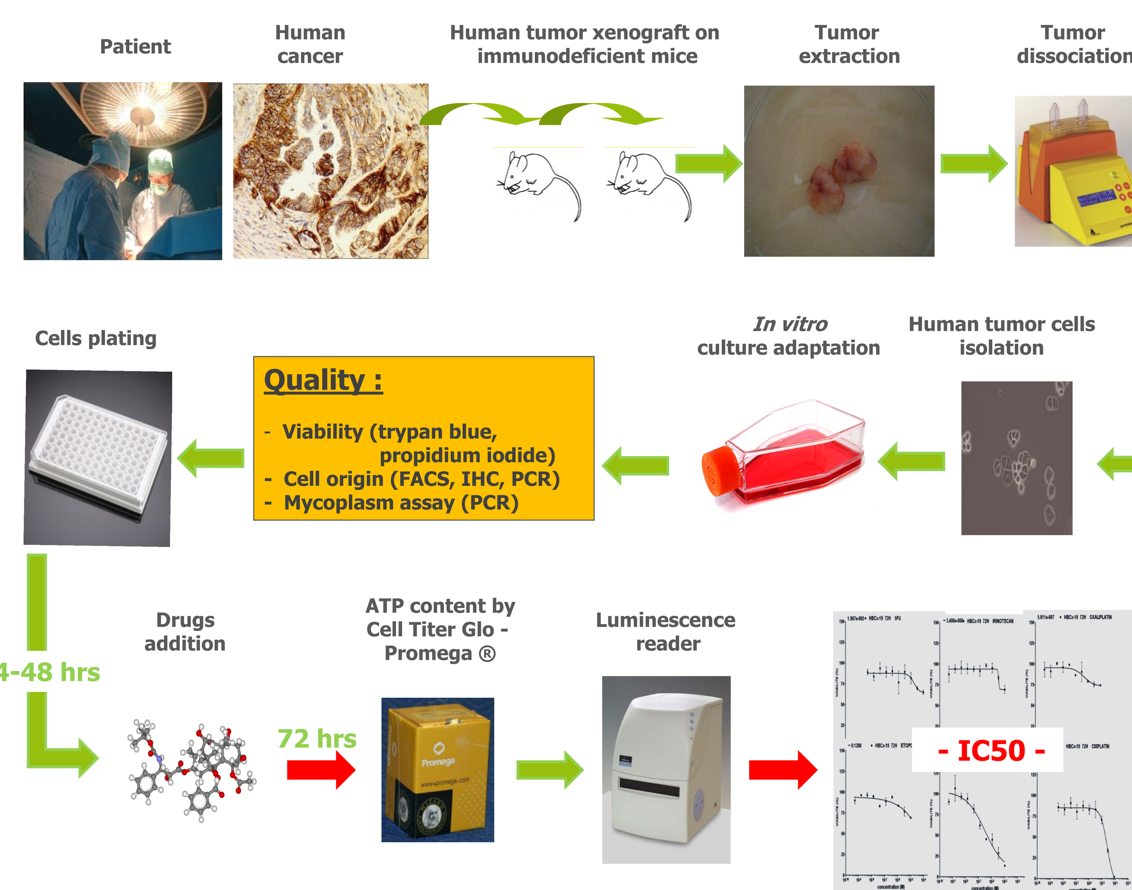
This assay system allows classifying the tumor models according to drug-specific IC50 along a scale of sensitivity. When it was possible, the same was done with *in vivo* response parameters of original tumorgrafts and both in vivo and in vitro responses were compared for each tumor model.

- for the panel of colorectal tumorgrafts, a good correlation was observed between *in vitro* and *in vivo* response parameters for 5-fluorouracil and its in vivo prodrug, capecitabine.
- to do the same correlation study with breast models, we need to increase the panel with cells derived from *in vivo* capecitabine-sensitive xenografts, since in presented models, only one is responsive to capecitabine. But, if we pooled together breast an colon models, good response correlation is maintained for 5-FU and capecitabine.

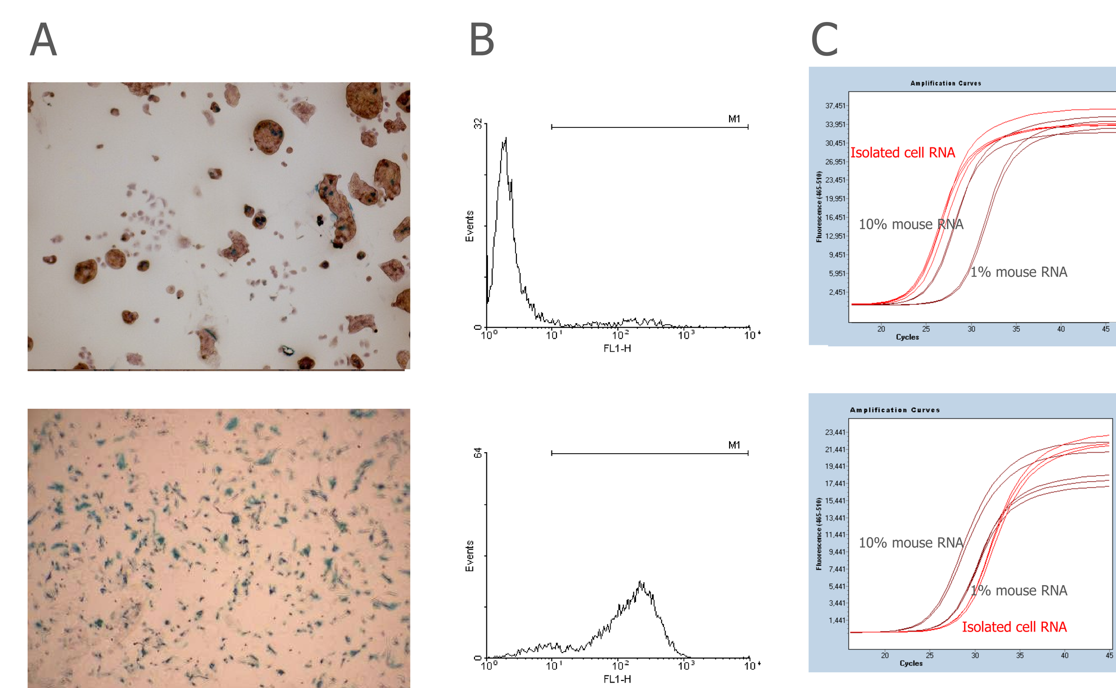
Work is in progress to study *in vitro/in vivo* drug response correlations with other tumor types, molecular targeted agents and drug combinations, which are often used in the clinic as well as in the majority of our *in vivo* studies.

The main objective of this project is to offer validated *in vitro* and *in vivo* drug efficacy assays based on a large collection of tumorgraft models, thus enhancing its value for preclinical drug development programs.

Tumorgraft *ex vivo* drug screening platform



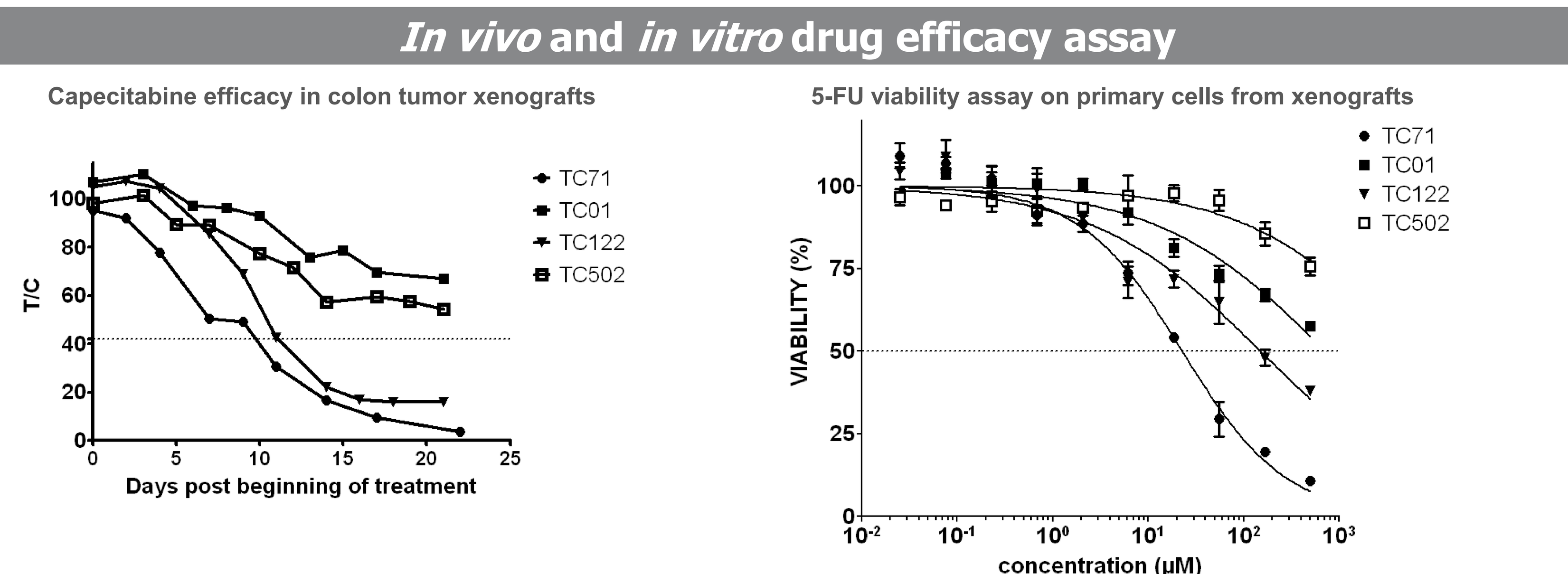
Evaluation of murine cell contamination in tumorgraft cultures



Upper panels: minor murine cell contamination observed. Lower panels: the majority of cells have a murine origin .

This was assessed by (A) IHC with anti- murine MHC class I H-2Kd and by (B) flow cytometry analysis of cells positive for CD90.2, a mouse fibroblast marker. (C) Mouse content contamination could by assessed by RT-PCR using specific primers (B2Microglobulin shown). Amplification curves are shown. In the upper figure mouse contamination was less than 1% and more than 10% in the lower one.

In vivo and *in vitro* drug efficacy assay



Capecitabine was administered *per os* at 540mg/kg five days a week, for two weeks.

Cells were incubated for 72H with 5-FU and viability was assessed by ATP content

RESULTS

Primary cell culture panel obtained from patient-derived tumorgrafts growing onto immunodeficient mice

Yield of cell extraction and doubling time of isolated cells were determined for all xenograft models. Then, depending on these parameters, models were used or not for *in vitro* assays.
In some cases, only few cells were collected from explanted tumors and the number of xenografts needed to produce enough cells was too important.
When doubling time was too long (>120 hours) the model was not used for *in vitro* testing.
The 19 validated models are tagged in yellow.

Tumor model	TC01	TC329	TC503	TC71	TC303	TC122	TC33	TC302	TC502	TC07	TC118	TC306	TC307	TC316	TC37	TC116	TC314	TC308	TC124	TC82
Tumor origin	colon	colon	colon	colon	colon	colon	colon	colon	colon	colon	colon	colon	colon	colon	colon	colon	colon	colon	colon	colon
Cell type	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated
Extraction yield	+++	+++	+++	+++	++	+++	+++	+	+++	+	+	+++	+++	+++	+	++	+++	++		
Doubling time (hrs)	40	35	45	45	35	60	70	44	100	50	45	50	95	60	50	60				
Cytotox assay screening	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing				

Tumor model	HBCx-19	HBCx-8	HBCx-3	HBCx-16	HBCx-23	HBCx-14	HBCx-1	HBCx-17	HBCx-15	HBCx-6	HBCx-9	HBCx-2	HBCx-12	HBCx-5	HBCx-10	HBCx-7	HBCx-11	HBCx-13
Tumor origin	breast	breast	breast	breast	breast	breast	breast	breast	breast	breast	breast	breast	breast	breast	breast	breast	breast	breast
Cell type	Isolated	Isolated	Isolated	Isolated	Isolated	Spheroid	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Spheroid	Spheroid	Isolated	Isolated	Isolated	Isolated
Extraction yield	++++	++	+	+	+	++	+	+	+++	+	+	+	+++	++	+			
Doubling time (hrs)	75	100	80	80	120	120	120	90										
Cytotox assay screening	Yes	Yes	Yes	Yes	Yes	Ongoing	Ongoing	Ongoing										

Tumor model	SC131	SC108	IC20	IC8	IC9	IC1	SC91	SC74	SC61	IC14	SC96	IC11	ML8	ML1	ML5
Tumor origin	lung	lung	lung	lung	lung	lung	lung	lung	lung	lung	lung	lung	lung	lung	lung
Cell type	Spheroid	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Spheroid	Isolated	Isolated
Extraction yield	++	+++	++	++	+	+	++	+	+/-	+	++	+	+		
Doubling time (hrs)	82	110													
Cytotox assay screening	Yes	Yes													

Tumor model	MCM1	MCM2	TPAN1	PANC2	GBM14	ODA4	HID28	PAC120	SC101	OVA2
Tumor origin	melanoma	melanoma	pancreas	pancreas	glioblastoma	glioma	prostate	prostate	lung	ovary
Cell type	Isolated	Isolated	Isolated	Isolated	Isolated	Isolated	Spheroid	Spheroid	Spheroid	Isolated
Extraction yield	++++	++++	+	+	++++	+++	+++	+++	+++	
Doubling time (hrs)	50	100	39							
Cytotox assay screening	Yes	Yes	Yes							

Legend:

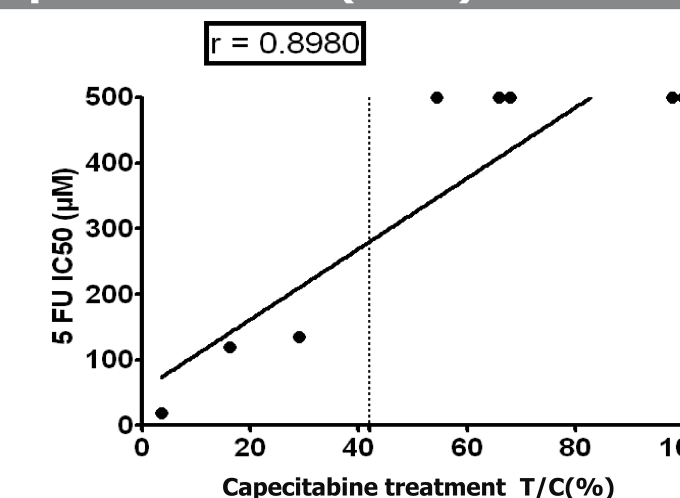
Extraction yield	+	++	+++	++++
Cells by gram of tumor	10e6	2.10e6	5.10e6	>10e7

Summary of IC50 data

IC50 (µM)	5-FU	Adriamycin	Irinotecan	Oxaliplatin	Etoposide	Topotecan	Cisplatin
Concentration Range (µM)	0.03 to 500	5.10^{-4} to 10	0.005 to 100	0.005 to 100	0.005 to 100	0.001 to 20	0.01 to 200
Fibroblastes	N/C	0,15	N/C	5,97	12,4	3,68	64,9
HBCx-16	276,6	1,2	N/T	N/C	N/C	N/C	63,79
HBCx-19	259,1	0,14	N/T	93,31	34,68	0,77	49,31
HBCx-23	N/C	0,97	N/T	63,9	N/C	11,1	79,5
HBCx-3	N/C	0,74	N/T	N/C	65,76	3,31	72,85
HBCx-8	N/C	0,88	N/T	38,77	N/C	4,22	45,44
MCM1	327,5	0,36	N/T	N/C	N/C	0,46	60,12
MCM2	N/C	0,1	N/T	23,9	32,6	4,9	57,6
SC108	N/C	0,12	N/T	7,48	14,6	3,61	39,3
SC131	N/C	3,44	N/T	N/C	N/C	8,07	44,78
TC01	N/C	4,25	N/C	55,71	N/C	4,22	49,11
TC122	134,7	2,14	67,6	8,49	67,79	1,95	45,65
TC302	N/C	0,31	89,22	15,33	14,6	6,02	59,04
TC303	N/C	2,87	N/T	N/T	6,25	5,29	85,6
TC329	N/C	5,31	N/C	N/C	27,82	1,12	N/T
TC33	N/C	0,85	N/C	38,42	15,48	2,99	64,23
TC502	N/C	0,28	62,13	4,24	39,02	1,58	56,85
TC503	119,2	2,47	9,97	4,65	3,75	2,75	46,76
TC71	22,88	0,05	3,78	19,97	3,99	0,04	21,28
TPAN1	69,49	0,18	21,47	N/T	10,62	0,57	27,92

N/C - Unable to accurately fit curve to calculate IC50 : 50% inhibition of viability was not obtained with the greatest concentration
N/T - Not tested

Correlation analysis between *in vivo* response to capecitabine and *in vitro* response to 5-FU (IC50) in colorectal tumorgrafts



Correlation coefficient (r) for comparison of *in vivo* capecitabine T/C% and *in vitro* 5-FU IC50 (Pearson correlation) using GraphPad Prism 5 software (p<0.005)