DEVELOPMENT PROGRAM OF PATIENT TUMOR TISSUE BANK TO SUPPORT THE DRUG AND TARGET DISCOVERY

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Introduction

The humanization of mice with various tissues named Chi-mice® aimed to reproduce the human situation to be more predictive than conventional models. Despite significant progress in identifying malignancy of cancer cells, a more detailed understanding of tumor generation is needed. Xenograft of tumor cells into immunodeficient rodents has constituted the major preclinical screen for the development of new drugs. These models have identified efficacious agents, but their chemosensitivity, genetic drift and clonal selection induced by cell culture have been part of the high attrition rate observed in the clinical development. Patient-derived tumor xenograft (PDX) obtained in xenografting fresh patient tumor samples in mice are reported as being more predictive to the clinical situation in maintaining the histopathology and molecular diversity of the patient tumors.

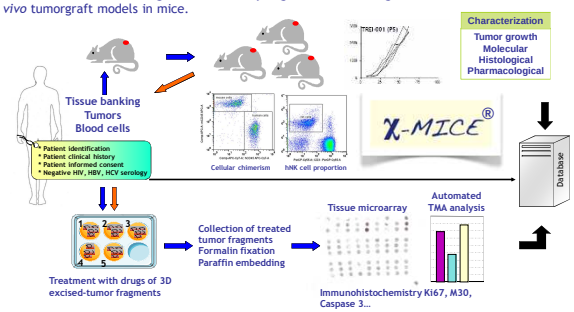
The PDXs collection has been set up under ethical agreement with informed consent of patients. The patients have been screened for absence of HIV, HBV and HCV. The anonymized patient's clinical history and tissue banking (including normal tissue when available) are centralized in our internal biological resource center. Tumor samples were freshly implanted in nude or SCID mice.

Cryopreservation of the PDX is performed at early passages allowing using these PDX only at low passage. The histopathology, HER, ER and PR statute for breast carcinoma and tumor growth characteristics of these PDX are being performed. Lymphoma characterization was performed using immunohistochemistry (hCD20, m/hKi67). Lymphoma detection, probably related to EBV infection, leads to switch to nude mice for xenografting.

To create a highly diversified panel of PDX, we organized a global process from multiple centers. These PDX are currently being used in preclinical development of new therapies and clinical positioning including biomarkers identification.

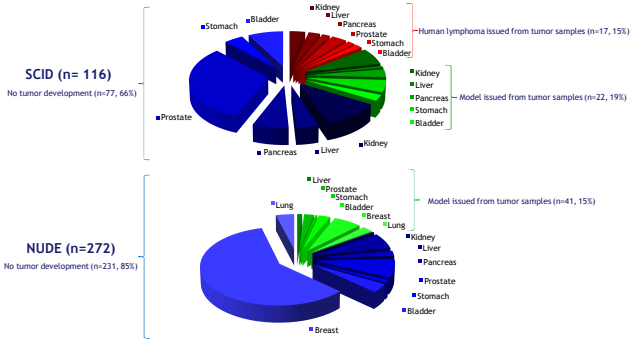
Material and Methods

Collection of human biological resources by registered Oncodesign BRC and establishment of *in vivo* tumorgraft models in mice.



- Patients were informed and gave their consent for providing surgical tumor samples to Onco*design*® BRC and for HIV1, HIV2, HBV and HCV serological status testing.
- Tumor samples were collected in Europe. Fresh tumor material was conditioned into AQIX® containing $NaHCO_3$ and nanomycoplutine.
- The human tumor fragments were xenografted in mice within 48 hours after specimen collection. Procedures were performed according to ethical guidelines for animal care and handling. 20-40 mg fragments were xenografted subcutaneously either in the flank or in the mammary fat pad area, in 2-4 immunodeficient SCID or nude mice. According to tumor model grafted mice were kept for a maximum of 12 months without tumor growth[1, 2].

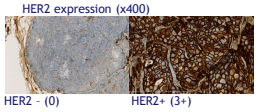
Results



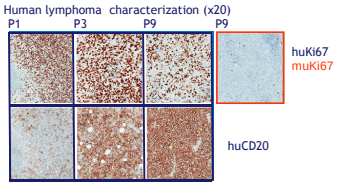
	PDX	Passage 0	Tu at Passage 1	
SCID	Kidney	25	8	32.0
	Liver	9	1	11.1
	Pancreas	14	4	28.6
	Prostate	41	0	0.0
	Stomach	13	6	46.2
	Bladder	14	3	21.4
	Breast	0	0	NA
	Lung	0	0	NA
	Lymphoma	17/39		43.6

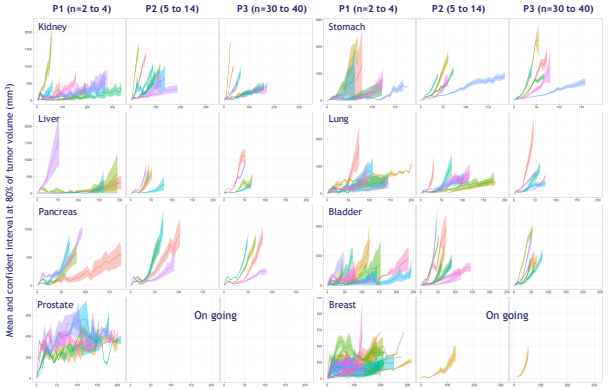
	PDX	Passage 0	Tumor growth at Passage 1	Take rate (%)
Nude	Kidney	1	0	0.0
	Liver	21	3	14.3
	Pancreas	6	0	0.0
	Prostate	27	6	22.2
	Stomach	4	1	25.0
	Bladder	17	7	41.2
	Breast	178	17	9.6
	Lung	18	7	38.9
	Lymphoma	0/41		0.0

- 388 samples (116 SCID mice and 272 nude mice) were xenografted with 26 kidney tumor samples (25 on SCID mice, 1 on nude mice, 25/1), 30 liver tumor samples (9/21), 20 pancreas tumor samples (14/6), 68 prostate tumor samples (41/27), 17 stomach tumor samples (13/4), 31 bladder tumor samples (14/17), 178 breast tumor samples (0/178) and 18 lung tumor samples (0/18).
- A tumor take rate of 30.8% was obtained for kidney, 13.3% for liver, 20.0% for pancreas, 41.2% for stomach, 32.3% for bladder, 9.6% for breast and 38.9% for lung on immunodeficient mice.
- Among 39 tumor growth after human tumor fragment engraftment in SCID mice, 17 (44%) human lymphoma were characterized using human CD20 and murine *vs* human Ki67 antibodies. No human lymphoma detection was observed among 41 tumor growth after human tumor fragment engraftment in nude mice.



Example of breast characterization for HER2 determination at P1. For human lymphoma detection, human Ki67 labeling was observed with an increase of human CD20 labeling between P1 to P9, and absence of murine Ki67 labeling.





Mean and confident interval (80%) of tumorgraft. At P1, 2 to 4 mice were xenografted. After validation of growth, the tumors were serially implanted at P2 (5 to 14 mice) and at P3 (30 to 40 mice). At P3 a tumor banking was performed.

An increase of the tumor growth homogeneity and a faster tumor growth was observed during the passages.

Pathology	Median age (range)	Male/Female	Grade 1	Grade 2	Grade 3	Grade 4	Anatompathology
Kidney	62 (18-81)	64/32	10.00%	30.00%	30.00%	30.00%	95.2% carcinoma 4.8% oncocytoma
Liver	67 (47-82)	90/10	13.60%	36.40%	40.90%	9.10%	89.7% hepatocarcinoma 10.3% hepatocholangiocarcinoma
Pancreas	64 (42-85)	47/53	-	-	-	-	92.9% adenocarcinoma 7.1% carcinoma
Stomach	65 (45-81)	81/19	0.00%	0.00%	0.00%	100%	92.9% adenocarcinoma 7.1% carcinosarcoma
Bladder	65 (42-80)	77/23	4.20%	37.50%	50.00%	8.30%	96% transitional carcinoma 4% adenocarcinoma
Breast	60 (26-90)	0.6/99.4	0.90%	85.90%	7.90%	5.30%	6% (ER-/PR-/Her2-) 55% (ER+/PR+/Her2+) 27% (ER+/PR+/Her2- or ER+/PR-/Her2+) 12% (ER+/PR-/Her2- or ER-/PR-/Her2+)
Lung	64 (55-77)	61/39	23.50%	47.10%	29.40%	0.00%	30% Her2-/50% Her2 1+/30% Her2 2+/4% Her2 3+ 93.3% non small cell lung cancer 6.7% small cell lung cancer
Prostate	64 (44-85) 44-64	Median prostate volume (%) (range) 12 (1-60) 5-125	Gleason score 4	5	6	7	8	9	98.7% adenocarcinoma 1.3% carcinoma
			1.3%	5.3%	9.2%	71.1%	1.3%	11.8%	

Characterization of collected tumors at P0. Age of patient, gender, tumor grade, TNM classification, histology results and anterior treatments are available.

Identified criterias among grade, age, volume of tumor, scoring impact on tumor take-rate in mice. Examples of patient age (44-64 years) and tumor volume (5 to 12% of prostate gland) lead to a better prostate tumor take-rate.

Conclusions

- Lymphomagenesis probably related to EBV infection[3] appears in SCID mice for all the 6 tested pathologies while it did not happens in nude strains.
- The use of nude mouse strain has inhibited the occurrence of lymphoproliferative malignancies[3].
- We are creating a highly diversified and broad range of PDX tumor models representative of human pathologies.
- These PDX are currently being used in drug discovery, preclinical development of new therapies and clinical positioning including biomarkers identification.

(1). Principe d'éthique de l'expérimentation animale. Directive n°86/609 CEE du 24 Nov. 1986, Décrêt n°87/848 du 19 Oct. 1987, Arrêté d'Application du 19 Avril 1988.
(2). Workman *et al*. Guidelines for the welfare and use of animals in cancer research. Br J Cancer. 2010;102(11):1555-1577.
(3). Chen *et al*. Plosone. 2012; 7: 1-8.