Liver cancer patient-derived xenografts to improve disease management in childhood and adolescence: perspectives and challenges of personalized medicine.

Stefano Cairo[1], Aurore Gorse[1], Delphine Nicolle[1], Erwan Selingue[1], Katell Mevel[1], Frederic Gauthier[2], Christophe Chardot[3], Elie Fadel[4], Dominique Elias[5], Daniel Orbach[6], Catherine Guettier[2], Arnaud Beurdeley[1], Vanessa Yvonnet[1], Olivier Deas[1], Monique Fabre [5], Laurence Brugières [5], Sophie Branchereau[2] and Jean-Gabriel Judde[1]. [1]XenTech, Evry, France; [2]Hôpital de Bicêtre, Le Kremlin Bicêtre, France; [3]Hôpital de Necker-Enfants Malades, Paris, France; [4]Centre Chirurgical Marie Lannelongue, Le Plessis Robinson, France; [5]Institut Gustave Roussy, Villejuif, France; [6] Institut Curie, France.

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INTRODUCTION

Despite the rarity of each individual cancer type, about 200 different rare cancers constitute in total about 20% of all cancer cases, including pediatric cancers. In Europe, nearly half a million people live with a rare cancer. Like other rare diseases, rare cancers are particular challenging due to their low incidence, particularly for the identification of novel therapies that could improve patient survival.

In spite of being the predominant type of pediatric liver malignancies, hepatoblastoma (HB), with a world-wide incidence of 1 case per million persons per year, is a rare tumor. Differently from adult hepatocellular carcinoma (HCC) that develops on a cirrhotic or chronically-infected background, liver tumors in children and adolescents occur on apparently normal liver. The high rate (> 60 %) of β-catenin activating mutations places HB as the human tumor most tightly associated with activation of the Wnt/β-catenin pathway. Evidence for (epi)genetic origin of HB is provided by its association with congenital anomalies, Beckwith-Wiedemann syndrome, and familial adenomatous polyposis, a disorder caused by germline mutation of APC, involved in β-catenin degradation. HCC, fibrolamellar carcinoma (FLC), and transitional liver cell tumors (TLCT), which combines histological features of HB and HCC, also arise in children and adolescents, at a lower extent though. Sporadically, very rare forms of liver tumor likely of non-epithelial origin such as rhabdoid tumor or hepatic sarcoma also occur.

RATIONALE AND METHODS

In order to assist medical decision on the management of liver cancer in childhood and adolescence, we have launched a program aimed at the constitution of liver cancer patient-derived xenografts (PDXs). HB PDXs could be used as a preclinical cohort for phase II-like studies. This would allow the pre-screen of therapeutic solutions that would require years when not decades to be put in place via standard clinical assays (**Figure 1**). In collaboration with pediatric oncologists and surgeons of the International Childhood Liver Tumour Strategy Group (SIOPEL), post-surgery tumor specimens were transplanted in the interscapular region of immunocompromised nude mice, and growing tumors were amplified by serial transplantation. Xenograft tumor histology was compared with that of the tumor of origin and reviewed by a human pathologist specialized in pediatric liver cancer (MF).

At present, 9/25 HBs, 2/2 TLCTs (one HB/HCC and one lung metastasis resembling an HCC developed that recurred from an HB/HCC), 0/2 FLCs, 1/1 rhabdoid tumor and 0/1 hepatic sarcoma have been successfully grown in immunocompromised mice (**Table I**).

RESULTS AND PERSPECTIVES

As shown by hematoxylin-eosin-safran staining, PDXs maintain the main histological features of primary human tumors (**Figure 2A**). Alpha-fetoprotein (AFP) protein, which is a diagnostic marker of HB, is expressed in HB PDXs, and its expression correlates with the circulating levels in mouse blood (**Figure 2B**). Also, several HB PDXs harbor activating mutation of β-catenin (**Figure 2C**), suggesting that they could also be useful tools for the development of efficacious Wnt/β-catenin inhibitors. In order to evaluate how established HB PDX models encompass the heterogeneity of clinical HB, a number of clinical parameters were investigated by comparing tumors that did not give rise to a PDX to those that were successfully xenografted. As shown in **Table II**, no specific clinical or histological features seem to be associated with PDX development. By contrast, the only data that associate to successful tumor grafting is a low percentage of necrotic tumor after chemotherapy, and the post-treatment serum AFP level. As these two parameters are indicators of tumor viability upon treatment, it seems from these data that the main parameter associated with tumor take is a suboptimal response to chemotherapy; otherwise the heterogeneity of the clinical population is well represented by the PDXs. Among the models established, 3 PDXs representing an HB, a TLCT (HB/HCC) and an HCC were selected to perform an anti-tumor treatment screening (**Section 3**). Upon treatment with different chemotherapy agents, the three models showed unique response profiles, indicating a tumor-specific sensitivity (labeled in green, orange and yellow). Moreover, all models were sensitive to treatment with combined irinotecan and temozolomide (labeled in blue), even if the efficacy was less strong in the HCC PDX.

- The results from this study strongly support the usefulness of PDX models to assist treatment decision in rare pediatric and non pediatric cancers. The creation of a robust preclinical cohort of HB models will help identifying the best working treatments for translation into the clinical setting. In addition, for sporadic liver tumors like TLCT and HCC where the creation of a preclinical cohort is hard to propose, systematic PDX establishment and comprehensive drug screening in vivo could orientate adjuvant therapy in a personalized treatment approach, or contribute to accumulate evidence on the usefulness of the tested drugs in such types of liver malignancies.

STRATEGY

1. The choice of phase-II studies for rare cancers

Figure 1.

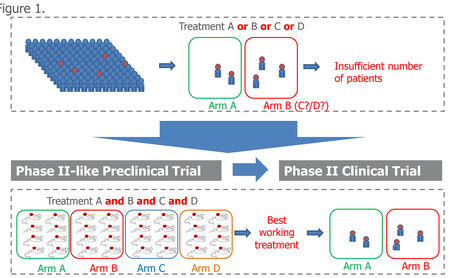


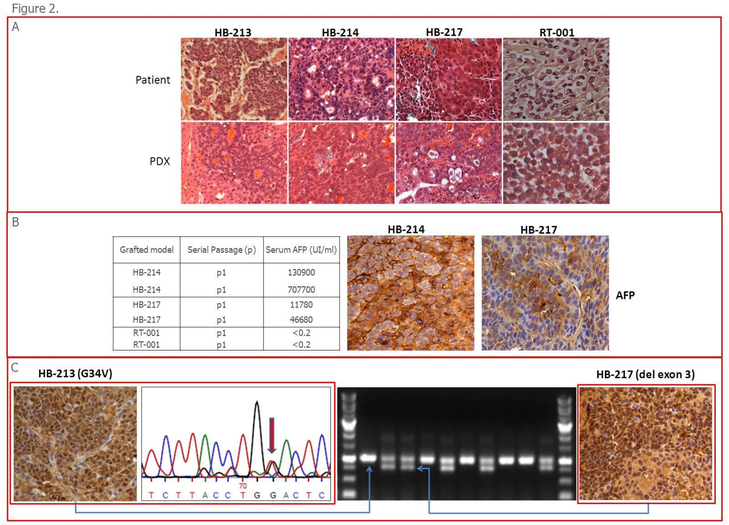
Table I. List of human HB xenografted.

Tumor ID	Age (months)	Sex	Tumor Type	Tumor origin	PDX (latency)	β-catenin status
HB-211	10	F	HB	Primary	N	
HB-212	8	M	HB	Primary	N	
HB-213	19	F	HB	Primary	Y	MUT
HB-214	30	F	HB	Primary	Y	MUT
HB-215	6	F	HB	Primary	N	
HB-216	24	F	HB	Primary	N	
HB-217	24	M	HB	Recurrence	Y	MUT
HB-218	24	F	HB	Primary	N	
RT-001	24	F	Rhabdoid	Primary	Y	WT
HB-220	12	M	HB	Primary	N	
HB-221	90	F	HB	Primary	N	
HS-001	145	M	Hepatic Sarcoma	Primary	N	
HB-223	23	M	HB	Primary	N	
HB-224	38	M	HB	Primary	N	
HB-225	39	M	HB	Primary	N	
HB-226	40	M	HB	Primary	N	
HB-227	23	F	HB	Primary	N	
HB-228	39	F	HB	Primary	N	
HB-229	54	M	HB	Recurrence	Y	MUT
HB-230	27	F	HB	Lung metastasis	N	
FLC-001	204	F	FLC	Peritoneal metastasis	N	
HB-231	104	F	HB	Primary	N	
HB-232	6	M	HB	Primary	Y	WT
HB-233	16	M	HB	Primary	Y	MUT
HB-234	276	M	HCC	Lung metastasis	Y	WT
HB-235	42	F	HB/HCC	Primary	Y	MUT
HB-236	8	F	HB	Primary	Y	MUT
FLC-002	180	F	FLC	Primary	N	
HB-237	15	M	HB	Primary	N	
HB-238	110	F	HB	Recurrence of HB-231	Y	MUT
HB-239	113	M	HB	Primary	Y	MUT
HB-240			HB	Primary	N (3 months)	
HB-241	121	F	HB	Primary	N (3 months)	
HB-242	27	M	HB	Primary	N (2 months)	
HB-243	52	M	HB	Primary	N (2 months)	
HB-244	114	M	HB	Recurrence of HB-239	Y	

RESULTS

2. Characterization of PDX cohort for preclinical phase 2-like trials

Figure 2.



Grafted model	Serial Passage (p)	Serum AFP (UI/ml)
HB-214	p1	130900
HB-214	p1	707700
HB-217	p1	11780
HB-217	p1	46680
RT-001	p1	<0.2
RT-001	p1	<0.2

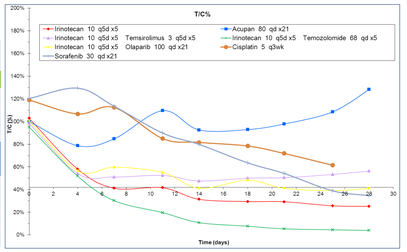
Patient tumor	HB-211	HB-212	HB-215	HB-216	HB-218	HB-220	HB-221	HB-223	HB-224	HB-225	HB-226	HB-227	HB-228	HB-230	HB-231	HB-237	HB-213	HB-214	HB-217	HB-229	HB-232	HB-233	HB-235	HB-236	HB-238	HB-239	p value
PDX established (Y/N)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	-
AGE (0=<36m; 1=>36m)	0	0	0	0	0	0	1	0	1	1	1	0	0	0	1	0	0	0	1	1	0	0	1	0	1	1	ns
Sample type I (T=primary tumor; R=recurrence; M=metastasis)	T	T	T	T	T	T	T	T	T	T	T	T	T	M	T	T	T	T	R	R	T	T	T	T	R	R	ns
Sample type II (R=resection; O= orthotopic liver transplantation)	R	R	R	R	R	R	O	R	O	R	R	R	R	R	R	R	R	R	R	R	R	R	O	R	R	R	ns
Sex	F	M	F	F	F	M	F	M	M	M	M	F	F	F	F	M	F	F	M	M	M	M	F	F	F	M	ns
Vascular invasion (Y/N)	N	N	N	Y	N	N	Y	N	N	Y	Y	N	Y		Y	N	Y	Y	N	Y		N	Y	N	Y	Y	ns
Solitary (S)/multiple (M) nodules	S	S	S	M	M	M	M	S	M	M	M	M			M	M	M	M	S	M	S	S	M		M	M	ns
Metastasis (Y/N)	N	N	N	N	N	N	Y	N	N	N		N	N		N	N	Y	Y	N	Y	N	N	Y	N	N	N	ns
Histology (M=mixed; E=epithelial)	M	E	M	M	E	M	E	E		M	E	E	E	M		M	M	M	E	E	E	M		M	E	E	ns
MAIN epithelial component (F=fetal; E=embryonal)	E	F	F	E	E	F	F	F		F	F	F	F	F		F	E	E	F	E	F	F		F	F	E	ns
Small cell component (Y/N)	Y	Y	N	N	N	N	N	N		N	N	N	N	N	N	N	Y	Y	N		N	Y		N		Y	ns
PRETEXT 3-4 (Y/N)	Y	Y	Y	N	Y		Y	N	Y	Y	Y	N	Y		Y		Y	Y			Y	N	Y	N			ns
High (H)/Standard (S) risk	S	S	S	S	H		H	S	H	H	H	S	S	H	H	S	H	H	H	S	S	S	H	S		H	ns
Tumor necrosis (0=<50%; 1=>50%)	0	1	1	0	1	1	1	1		0	0	0	1		1	0	0	0	0	0	0	0	0	0	0	0	0.01
AFP at diagnosis >5x 10^5ng/mL (Y/N)		Y	N	Y	N			N	Y	N	N	N	N		Y	N	Y	Y			Y	Y	Y	Y	Y	Y	0.01
AFP post-chemotherapy >5,000 ng/mL	N	N		N	N			N	N	N	N	N	N		N	N	N		N	Y	Y	Y	N		Y	0.03
LOG10 AFP reduction >3 (Y/N)		Y		N	N			Y	N	Y	Y	N	N		Y	N	Y	Y		N	N	N	N		N	ns

Table II. Comparative analysis of clinical annotation of tumors that differed in their capacity to grow in nude mice (Chi-square test).

3. Tumor response profiles identify common and personalized treatments

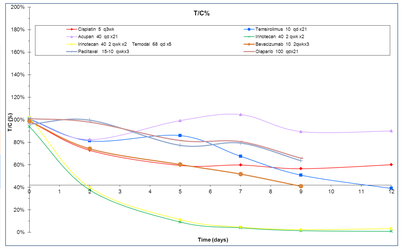
HB-229 (HB)

Treatment efficacy	Day 18
No Treatment vs Irinotecan 10	*
No Treatment vs Acupan	ns
No Treatment vs Irinotecan/Temsirolimus	ns
No Treatment vs Irinotecan/Temozolomide	**
No Treatment vs Irinotecan/Olaparib	ns
No Treatment vs Cisplatin	ns
No Treatment vs Sorafenib	ns



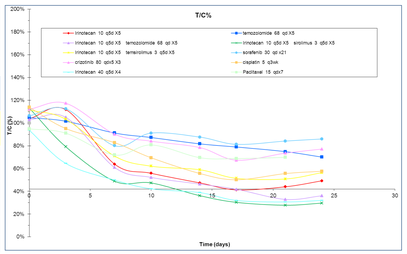
HB-235 (HB/HCC)

Treatment efficacy	Day 9
No Treatment vs Cisplatin	*
No Treatment vs Temsirolimus	**
No Treatment vs Acupan	ns
No Treatment vs Irinotecan 40	**
No Treatment vs Irinotecan/Temozolomide	**
No Treatment vs Bevacizumab	*
No Treatment vs Paclitaxel	ns
No Treatment vs Olaparib	ns



HB-234 (HCC)

Treatment efficacy	Day 17
No treatment vs Irinotecan 10	*
No treatment vs Temozolomide	ns
No treatment vs Irinotecan/Temozolomide	**
No treatment vs Irinotecan/Sirolimus	**
No treatment vs Irinotecan/Temsirolimus	*
No treatment vs Sorafenib	ns
No treatment vs Crizotinib	
No treatment vs Cisplatin	*
No treatment vs Irinotecan 40	**



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