A preclinical platform of mouse-transplanted rare human pediatric liver tumors to evaluate the efficacy of conventional and innovative anticancer therapy.

Stefano Cairo[1], Delphine Nicolle[1], Marie-Emmanuelle Legrier[1], Arnaud Beurdeley[1], Aurore Gorse[1], Monique Fabre[2], Laurence Brugières[2], Sophie Branchereau[3], Frédéric Gauthier[3], Jean-Gabriel Judde[1].
[1]Xentech, Evry, France; [2]Institut Gustave Roussy, Villejuif, France; [3]Hôpital de Bicêtre-APHP, Le Kremlin Bicêtre, France.

INTRODUCTION

Hepatoblastoma (HB) is a pediatric liver tumor characterized by the proliferation of immature hepatoblasts frequently associated to malignant mesenchymal tissue, suggesting that it derives from uncommitted progenitor cells. Although the etiology of HB is at present unknown, an association with congenital abnormalities such as Familial Adenomatous Polyposis and Beckwith-Wiedemann syndrome is well established. Importantly, deregulation of the Wnt pathway through activating mutations of b-catenin plays a crucial role in the development of this tumor. In most cases, cure of HB patients is achieved by pre and post-operative chemotherapy and surgery. However, approximately 30% of the children do not survive the disease, and new treatments are urgently needed.

Although being the predominant type of malignant liver tumor in childhood, as it accounts for between 60 and 85% of all hepatic tumors, HB is a rare tumor, with a world-wide incidence of 1.5 cases per million children per year. The low rate of HB occurrence renders the constitution of patient cohorts for clinical trials problematic, as the number of patients enrolled in a treatment program is in most of cases not wide enough to provide clinicians with statistically significant results.

PROJECT AIM

Tumor xenografts are a suitable supportive tool to overcome two main problems related to a rare cancer as HB :

- low number of patients => uneasy to set up clinical assays
- treat kids with drugs of unknown toxicity and efficacy

The aim of this study is to provide preliminary supportive evidence of the robustness of the human tumor xenograft approach in HB.

The expected advantages from patient-derived xenograft panels are:

- Maintain the phenotypic and genotypic features of patient's tumor
- Cover molecular diversity of human tumors
- Allow identification and validation of challenging therapeutic approaches

STRATEGY AND METHODS

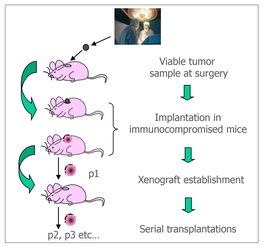

Figure 1. Overall experimental strategy to establish human tumor xenografts (p=passage)

- In collaboration with pediatric oncologists and surgeons of the International Childhood Liver Tumour Strategy Group (SIOPEL), we have launched a program aimed at the constitution of a large panel of human HB transplanted on immunocompromised mice.
- Post-surgery tumor specimens were transplanted in the interscapular region or into the renal capsule of nude or NOD/SCID mice (Figure 1). Tumor growth was observed with a latency period of 1 to 6 months. Tumor xenografts were amplified by serial transplantation, and tissue samples were retained at each passage for comparison with the patient's tumor (MF).
- Xenograft tumor histology was compared with that of the tumor of origin and reviewed by a human pathologist specialised in HB.
- Pharmacological response of tumorgrafts was evaluated by administration of reference chemotherapy protocols currently in use for the treatment of HB in children.

CONCLUSIONS AND PERSPECTIVES

- HB xenografts sharing histological characteristics with the human tumor of origin could be successfully established in immunocompromised mice.
- Heterogeneous response profile of HB xenografts to chemotherapy indicates that tumor subtypes with different sensitivity to treatment can be generated.
- Increased percentage of immature tumor components is observed in human tumorgrafts. As undifferentiated tumor components are associated with adverse prognosis, enrichment of tumor compartment associated with aggressive tumor phenotype will allow a more informative drug efficiency profiling and help identify appropriate treatment strategies for poor-prognosis patients.

Establishment of a large collection of HB models will provide a robust preclinical panel to perform studies that will contribute to:

- Improvement of current treatment
- test new treatments

The results from this study strongly support the usefulness of tumorgraft models to assist treatment decision in rare pediatric and non pediatric cancers.

RESULTS

List of xenografted HB

Eight HBs have been transplanted in immunocompromised mice in the last 10 months. Three of them (marked in green) gave rise to ectopic tumor growth. As shown in the table, of the three tumor models, two are derived from metastatic HBs, and one is derived from an intrahepatic tumor recurrence.

Tumor ID	Age (months)	Sex	Tumor type	Pretext stage	Metastasis	Treatment protocol	Risk	Xenograft
HB-211	10	F	Primary	III	N	SIOPEL6	S	N (9 months)
HB-212	8	M	Primary	III	N	SIOPEL6	S	N (8 months)
HB-213	19	F	Primary	III	Y	SIOPEL3	H	Y (5 months)
HB-214	30	F	Primary	II	Y	SIOPEL3	H	Y (4 months)
HB-215	6	F	Primary	III	N	SIOPEL6	S	N (3 months)
HB-216	24	F	Primary	II	N	SIOPEL6	S	N (3 months)
HB-217	24	M	Recurrence		N	SIOPEL6	S	Y (1 month)
HB-218	27	F	Primary	IV	N	SIOPEL 3	H	N (2 months)

Table I. List of human HB xenografted. The table recapitulates the main clinical features of the tumors xenotransplanted in the last 10 months. All tumors received preoperative chemotherapy. SIOPEL 6 protocol consists of cycles of Cisplatin; SIOPEL 3 high risk protocol consists of alternating cycles of cisplatin and the combination of carboplatin plus doxorubicin. Pretext (Pretreatment Extension) stage indicates tumor extension in the liver at diagnosis. Abbreviations: Y= yes, N= no, F= female, M= male. Metastasis column indicates the presence of distant metastasis at diagnosis. The time frame in the Xenograft column indicates the observation period.

Tumor of origin and xenograft share main histological features

Analysis of tumor histology and of HB markers expression indicates that the overall tumor phenotype of patient's tumor is retained in the xenograft model (Figure 1 and Table II). An increase of poorly differentiated cells is observed in the xenograft models, where the embryonal and small undifferentiated cell (SCUD) compartment augment at the expense of the more differentiated fetal compartment (Table II).

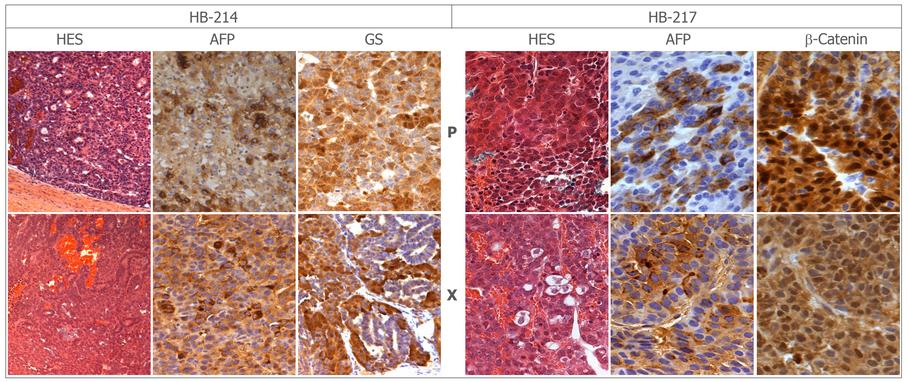

Figure 1. Analysis of patient's tumor and xenograft models by histological staining and immunochemistry. HES: Hematoxylin–eosin staining of the embryonal component of HB-214 and HB-217 tumors in patient (P), and xenograft (X). AFP: alfa-fetoprotein; GS: Glutamine Synthetase. Magnification: HB-214, 20x; HB-217, 40x.

Tumor ID	Main histological component		Main cellular component	
	Patient	Xenograft	Patient	Xenograft
HB-213	Epithelial and teratoid	Epithelial and teratoid	Fetal 40%, Crowded F etal 40%, Embryonal 1%, SCUD 20%	Embryonal 45%, Fetal 5%, SCUD 50%
HB-214	Epithelial	Epithelial	Fetal 50%, E mbryonal 40%, Osteoid 10%	Embryonal with calcifications
HB-217	Epithelial	Epithelial	Fetal 60%, Embryonal 30%, SCUD 10%	Embryonal 80%, Fetal 10%, SCUD 10%

Table II. Comparative histology of patient's tumor and xenograft model. The table illustrates the results of histological analysis of tumor after surgical resection and of derived xenograft model. Crowded fetal indicates a subtype of fetal component endowed with high proliferation rate.

Response of HB tumorgraft to conventional chemotherapy

Response of xenograft models to chemotherapy was investigated by administration of reference chemotherapeutic agents used in the clinical practice. As shown in Figure 2, tumors display different drug response profiles. HB-213 shows resistance to cisplatin and doxorubicin administered as single agents as well as in combination. HB-214 shows a weak but significant response when cisplatin and doxorubicin are administered together. In HB-217, administration of cisplatin alone is sufficient to induce strong inhibition of tumor growth. These results are consistent with the degree of response of patient tumor tumors scored by the pathologist upon tumor examination post-surgery (Table III).

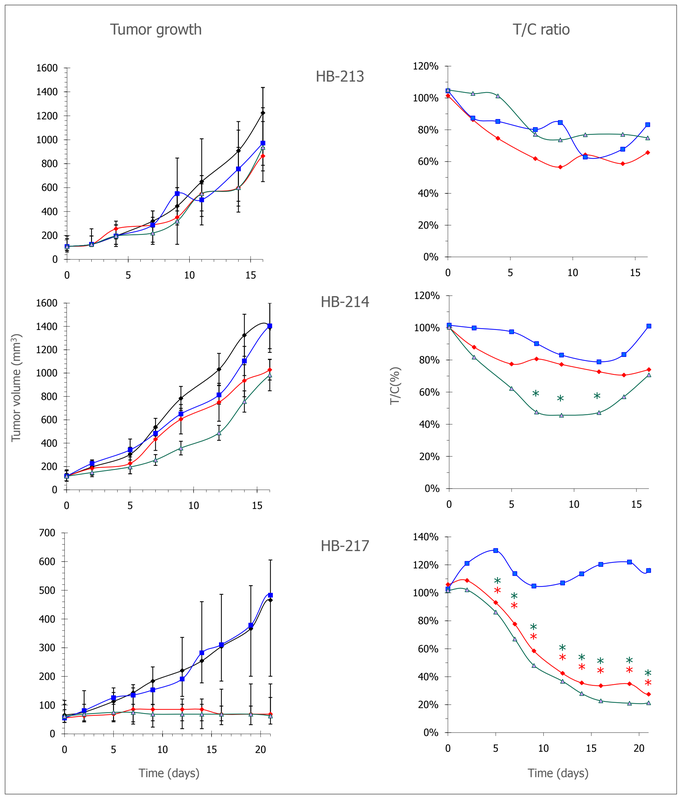

Figure 2. Graphs describe tumor growth rate (left side) and tumor growth inhibition (right side). T/C indicates the percentage ratio between the mean tumor volume of a treated group (T) and the mean tumor volume of the control Group. Each group consists of ten animals bearing comparable tumor size at the time of treatment. Dark line: untreated animals; blue line: animals treated with a single dose of doxorubicin (2mg/kg, administered at day 0); red line: animals treated with a single dose of cisplatin (5mg/kg, administered at day 0); green line: animals treated with a single dose of doxorubicin and cisplatin at the concentration reported above, at day 0. Asterisk indicates significant inhibition of tumor growth, and the color code corresponds to the treatment .

Tumor ID	Patient treatment	Patient's tumor response (histology post-surgery)	Xenograft response 14 days post-treatment (T/C)
HB-213	Cisplatin + Doxorubicin	20%	23%
HB-214	Cisplatin + Doxorubicin	50-60%	43%
HB-217	Cisplatin	70%	66%

Table III. Comparison of histological evaluation of patient's tumor response in post-surgical tumor specimens with tumor growth inhibition observed in xenograft models. The percentage indicated for patient's tumor response refers to the degree of fibrosis/necrosis found by the pathologist after examination of the resected tumor.