#4169 CReMEC initiative: Characterization of patient-derived colorectal tumor models and correlation with patient profile

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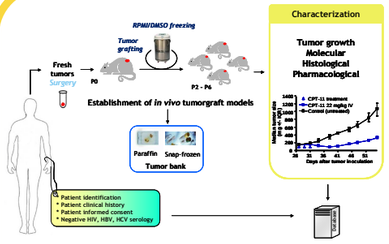
INTRODUCTION

Well characterized models representing the heterogeneity of human colorectal cancers (CRC) are needed to develop effective therapeutic agents. Establishment of such tools will allow a better prediction of the clinical outcome, taking into account the diversity of each patient tumor phenotype and genotype. For this purpose, we have associated efforts from hospitals, academic groups, biotech and pharmaceutical companies. The goal of this consortium is to create an experimental tumor model resource center to improve or strengthen drug development. **From May 2007 to January 2009, 86 surgical specimens [59 primary (P) tumors, 19 metastasis (M), and 8 peritoneal carcinomatosis (C)] were collected from CRC patients (with informed consents and negative HBV, HCV, and HIVs serologies).** Tumor samples were subcutaneously xenografted in *nude* mice and characterized as described below. We report here the results on our panel of models.

MATERIAL AND METHODS



- **Patients were informed and gave their consent for providing surgical tumor samples to CReMEC and for HIV1, HIV2, HBV and HCV serological status testing.**
- **Tumor samples were collected in 3 medical centers of the Paris-Ile-de-France area: Institut Curie, Institut Gustave Roussy, Hôpital Lariboisière.**
- **Fresh tumor material was conditioned into RPMI 1640 with 200 U/mL penicillin, 200 µg/mL streptomycin and 2.5 µg/mL fungizone.**
- **Initial xenografting was carried out at the site of sampling, within 12 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 interscapular area, in 3-5 immunodeficient SWISS *nude* mice**
- **Grafted mice were kept for a maximum of 4 months without tumor growth.**

Clinical data collection

- **Relevant clinical information was collected by the attending physician and included in a standardized data sheet.**
- **Identification of the data sheet is anonymous.**

Histological characterization

- **Samples were fixed for a maximum of 48 hours in alcohol-formalin-acetic acid (AFA) and embedded in paraffin. 5 µm sections were stained with hematoxylin-eosin-saffron.**

Molecular characterization – sequencing

- ***CGH array analysis:*** **Evaluation of genome-wide, gene copy number was evaluated using a 244k CGH array Agilent technology. It was carried out on DNA from the patient sample or at an early passages (P0/P1), and at the passage used for the pharmacological evaluation (P8-9).**
- ***DNA sequencing:*** **The following genetic markers, relevant in CRC, were selected for sequencing: *APC* (exons 9 & 16), *KRAS* (exons 2 & 3), *BRAF* (exons 11 & 15), *TP53* (exons 2 to 11), *CTNNB1* (exon 3), *PIK3CA* (exons 10 and 21), *FBXW7* (exons 4 to 11), *EGFR* (exons 18 to 21) and *AKT1* (exon 4). Sanger direct sequencing was performed after PCR amplification of exons of genes harboring hotspot mutations as described in Catalogue Of Somatic Mutations In Cancer (COSMIC: http://www.sanger.ac.uk/genetics/CGP/cosmic/).**
- ***Determination of Microsatellite Instability (MSI) status:*** **The MSI status was determined according to the Consensus Conference (or Revised Bethesda Guidelines) (*J. Natl Cancer Inst* 2004, 96, 261-268) recommendations using the following five quasimonomorphic markers: *NR21*, *BAT26*, *BAT25*, *NR24* and *NR22*.**

Pharmacological characterization

- ***Tested agents:*** **5-fluorouracil (5-FU), oxaliplatin (L-OHP), irinotecan (CPT-11) or cetuximab, were tested.**
- ***In vivo determination of antitumor activity:*** **Tumor fragments were subcutaneously xenografted in *SCID* mice or *nude* rats. Tumor-bearing animals were randomized when the mean tumor volume reached 100-200 mm³ in mice or 500-700 mm³ in rats. Compounds were formulated in glucose 5% in water. 3 cytotoxic drugs were IV administered, and tested at 70% of their respective highest non toxic dose and using the following regimen in mice: 5-FU at 56 mg/kg/adm Q4Dx2 (30 mg/kg/adm Q7Dx3 in rats) , L-OHP at 5 mg/kg/adm Q4Dx2 (4 mg/kg/adm Q4Dx3 in rats), CPT-11 at 22 mg/kg/adm, Q2Dx3 (40 mg/kg/adm, Q7Dx3in rats) ; 1 targeting therapy was IP administrated: cetuximab at 12.5 mg/kg/adm, (Q3Dx2)x2 (10 mg/kg/adm, IV, Q7Dx3 in rats). Antitumor activity was evaluated by calculating the Δ(T/C) *ratio*:**

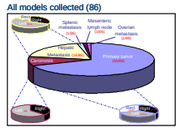
$$\Delta(T/C)\ (\%) = \frac{\text{median T}_{\text{VOL- DY}} - \text{median T}_{\text{VOL-DX}}}{\text{median C}_{\text{VOL- DY}} - \text{median C}_{\text{VOL-DX}}} \times 100$$

Scoring criteria:
- = Δ(T/C) > 42 %
+ = 10 < Δ(T/C) ≦ 42 %
++ = 0 ≦ Δ(T/C) ≦10 % (stable disease)
+++ = Δ(T/C) <0 % (tumor regression)

PR: Partial Response
CR: Complete Response
TFS: Tumor Free Survival

RESULTS

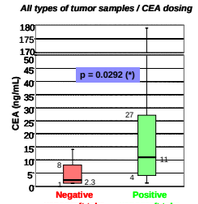
Clinical characteristics and *in vivo* tumorgraft take rate



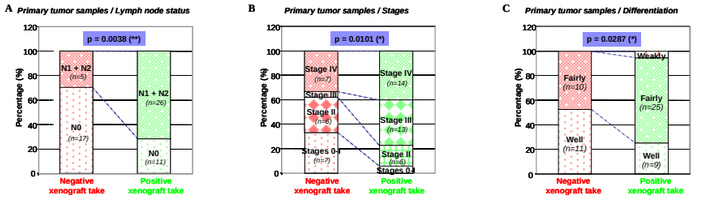
Patient characteristics in the collection

Parameter	Class	Number	Frequency (%)	Tumor take rate (%)
Site of origin	Primary	37	66.1	62.7
	Peritoneal carcinosis	5	8.9	62.5
	Metastasis#	14	25.0	73.7
Sex	Female	35	62.5	66.0
	Male	21	37.5	63.6
Age	(),-50y	11	20.4	78.6
	[50y ; 60y[	12	24.5	50.0
	[60y ; 70y[	12	20.4	60.0
	[70y ; 80y[	15	24.5	68.2
	>80y	6	10.2	100
Stage	0 - I	2	3.7	22.2
	II	8	14.8	57.1
	III	13	24.1	92.9
	IV	31	57.4	70.4

hepatic, splenic and mesenteric lymph node metastasis



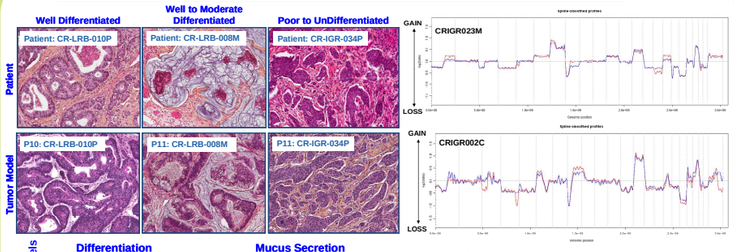
- **Significant correlation* between CEA serum level of patient and tumor take of all types**

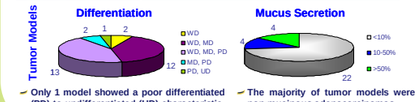


- **Significant distribution* of primary tumors in regards of lymph node status (panel A, N1 = 1 to 6, N2 = 7 to 15 positive regional lymph nodes), stages (panel B) and differentiation status (panel C).**
- **No other significant correlation were found among the following parameters: gender, age, resection extent, lymphatic embolies, perinervous invasion, initial treatment, genotype**

*** p values were calculated with logical regression test**

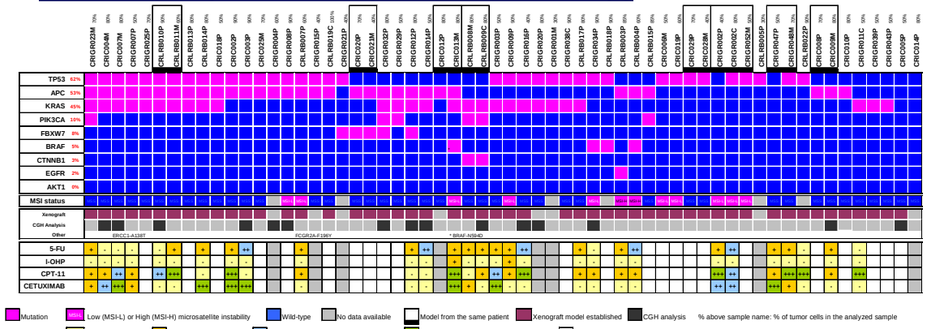
Preservation of the tumor phenotype and genotype





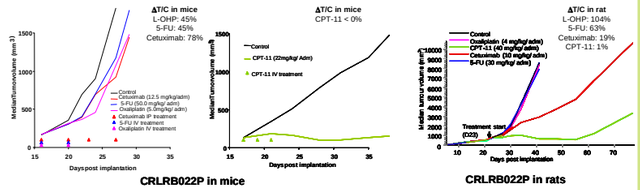
- **Only 1 model showed a poor differentiated (PD) to undifferentiated (UD) characteristic, as well as its correspondent patient**
- **The majority of tumor models were non-mucinous adenocarcinomas**
- **Histopathological analyses completed for 30 colon tumor models were in concordance to those observed in the corresponding patient's tumor.**
- **CGH analysis showed very similar profile between early and advance passages**
- **The expression and localization of β-catenin, CEA, CAIX, EGFR, CD105 and LYVE-1 is ongoing.**

Gene mutation profile and response to chemotherapy



Activity score	-	+	++	+++
5-FU	7	17	5	0
L-OHP	27	2	0	0
CPT-11	5	12	4	8
Cetuximab	15	4	3	7

- **Multiple mutation profiles are observed in our tumor panel (n=60), matching the human tumor genetic heterogeneity**
- **Pharmacological studies exhibit diversity in the response to chemotherapy**
- **13/29 tumorgrafts show tumor regression after treatment with at least one standard monotherapy**



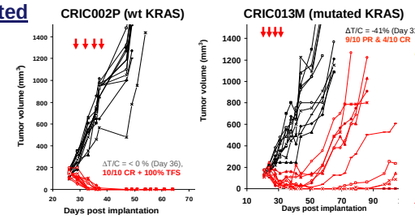
CRLRB022P in mice **CRLRB022P in rats**

- **Similar tumor response ranges have been observed in mice and rats**

Sensitivity to Cetuximab versus mutated genes involved in EGFR pathway

	Activity score	-	+	++	+++
Mutations	KRAS	8	1	1	3
	KRAS/PIK3CA	1	2	0	0
	KRAS/BRAF	0	0	0	1
	BRAF	2	0	0	0
EGFR mutated pathway		11	3	1	4
EGFR wild-type pathway*		3	1	2	3

* Mutations of PTEN or other genes related to EGFR pathway have not been yet characterized



- **Most models with EGFR mutated pathway are resistant to Cetuximab, nevertheless several Cetuximab sensitive KRAS-mutated models have been identified.**

CONCLUSION

- *Diversity of colorectal cancers is fully addressed in this collection of patient-derived tumor models.*
- *Genotype and phenotype are largely preserved throughout the model establishment process.*
- *Difference of gene mutation and drug sensitivity profiles are observed between the models.*
- *Plan to complete the full correlation analysis between clinical data, gene mutations, transcriptome profile, ex-vivo and in vivo drug sensitivity.*
- *Perspectives to fully exploit this new collection for new drug candidate selection.*