Extensive use of fresh patient-derived tumor samples for drug discovery and drug positioning in oncology

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Jean-François Mirjolet, Nicolas Hoffmann, Olivier Duchamp, Marc Hillairet de Boisferon, Cyril Berthet, Francis Bichat, Jan Hoflack
Oncodesign®, Dijon, France

Introduction / Abstract

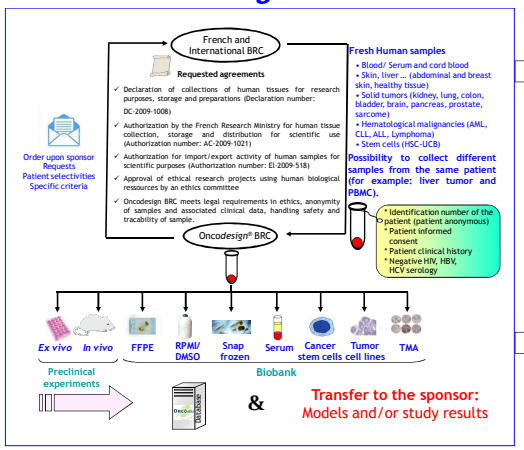
Most preclinical investigations in Oncology research are performed using permanent cancer cell lines that have been kept in continuous *in vitro* passages since decades. These plastic-cultured cells became very different from their original tumors since accumulated genetic abnormalities and multiple selections of subclones occurred over time. The use of fresh patient-derived tumor samples for *ex vivo* assays and for establishment of new relevant *in vivo* tumor models allows investigating the anti-tumor activity of new therapies directly linked with clinical reality. In this respect, Oncodesign® has developed a large and international network of clinical centers for the collection of a large number of fresh patient-derived tumor biopsies from all cancer pathologies and from healthy tissues. The collection of these samples is done under ethically approved master agreements and with the signed consent of each patient. The patient's clinical history, the serology results (HIV, HBV and HCV) and tissue banking are centralized in our internal approved biological resource center.

Examples of *ex vivo* assays will be presented based on patient-derived acute lymphoblastic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia and other hemopathologies using chromium release and Annexin V FACS assays. These studies aimed to demonstrate the CDC, ADCC and apoptosis induction of new therapeutics antibodies whereby approved antibodies such as rituximab and alemtuzumab were used as positive controls. Similarly, these fresh patient-derived models were used to study the direct dose-response effect of new chemotherapeutic agents through apoptosis induction (bortezomib used as positive control).

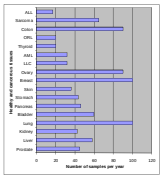
The freshly collected tumors were also used to establish new *in vivo* tumor models in different strains of mice and rats. The full characterization of the genetic patterns of the xenograft derived tumors was compared with the original collected human tumors and with the clinical data of the patient. A panel of "standard of care" compounds was tested in these new tumor models for pharmacological characterization. The expansion of such tumors at early passages in mice was used to implement a reproducible *ex vivo* 3D assay. The assay was developed to investigate the potential anti-tumor activity of conventional and targeted therapies, and allows sufficient throughput for use during drug discovery lead optimisation. *Ex vivo* drug effects were then correlated with *in vivo* results using the same panel of tumorgrafts.

The use of fresh patient-derived tumors in drug discovery and early preclinical development of new therapies aimed at corroborating results with clinical reality. Altogether, these processes from the clinical tumor collection to the *in vivo* drug efficacy study through *ex vivo* assays should help the preclinical drug selection, development and clinical positioning as well as companion biomarker identification.

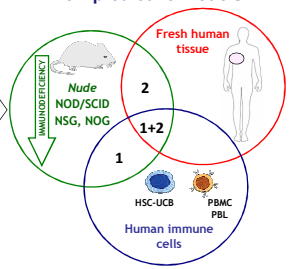
Oncodesign® BRC



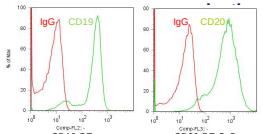
Collection opportunities



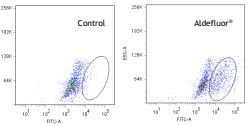
Chi-mice® platform to establish new predictive models



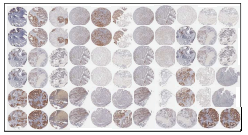
Target identification and patient



FACS analysis of CD19 expression on PBMCs from a fresh patient-derived chronic lymphocytic leukemia (CLL). PBMCs were sorted using magnetic beads and then analysed for CD20 expression by FACS.



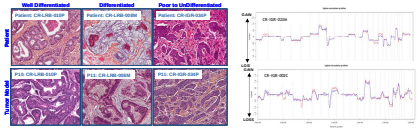
FACS analysis of ALDH positive cells isolated from fresh patient-derived lung tumor tissue using Aldefluor® kit (StemCell Technologies). These ALDH+ cells were sorted on a BD FACS Aria® and derived cell line was established.



Tissue microarrays are used to validate clinical relevance of potential biological targets in the development of diagnostics, therapeutics and study new protein markers and genes.

Histological and molecular characterization

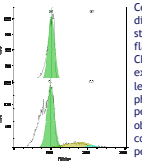
Histopathological analyses completed for 53 colon tumor models (#) were in concordance to those observed in the corresponding patient's tumor.
CGH analysis showed very similar profile between early (blue) and advance (red) passages *in vivo*.



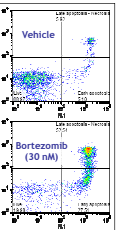
Conclusions

- Oncodesign® has built up an unique private approved biologic resource center in accordance with European legal and ethical rules,
- Oncodesign® BRC has developed an international network to provide (all fresh) biological samples needed for preclinical investigations,
- The BRC associated to the Chi-mice® platform allows the establishment of new advanced and predictive experimental models,
- The combination of patient-derived models with reconstituted human immune system in mice aims to improve efficient translational drug discovery and drug-positioning in Oncology and other related pathologies.

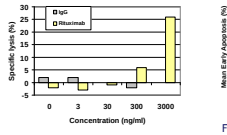
Ex vivo drug evaluation in hematological malignancies



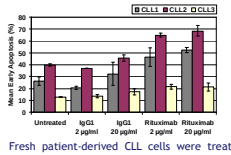
Cell cycle analysis (monovariate distribution of propidium iodide staining) after a 24hr treatment with flavopiridol on fresh patient-derived CLL cells. Cells from CLL samples exhibit a non-proliferative state with less than 5% of cells in S and G2/M phases of cell cycle. An increase in the percentage of cells in sub-G1 was observed after treatment with a corresponding decrease in the percentage of cells in G0/G1 phase.



Fresh patient-derived CLL cells were treated for 24hr with bortezomib or vehicle and analysed by FACS for apoptosis induction. Results are presented as bivariate distribution of Annexin V-FITC (x axis) *versus* 7-AAD (y axis) labeling.
Apoptosis was induced on CLL cells after bortezomib treatment.

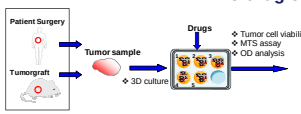


Analysis of antibody-dependent cell cytotoxicity (ADCC) of rituximab or irrelevant IgG by ^{51}Cr release assay on fresh patient-derived CLL cells. ^{51}Cr loaded cells were treated for 30 min with various concentrations of each antibody. Thereafter, purified and IL-2 activated NK cells (effector cells) were added for 3hr.
Dose-dependent ADCC of rituximab was specifically demonstrated using CLL cells.

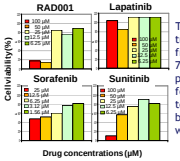


Fresh patient-derived CLL cells were treated for 24hr with rituximab or isotype control and analysed by FACS for apoptosis induction using 7-AAD and FITC-conjugated Annexin V. Heterogeneous early apoptosis induction was evidenced on CLL cells after rituximab treatment.

Ex vivo drug evaluation in solid tumors

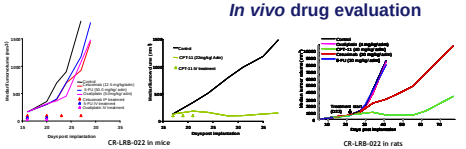


In this example, the TREI-002 renal patient-derived xenograft tumor was sensitive to RAD001, sorafenib and sunitinib and was not sensitive to lapatinib *ex vivo* treatments.

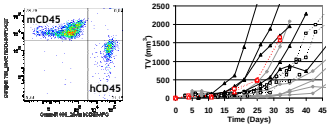


Tumors, collected in patient or in tumor-bearing mice, were cut in small fragments of 1-2 mm and cultured for 72hr in 96-well poly-HEMA coated plates. Tumor fragments were treated for 24hr with either vehicle (DMSO) or tested drugs (5 concentrations between 0-100 µM). MTS/collagenase was used before OD analysis.

In vivo drug evaluation



Similar tumor response ranges have been observed in mice and rats in 53 colorectal patient-derived models (#) with some advantages for rat models as seen in this example (CR-LRB-022 model is sensitive to CPT-11 both in mice and rats and sensitivity to cetuximab was only seen in rats).



Whole body irradiated NSG newborn mice were inoculated *via* the intracardiac route with CD34+ cells isolated from UCB samples. Peripheral blood chimerism was analysed by FACS on week 12 by detecting both mouse and human leukocytes. Twenty six weeks after humanization, subcutaneous tumours were induced by xenografting human patient-derived ovarian tumor fragment (TOVA-002) into the right flank of humanized NSG mice (D0).
Take and growth-rate of TOVA-002 patient-derived tumor was not modified when xenografted on humanized NSG mice.

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