

Foreign Correspondent – December 2009

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The French programme CIT “carte d’identité des tumeurs” (<http://cit.ligue-cancer.net/>) is piloted by the French charity “Ligue Nationale contre le Cancer” (LNCC), a non-profit association and first non-governmental financial resource for cancer research in France, represented throughout France by committees which finance the program through donations and membership. The CIT program, the leading national cancer genomics program in France, is also supported in part by the INCa (French National Cancer Institute, part of the “plan cancer” which was set up by the former President Jacques Chirac, and coordinates the national policy against cancer, <http://www.e-cancer.fr/>).

CIT program aims at characterizing multiple types of tumors through the coupled genomic analysis of gene expression and chromosomal alterations. The CIT program involves 60 teams, including clinicians and researchers, and offers one of the largest tumor databases in Europe containing approximately 7,000 annotated tumor samples and 9,000 micro-array experiments. The brain tumor collection, which represents 12% of the total database, ranks third after breast and colorectal cancer.

Tumor samples, obtained with the informed consent of the patients, are immediately cryoconserved at -80° C following surgery. The database contains the clinical follow-up, the data generated by each of the technological platforms and the results of the analyses. In order to ensure a high standard of quality and reproducibility of results, the CIT program has set up at the Hôpital Saint-Louis (Paris) a platform dedicated to the extraction and qualification of RNA and DNA (Qiagen QIAmp DNA Mini and RNeasy Mini kits, and more recently AllPrep DNA/RNA Mini kit for simultaneous RNA and DNA extraction, the quality of the samples is assessed using agarose gels and electrophoregram profiles with Agilent Bioanalyzer 2100). The samples are processed on the same platform, with validated and standardized protocols, to optimize the yield of the platform and implement precise quality controls (as a consequence 25% of RNAs sample are discarded).

At the start of the program, the products on the market did not meet the CIT requirements and the program therefore developed a CGH (“Comparative Genomic Hybridization”) BAC chip. It is the result of a partnership between the “Ligue nationale contre le Cancer”, the Genoscope, the Curie Institute (Dr O Delattre) and Integragen company. The chip was enriched through the years: the final version including over 6,000 BACs in quadruplicate and distributed on average every 500,000 bases throughout the genome. The density of the

coverage is increased in genomic regions known to contain genes involved in cancers. The technique has been transferred to the Pitié-Salpêtrière Hospital and nearly 800 gliomas have been genotyped with this technique in Paris (see below).

Since 2008, the genome analyses have been carried out with the high resolution "Bead Array" technology by Illumina ("Human CNV 370 Quad" chip) that contains over 370,000 probes throughout the genome with a median coverage of one probe every 5,000 bases (vs 500 000 bases for the CGH BAC Chip). The hybridizations are carried out on the Integragen commercial platform. In addition to the greater density of coverage and the availability of a large set of "Copy Number Variants" (CNV), this chip brings a new dimension to the genome studies by allowing the determination of allelotypes. The power of this technique has been recently illustrated by the work performed by an international consortium that included a substantial contribution of the Pitié-Salpêtrière group. The genome wide analysis of 4500 patients with gliomas and over 6500 controls identified five risk loci for glioma, demonstrating that common low-penetrance susceptibility alleles contribute to the risk of developing glioma. In addition, the DNA of 160 gliomas have been analysed to date (mainly oligodendroglial tumors from the French network "POLA", see below)

The micro-arrays used for the transcriptome analysis are the Affymetrix GeneChip® (currently HG U133 Plus 2.0). These chips allow the study of over 47,000 transcripts which corresponds to 38,000 genes in each tumor. The hybridizations of the tumor samples are carried out on the IGBMC genomic platform in Strasbourg. Standardized protocols are set up at all stages of the process (preparation of the samples, hybridization, scanning, image analysis etc). A key objective of the CIT program is to process the greatest possible number of samples on the same platform to minimize bias related to the experiments, to optimize the parameters of the different stages and to enable comprehensive quality control checks.

To date, nearly 1,000 brain tumors (mainly gliomas) have been analysed by CGH-array in the CIT program. First CGH-arrays were based on BAC array: nearly 800 tumors were analyzed in the program "CGH-array at bedside", sponsored by the LNCC and carried out at the Pitié-Salpêtrière hospital, Paris, from 2006 to 2008. CGH-array was performed within 15 days from surgery in order to propose a personalized management based on clinical, histological and genetic profile. In 2008, the technique switched from BAC array to the much more resolutive "Human CNV 370 Quad" chip (see above) and CGH-array is performed electively on anaplastic oligodendroglial tumor (no longer in GBM) and is integrated in the POLA project, the French National Network devoted to clinical and translational research on anaplastic oligodendroglial tumors. Taken together the data from nearly 1,000 gliomas of

different grade with clinical annotations and follow-up data are available for current and further analysis.

Transcriptome analysis has been performed for more than 200 gliomas, and has been correlated with chemosensitivity and with genomic profile. An ANOCEF (Association des Neuro-oncologues d'Expression Française) consortium (<http://www.anocef.org>) analysed the correlation between GBM transcriptomic profile and response to treatment (Ducray *et. al.* Mol Cancer in press).