

- 1. Why use blood since only a small number of CTC's are found in the blood?**
- 2. Making sure of the purity of CTC's harvested.**
- 3. Relevance between the analyzed cells and the risk of relapse in clinical reality.**
- 4. Change of phenotype-genotype during expansion-cultivation.**
- 5. Non linearity between genotype and phenotype.**

I. Our first innovation is selecting the most appropriate sample: It is well known and widely accepted in the scientific community that the primary tumor consists of the stroma [fibroblast, monocytes, lymphocytes, vessels, etc.] and the malignant cells which [are in-homogenous] since they are composed from different subgroups and subclones with different features and abilities. Only very few populations will develop metastatic ability which will allow them to invade the surrounding tissue and pass into the circulation and perform the epithelial to mesenchymal transition (EMT). These are known as the circulating tumor cells [CTC's]. A large proportion of the CTC's are actually cancer stem cells. These cells have all the necessary information and ability for micro colonization and micrometastases and will later develop into potent micrometastases. For this reason RGCC has selected blood samples as the most appropriate form for analysis since it includes the cancer cells with the relevant information for both calculating the risk and for the potent metastases.

II. Innovation in finding and isolating the CTC's: RGCC uses powerful sorters and flow cytometers as well as negative selection based interrogation. We are able to actually isolate the relevant CTC's and not enrich them. Hence, we manage to have a pure sample of CTC's and simultaneously harvest all the group of CTC's from a single blood sample.

III. Appropriate expansion of the CTC's: The CTC's will expand as the cancer stem cell like cell and then enter in exponential phase of growth which will generate a respectful number of circulating stem cells [CSC's] in a very short period of time. At the same time we manage to keep intact the both genotype and phenotype of the cells and avoid any changes to the primary [CSC]. Therefore, after the final expansion we have maintained the identical genotype.

IV. Gene-expression profile of the CSC's: The expanded cells will be analyzed for one expression profile in a hall genome micro-array analysis. Hence, we now have all the information concerning epigenetic screening of CSC's. This profile will indicate to us which therapy the CSC's are sensitive or resistant to.

V. Verification of chemosensitivity/chemoresistance in viability assays: The information obtained from the gene expression analysis will be validated in a micro culture where the effect of each indicated substance will be tested. These assays overcome the problem of linearity between gene expression and protein expression levels.

I hope this information is sufficient and will help you deal with any concerns and/or misinformation that you may have.

In case you may have any additional inquiries, please feel free to contact me at any time. It is always my pleasure to help and assist you anyway we can.

Sincerely,
Dr Ioannis Papatiriu MD
Medical Director of
R.G.C.C. Ltd-Research Genetic Cancer Centre Ltd

RGCC TESTS INFORMATION :

ONCOCOUNT TEST: This test will report **only the number of Circulating Cancer Tumor cells (CTC's) isolating and quantification**. It does not include any other information concerning the CTC's or Circulating Cancer Stem Cells (CSC's) Immunophenotyping.

This test helps out in the detection of CTC's for the early detection of relapse or remission condition or Recurrence case and sometimes helps in early detection of cancers.

ONCOTRACE TEST: This test involves the isolation and enumeration of CTC's and the information on **immunophenotyping** (studying the markers on CTC) in order to predict the origin of CTC's from the unknown tumor.

This test is a follow up test for the unknown tumor origin. This test is a control diagnostic analysis and is suitable to exclude and active tumor process.

ONCOTRAIL TEST : This test is a tailor made test for specific type of malignancy such as breast cancer (Oncotrail Breast), prostate (Onco trail prostate) , Colon, Melanoma, lung, Sarcoma, Gastrointestinal for follow up control.

ONCOSTAT TEST/ONCONOMICS TEST This test includes the isolation and enumeration of CTC's and the chemosensitivity/chemoresistance assessment for **cytotoxic drugs, monoclonal antibodies**, and small molecular weight molecules that inhibit specific targets eg. (TKI, etc.,) drugs list include all the available drugs in the market including monoclonal antibodies (80 to 100 drugs). Along with these the **Gene Profiling** of cancer related genes of nearly 72 genes at epigenetic level.

For drugs list and genes list please go through the website www.neeligenetics.com/www.rgcc-group.com .

ONCOSTAT EXTRACTS TEST/ ONCONOMICS EXTRACTS TEST : This test includes the isolation and enumeration of CTC's and the chemosensitivity/chemoresistance assessment for the **natural substance and natural extracts** for anticancer potency on the CTC's isolated from the provided blood sample. (Gene profiling will not be provided)

ONCOSTAT PLUS TEST/ ONCONOMICS PLUS TEST This test is the combination of **ONCOCOUNT TEST**, **ONCOSTAT TEST** and **ONCOSTAT EXTRACTS TEST**.

This test includes the isolation and enumeration of CTC's and the chemosensitivity/chemoresistance assessment for **cytotoxic drugs, monoclonal antibodies**, and small molecules that inhibit specific targets eg. (TKI, etc.,).

The assessment of **natural substances and natural extracts** for anticancer potency.

This test also includes the report of the **Genetic Profiling** of each and every Tumor related Genes at epigenetic level.

IMMUNOSTAT TEST: Profile of humoral and cellular immunity and cachexia. This test uses specific cellular markers and cytokine production to detect the types/types of cells that are responsible for activation / repression of immune system of host.

METASTAT TEST : Markers on CTC's that point out the potential organ for relapse. It can help practitioners in the prognosis of metastasis trends in cancer patients and guide them in the choice of appropriate chemotherapy.