NutriPATH

INTEGRATIVE PATHOLOGY SERVICES

RGCC

Circulating Tumour Cells

PRACTITIONER MANUAL

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CIRCULATING TUMOUR CELLS

Circulating Tumour Cells (CTC) are cancer cells which have broken away from the primary tumour and have entered the blood stream where they circulate and have the potential to generate metastatic disease.

These cells can be isolated and identified, and there is growing interest in their detection for the following purposes:

- The early detection and diagnosis of new cancers;
- Monitoring of existing cancers;
- Prognosis – providing information about the risk of recurrence of a current or previous.

THERAPEUTIC CONCEPT

The tumour is made up from several subgroups of cells (subpopulations) with different features. One of the subset actually drives the progress of the disease, the resistance to therapy and the relapse. This subset is called cancer stem cell-like cells or tumour initiating cells.

When a patient is treated and the cancer cells may be destroyed, then when the compatible diagnostic does not discover any signal, the cancer cells may consist of a population of 10^9 to 10^{12} cells. This limit defines the remission stage of a patient. At that stage only the cancer stem cells may survive and colonise into distant organs and generate metastases in time.

Hence the usage of this test is to detect which therapeutic approach the cancer cell may respond to. Also during remission it is essential to detect, discover and explore the features of the disseminated cancer stem cell-like cells in order to delay the risk of relapse and to generate options to treat these kinds of cells. The main goal is to discover, analyse and screen the cancer cells in every step of the disease.

Personalised Cancer Testing

Early detection offers chemosensitivity testing and therefore, personalised treatment of cancer.

R.G.C.C. Ltd. offers a range of individualised tests from a world-class molecular oncology laboratory in Greece.

These tests can:

- Detect early signs of a developing cancer;
- Help to monitor existing cancers; and
- Produce an individual profile of which cancer drugs and which natural substances can be used to achieve the best treatment outcome.

Chemo-sensitivity testing

Oncologists principally rely on the statistical analysis of large treatment trials to decide which drugs to use for specific cancers. There is a growing interest in personalised cancer therapy which involves identifying those treatments which may work best for an individual's cancer.

Chemo-sensitivity testing is one method of doing this. Chemo-sensitivity testing involves testing an individual's cancer cells in the laboratory to see which drugs demonstrate the best response. It therefore provides guidance about which treatments may be best for the individual in clinical practice.

Tumour cells are identified and isolated from the sample for the following analysis:

- Viability testing of chemotherapy drugs;
- Genetic profiling for guidance about targeted therapies e.g. monoclonal antibodies;
- Viability testing (and identification of mechanisms of action) of natural substances which may be used as part of a complementary treatment strategy.

The results are presented in a written report which can be used to help guide treatment options and choices.

In addition, R.G.C.C. International can provide information to the practitioner about how an individual will 'handle' specific chemotherapy agents. Our genetic makeup determines whether we are 'accumulators' or 'rapid metabolisers' of certain drugs. This can play a critical role in determining how effective a specific treatment is likely to be for us, and how significant the side effects will be.

WHO ARE THESE TESTS FOR?

- Clients who want to actively engage in reducing their risk of developing cancer in the future.
- Clients with an increased risk of cancer e.g. due to family history or lifestyle / environmental issues who want the opportunity to engage in a screening program for early detection and diagnosis.
- Clients with a current diagnosis of cancer who want more information about treatment options for them as an individual – including natural treatments.

SCIENTIFIC EVIDENCE

It is well established by literature and validation that the overall response rate from empirical chemotherapy varies from 5 to 7.5%. *Royal North Shore Hospital, Clin Oncol (R Coll Radiol) 2005 Jun;17(4):294.*

Pharmaceutical industry assesses and validates candidate drugs by utilising extensive chemosensitivity assays under the term of High Throughput Screening (HTS).

Cancer is caused by severe damaged genetic material which leads to random genetic instability, so each malignancy behaves differently to each individual.

Each person has different genetic fingerprinting from the others. This classifies people to:

- Rapid metabolisers (individuals that metabolize fast the drug without receive any benefit from it).
- Accumulators (individuals that cannot excrete the byproducts of a drug that causes severe side effects and toxicities).
- Normal metabolisers (these individuals can normally metabolise a drug to its active form and excrete the byproducts normally).

Hence based on the above, each patient and each malignancy has its own identity and behaves individually and differently from person to person.

Therefore personalised treatment becomes essential in order to generate better rates of successful treatments in cancer.

For adjuvant chemotherapy (chemotherapy given in addition usually to surgery and/or radiation) the success for 5 yr survival rate for the 5 major types of malignancy (breast, colon, lung, prostate, skin) varies from 2.1% to 2.3%. *Royal North Shore Hospital Clin Oncol (R Coll Radiol) 2005 Jun;17 (4):294*

For the curative stage of disease the success rate varies between 5-7.5% for the same 5 types of malignancies.

The overall contribution of curative and adjuvant cytotoxic chemotherapy to 5-year survival in adults was estimated to be 2.3% in Australia and 2.1% in the USA.

Various downloadable pdfs are available at:
http://www.rgcc-genlab.com/?page=booklets&section=Physisians

COMPARATIVE METHODS

	Beads-based method	PCR-based method	R.G.C.C. Ltd
Method of isolation	Magnetic Beads (antibodies with iron particles)	PCR based method which need to destroy the cells in order to identify one marker (mainly panCK or Epcam)	Flow cytometric sorting with interrogation in droplets in ratio of droplet per cell (1:1)
Purity of CTCs	Enrichment method and not isolation method	There are no cells left	Purity is higher than 97 to 99% (isolation
Viability of the isolated cells	70-85%	No cells	Viability >99%
Quality of CTCs for further analysis	Inappropriate for further molecular analysis due to lymphocyte contamination	Limited for further molecular analysis	Appropriate for further molecular analysis since there is no noise
Selection of CTCs	Based mainly in positive selection of CTCs in a few number of markers	Based on positive selection	Based on negative and positive selection in order to identify and secondly immunophenotyping CTCs
Additional features	Method only to enumerate CTCs	Method to enumerate CTCs and identify only very limited features of CTCs	Method which allows performing gene expression assays and determining features vital for therapy scheduling.
Further abilities			Identification of heterogeneity of CTCs

Further Comments

"Evidence for the existence of biologically distinct CSC's, first demonstrated in a haematological malignancy and in the past 5 years in several solid tumours, has shaped a new paradigm of human cancer as a hierarchical disease whose growth is sustained by a population of CSC's. This conceptual shift has important implications not only for researchers seeking to understand mechanisms of tumour initiation and progression, but also for the development and evaluation of effective anticancer therapies".

"Thus, research must be directed at the relevant cell populations as identified through functional assays, the ultimate goal being the rational development of therapies that interfere with the oncogenic program within the CSC's."

"In contrast, the CSC model postulates that with an appropriate purification strategy, the CSC's with the capacity to initiate and sustain tumour growth in vivo can be identified and isolated from the bulk cells that do not have tumour-initiating activity."

Vincent T., Jr. DeVita, Theodore S. Lawrence, Steven A. Rosenberg - Cancer: Principles & Practice of Oncology: Primer of the Molecular Biology of Cancer, Lippincott Williams & Wilkins; 1 Pap/Psc edition May 2011, Page 163, 164.

"Here we show that a small population of cancer stem cells is critical for metastatic colonization, that is, the initial expansion of cancer cells at the secondary site, and that stromal niche signals are crucial to this expansion process". *Malanchi I, Martinez-Santamaria A, Susanto E, Peng H, Lehr HA. Interactions between cancer stem cells and their niche govern metastatic colonization. Nature 481,85–89 (05 January 2012) doi:10.1038/nature10694.*

"Cancer lethality is mainly due to the onset of distant metastases and refractoriness to chemotherapy. Growing evidence indicates that a cellular subpopulation with stem cell-like features, commonly referred to as cancer stem cells (CSC's), is critical for tumour generation and maintenance". *Maugeri-Saccal M, Vigneri PG, De Maria R. Cancer Stem Cells and Chemosensitivity. Clin Cancer Res. 2011 Aug 1;17(15):4942-7. doi: 10.1158/1078-0432.CCR-10-2538.*

…most primary solid tumours probably go through a prolonged state of avascular, and apparently dormant, growth in which the maximum size attainable is ~1–2 mm in diameter. Up to this size, tumour cells can obtain the necessary oxygen and nutrient supplies they require for growth and survival by simple passive diffusion; (ii) these microscopic tumour masses can, in some way, eventually switch on angiogenesis by recruiting surrounding mature host blood vessels to begin sprouting new blood vessel capillaries which grow toward, and eventually infiltrate the tumour mass, thus setting in motion the potential for relentless expansion of the tumour mass and haematogenous metastatic spread as well… *Robert S. Kerbel ,Tumour angiogenesis: past, present and the near future, Carcinogenesis (2000) 21(3): 505-515 doi:10.1093/carcin/21.3.505*

"The problem is, when we treat cancer cells with chemotherapy, the cancer stem cells are being stimulated to grow too….When we take mesenchymal stem cells and mix them with tumour cells, the tumours grow much more quickly in animals." Dr. Wicha's lab has found that inflammatory molecules secreted by dying tumour cells can hook up with the stem cells and cause them in effect to come out of hibernation.

- Adult stem cells exist in most tissues, and go into action to repair damage from wounds or infections.
- In cancer, they can mutate and no longer obey normal bodily signals to stop growing, Dr. Wicha said.
- He and other researchers say that even when chemotherapy and radiation cause tumours to shrink dramatically, these stem cells can stay alive, living under the radar until they are once again spurred into action.
- They also believe stem cells are probably the ones that break away from an original tumour and cause cancer to spread elsewhere in the body.
- Chemo and radiation kill off the fastest-growing cells in the body, which applies to most cancer cells, but the cancer stem cells that create those rapidly dividing tumour cells actually grow much more slowly themselves, and are less susceptible to those therapies, he said.
- One tactic to address this problem is to kill off both types of cancer cells at once, Dr. Wicha said.
- A recent experimental trial with advanced breast cancer patients at the University of Michigan, Baylor University in Texas and the Dana-Farber Cancer Institute at Harvard University used standard chemotherapy along with a substance designed to block one of the biochemical pathways of stem cells.
- The approach killed off more than 90 percent of the cancer stem cells, Dr. Wicha said, and researchers now hope to expand the treatment to a much larger group of patients.
- Ultimately, he hopes cancer treatments can avoid general chemo altogether, with its debilitating side effects, and just use targeted therapies against the stem cells.
- There is still a long road ahead, he said, and "my feeling is, to really knock these stem cells out, we're probably going to have to use multiple inhibitors."

Max Wicha, M.D.
Distinguished Professor of Oncology
Director, University of Michigan, Comprehensive Cancer Centre

You Tube http://weeksmd.com/?p=5014 and
http://wn.com/professor_max_wicha__breast_cancer_stem_cell_regulation

Dr Bruce Zetter Professor of Cancer Biology in the Department of Surgery, Boston Children's Hospital

“As many as 90% of all cancer deaths can be attributed to metastatic disease. Cells from the primary tumour, after travel to regional or distant sites, cause failure of essential organs including the lungs, liver, brain and bone marrow. Significant advances in the field make this an exciting time for the study of metastasis. Genetic signatures in primary tumours as well as circulating tumour cells and oligonucleotide or protein biomarkers hold the promise of predicting cancer outcomes and allowing selection of optimal drug candidates. The isolation of circulating tumour cells has further improved our ability to analyse the tumour genotype. The ability to metastasize is influenced by the invasive potential of cells in the primary tumour and particularly by self-renewing tumour cells that have properties of cancer stem cells”.

http://usatoday30.usatoday.com/news/health/story/health/story/2012-02-27/Cancers-growing-burden-the-highcost-of-care/53271430/1

TESTS AVAILABLE

Screening/Follow up assessments

TEST NAME and CODE	ASSESSMENT
ONCOTRACE [7006]	Measures the concentration of CTCs and the immune-phenotype control of these cells.
ONCOTRAIL [7007]	Measures relevant markers for specific type of malignancies i.e. • BREAST Oncotrail • PROSTATE Oncotrail • COLON Oncotrail • MELANOMA Oncotrail • LUNG Oncotrail • SARCOMA Oncotrail • GASTROINTESTINAL Oncotrail
IMMUNOSTAT [7009]	Profile of humoral and cellular immunity and cachexia. This test uses specific cellular markers and cytokine production to detect the type or types of cells that are responsible for the activation or repression of the immune system of a patient.
METASTAT [7010]	Markers on CTCs that point out the potential organ for relapse. It can help practitioners in the prognosis of metastases trends in cancer patients and guide them in the choice of appropriate chemotherapy.

Primary assessments

TEST NAME and CODE	ASSESSMENT
ONCOCOUNT [7008]	Measures the quantity of CTCs only. *(It does not include any other information concerning CTCs)*
ONCOSTAT [7001]	Measures the chemo-sensitivity / chemo-resistance assessment for cytotoxic drugs, monoclonal antibodies, small molecules that inhibitor specific targets (TKI etc).
ONCOSTAT PLUS [7003] (former TU Profile Plus)	Measures the chemo-sensitivity / chemo-resistance assessment for cytotoxic drugs, monoclonal antibodies, small molecules that inhibit specific targets (TKI etc) *AND* the assessment of natural substances and extracts for anticancer potency.
ONCOSTAT EXTRACTS [7002] (former TU Profile Extracts)	Measures only the assessment of natural substances and plant extracts for anticancer potency.

CHEMOTHERAPEUTIC & NATURAL SUBSTANCES TESTED

ALKYLATING AGENTS	INHIBITORS OF TOPOISOMERASE	TUMOUR RELATED GENES	TUMOUR RELATED GENES	TUMOUR RELATED GENES
ACNU	Amrubicin HCl	5-LOX	DNA methyltransferase-1	NR3C4-A
BCNU	Dactinomycin	67LR	DPD	NR3C4-B
CCNU	Daunorubicin	6-methyl-DNA-tran	EGF	P16
Altretamine	Doxorubicin	ALK	EML-4-ALK	P180
Bendamustine	Liposomal Doxorubicin	ANG 1	EpCSM	P27
Bleomycin	Epirubicin	ANG 2	ERCC1	P53
Cisplatin	Etoposide	Bax	Estrogen recep.	PDGF
Carboplatin	Idarubicin	Bcl-2	FGF	Progesterone rec.
Chlorambucil	Mitoxandrone	Bcr-abl	Gamma GC	PTEN
Cyclophosfamide	**NUCLEUS SPINDLE STABILIZER I & II**	CD177 (c kit)	GARFT	Ras-Raf-MEK-Erk
Dacarbazine	Abraxane	CD20	HAT	RET
Estramustine	Cabazitaxel	CD33	Histone-deacetylase-dipeptide	Ribonucleoside reductase
Hydroxyurea	Docetaxel	CD52	HDAC	RRM1
Ifosfamide	Eribulin	CD95 (fas-r)	HSP27	SHMT
Melphalan	Paclitaxel	CDC6	HSP72	SS-r
Mitomycin	Vinblastine	c-erb-B1	HSP90	TGF-b
Nedaplatin	Vincristine	c-erb-B2	h-TERT	TP
Oxaliplatin	Vinorelbine	CES1-2	IGF-r 1	TS
Procarbazine	**NUCLEOSIDE ANALOGUES**	c-Fos	IGF-r 2	Up
Temozolomide	5FU	c-Jun	IkB (A,b,c,)	VEGF
Treosulfan	Capecitabine	C-MET	JAK1/2	
Trofosfamide	Cytarabine	COX2	KISS-1-r	
EPOTHILONES	Fludarabine	CXCL12	MMP	
Ixabepilone	Fudr	CXCR12	Mn23	
INHIBITORS OF TOPOISOMERASE I	Gemcitabine	CXCR4	MTor	
CPT11	MTX	CYPB1	NFkB	
Gimatecan	Pemetrexed	DHFR	Np	
Topotecan	Raltitrexed	DNA demethylase	NPM ALK	

Chemotherapeutic & Natural Substances Tested (contd)

BIOLOGICAL MODIFIERS	BIOLOGICAL MODIFIERS		NATURAL SUBSTANCES	NATURAL SUBSTANCES
5-azacytidine	Ipilimumab		AHCC – Active Hexose Correlated Compound	Metformin
Abiraterone	Lapatinib		Amygdalin (B17)	Mistletoe
Alemtuzumab	Leprolide		Anvirzel™	Naltrexone (LDN)
Anastrozol	Nilotinib		Arabinogalactan	Nrf2 Activator™
Anti-androgen	Octreotide		Aromat8-PN™	Micellized D3
Axitinib	Ofatumumab		Artecin®	OPC Synergy ™
Bevacizumab	Olaparib		Ascorbic Acid	Paw Paw Cell-Reg ™
Bortezomib	Panitumumab		Bio D Mulsion	Poly-MVA
Brentuximab vedotin	Pazopanib		Bio-A-Mulsion Forte®	Polymannan Extract (PME)
Catumaxumab	Pertuzumab		Cellular Vitality	ProteoXyme™
Cetuximab	Regorafenib		Cruciferous Complete	Quercetone®
Crizotinib	Rituximab		C-statin (Vascustatin)	Research Aloe Extract (New PME)
Dasatinib	Ruxolitinib		Curcumin	Resveratin™ PLUS
Erlotinib	Semaxanib		CV247	Retenzyme Forte
Everolimus / Temsirolimus	Sorafenib		Dextrol	Salicinium ™
Exemestane	Sunitinib		Epimune Complex	Salvestrol Platinum
Fulvestrant	Tamoxifen		Fermented Soy Extract	Sodium dichloroacetate (DCA)
Gemtuzumab	Tositumomab (Bexxar)		Genistein	Super Artemisinin
Getifinib	Trabectedin		Indol-3-carbinol	Superoxide Dismutase (SOD)
Goserelin	Transtuzumab		Intenzyme Forte	Thymex
Ibritumomab (Xevalin)	Vandetanib		Lycopene	Ukrain
Imatinib-mesylate	Vemurafenib		Mammary PMG	Virxcan
	Vorinostat		Melatonin	Vitanox

COLLECTION INSTRUCTIONS

IMPORTANT PRE-COLLECTION INFORMATION

Please note that this test relies on using live blood cells for performing the analysis. Ensuring that these collection instructions are followed accurately will ensure that you receive accurate and meaningful results.

If the patient has undergone chemotherapy treatment, then the collection of blood should only occur 7 days after the last chemotherapy.

The clear liquid in the 50ml collection tube should NOT be thrown out.

Kit Contents

1 x 50ml conical tube with preservative buffer

1 x RGCC Request/Consent form

1 x Collection Instructions

1 x specimen transport bag

1 x cardboard transport cylinder

1 x TNT freight satchel

3 x sheets absorbent paper

Blood Specimen Collection

1. **Collect specimen as early in the day as possible on a MONDAY or THURSDAY ONLY.**

   DO NOT collect blood on the day prior to a public holiday.

2. Prior to having the blood specimen collected ensure that the **RGCC Patient Request / Consent form** has been completed and **signed by BOTH patient and treating practitioner**.

NutriPATH has blood collection arrangements in place for this test through Lifescreen Australia. Lifescreen Australia provides collection services through ALL state capital cities and major regional centres.

The patient should confirm that Lifescreen Australia is able to collect the sample from their locality. The patient will need to call **Lifescreen Australia** on **1800 686 000** and arrange a collection time. Ensure that the collection time is on a **Monday or Thursday morning ONLY.**

If the patient resides in a remote area that Lifescreen is unable to service, the patient should then contact NutriPATH on 1300 688 522 to organise other collection arrangements.

Patient Instructions – part 1:

On the day of the blood collection, have the Lifescreen pathology collector read through these collection instructions in order to be familiar with the required procedure, before collecting the blood specimens.

LIFESCREEN Pathology Collector Instructions:

1. The pathology collector should collect the required specimens provided in the NutriPATH collection kit.
   The blood MUST be collected using the 50 ml tube provided inside this collection kit.
2. When blood has been added to the 50ml tube, please ensure it is thoroughly mixed by gentle inversion/rolling.
3. All samples should be labelled with the patient's details (full name and date of birth).
4. The pathology collector should hand all specimens over to the patient after they have been collected.

Patient Instructions – part 2:

1. Wrap the blood sample with the 3 sheets of absorbent paper towel.
2. Place the wrapped specimen into the resealable section of the specimen transport bag.
3. Place all paperwork in the other section of the specimen transport bag.
4. Insert the specimen bag into the plastic inner tube of the transport cylinder and screw the cap on tight.
5. Place the plastic inner container into the outer cardboard container.
6. **DO NOT** put cold packs or ice packs near blood samples. This will destroy the blood cells and render the samples useless for testing. The specimens should be transported at ambient temperature.
7. Sign and date the TNT satchel consignment note. **DO NOT** alter any of the other consignment note details.
8. Place transport cylinder and seal in the TNT satchel provided. **Call TNT on 13 11 50** for pick up.

FREQUENTLY ASKED QUESTIONS

1. *Can anyone order this test?*

This test can only be requested through a medical or complementary health practitioner. Direct public requests will not be processed.

2. *What is the difference between ONCOTRAIL and ONCOSTAT?*

The ONCOTRAI**L** test is a tailor-made test for specific type of malignancies such as breast cancer (Oncotrail for Breast), prostate (Oncotrail for prostate), sarcoma etc. This test includes only markers relevant for a specific type of malignancy which make the test a good tool for follow up control.

On the other hand, the ONCOSTAT test includes the chemosensitivity / chemoresistance assessment for cytotoxic drugs, monoclonal antibodies, small molecules that inhibitor specific targets (TKI etc) and also includes the assessment of natural substances and extracts for an anticancer potency.

3. *What is the difference between ONCOSTAT and ONCOSTAT PLUS?*

The ONCOSTAT test includes only the chemosensitivity for cytotoxic drugs.

The ONCOSTAT PLUS includes the chemosensitivity for cytotoxic and natural substances. Thalidomide is also tested with the Oncostat Plus test.

4. *Is blood as good as tissue sample? Are there limitations of using blood?*

The main cell of interest of analytic platform is CTCs which obtained by negative selection and sorting via flow cytometer (so that we will obtain viable CTCs from a blood sample). In cases like Glioblastoma, cancer cells cannot be detected in a blood sample due to blood-brain-barrier and we need a tissue sample in order to be able to proceed with the test.

See FAQs at http://www.rgcc-genlab.com/?page=faq&cperpage=0&section=General.

5. ***Is the testing method recognised in Australia or TGA approved?***

R.G.C.C. registers its products at an international level not only in Australia. Our methods are recognized By ISO (http://www.iso.org/iso/home.html) See certificate at http://www.rgcc-genlab.com/Media/pdf/RGCC.ISO-17025.pdf .

6. ***Do they measure cancer derived CTCs as part of the ONCOSTAT EXTRACTS or do I need to do the prostate ONCOTRAIL as well***

Yes, the laboratory measures CTCs as part of the ONCOSTAT EXTRACTS as well.

7. ***Is the lab able to provide results of natural sensitivities and natural therapies that may specifically complement prescribed chemotherapy drugs for maximum effectiveness.***

We can perform additional tests for substances that are required by patient or doctor. We cannot indicate specific substance's tests.

8. ***In terms of the natural sensitivities test performed under the ONCOSTAT PLUS, will the results provided on the blood tests taken prior to treatment commencing still be relevant after chemotherapy or would the test need to be repeated again?***

The results provided on the blood tests taken that sent to laboratory. It is the patient and/or doctor to decide if they will send us a sample prior or after the chemotherapy. Have in mind that if the patient is under a chemotherapy treatment the blood collection should be performed strictly seven days after the last chemotherapy. After that a follow up test (ONCOTRACE or ONCOCOUNT) can be performed to see his/her clinical progress.

9. ***Is there data regarding success rates of treatment administered on basis of sensitivity testing recommendation?***

See RGCC website. Most questions are referred to this link http://www.rgcc-genlab.com/. Statistics are available as well.

Further information may be found at:
http://www.rgcc-genlab.com/?page=faq&cperpage=0&section=Science#undefined

WHY WE RECOMMEND & USE THE RGCC TEST

MEMBER OF TEXAS HEALTH CARE ENTITIES (A PRIVATE MEMBERSHIP MEDICAL ASSOCIATION, 3105 MAIN STREET, ROWLETT, TX 75088

TEL: 214-299-9449

www.rgccusa.com

We have been using this test for about 9.5 years. We were the first clinic in the US and Canada to start looking at a better more Personalized Patient-Centred Cancer Care to help those with cancer. This has been in place in Europe for over 10 years.

This is not 'The Greece Cancer Cure' from Dr. Hariton-Tzannis Alivizatos of Athens, Greece. We do not profess to cure cancer.

Over the last 10 years this test has emerged to be one of the most accurate and complete test of its kind we have seen to date.

The test is performed in Greece by Ioannis Papasotiriou, M.D., PhD, medical director of RGCC-Ltd. It was good in the beginning and has consistently improved over the years to a great test, in my opinion and the opinion of many other physicians throughout the world. The use of a simple blood sample with RGCC-Ltd works with ALL cancers (solid tumours, blood cancers, sarcomas, etc.) except brain and central nervous system primary tumours (glioblastomas, astrocytoma, meningioma etc.).

RGCC Labs can still work with these cancers from a small live tissue sample from the tumour.

The field of oncology has become highly competitive over the past 2-3 years, due to a beginning paradigm shift, based on long standing lack of good predictable results. Mass General, Sloan Kettering, University of Texas M.D. Anderson Cancer Centre in Houston, and Dana-Faber Cancer Institute of Boston have all started using and developing similar test in the last 2 years. They estimated it may start being used in about 5-9 years. At this point, none of these centres or any others we have looked at can do what RGCC-labs of Greece can do from a simple blood test. We know that cancer has been metastasizing [spreading and with a vengeance] in most all cancers patients for many, many years with little or no change. Up until recently no one knew for sure how or why this happened, just that it happened with great frequency. Now many scientists throughout the world and saying, it is due to the circulating tumour and cancer stem cells (CTC's/CSC's). This is rapidly becoming the focus of much cancer research, how to stop these peripheral CTC's and CSC's from causing metastatic tumours which is responsible for at least 90% of all cancer related deaths. Furthermore, the CTC's and CSC's are suspected by many scientists to be the cause of almost if not all the metastases that do occur. With this new information what should be one of the main targets by oncologists? Both the tumour and circulating CTC's and CSC's for each individual.

The primary cancer tumour is bad enough, but the CTC's and CSC's are the real problem. These cells allow the metastasis and return of this chronic, systemic disease. After 42 years and well into the trillions of dollars spent on cancer research and treatment since the war on cancer was declared in 1971, there has been between 2.1-7.5% increase in the 5 yr. survival rate (see page 5, #1 for source and details). All this time, money, patient suffering and death, in my opinion, is not something one would be proud of, and definitely not a good return on your investment.

The personalized cancer test from RGCC-Labs in Greece is known as an ex vivo test. Ex vivo (Latin: "out of the living") means that which takes place outside an organism. In science, ex vivo refers to experimentations or measurements done in or on tissues (or in this case CTC's and CSC's) in an artificial environment outside the organism with the minimum alteration of natural conditions. Ex vivo conditions allow experimentation under more controlled conditions than is possible for in-vivo experiments (in the intact organism), at the expense of altering the "natural" environment. RGCC has developed a way to not change any of the genetics (genotype and phenotype) and more important to not change the epigenetics of the CTC's and CSC's this is very important.

In vivo (Latin: "within the living") is experimentation using a whole, living organism as opposed to a partial or dead organism, or an in vitro ("within the glass", i.e., in a test tube or Petri dish) controlled environment. Animal testing and clinical trials are two forms of in vivo research. In vivo testing is often employed over in vitro because it is better suited for observing the overall effects of an experiment on a living subject. In microbiology in vivo is often used to refer to experimentation done in live isolated cells rather than in a whole organism, for example, cultured cells derived from biopsies. In this situation, the more specific term is ex vivo. Once cells are disrupted and individual parts are tested or analysed, this is known as in vitro.

In microbiology in vivo is often used to refer to experimentation done in live isolated cells rather than in a whole organism, for example, cultured cells derived from biopsies or from the peripheral blood test (the RGCC Test).

In vitro (Latin: "within glass") refers to studies in experimental biology that are conducted using components of an organism that have been isolated from their usual biological context in order to permit a more detailed or more convenient analysis than can be done with whole organisms. Colloquially, these experiments are commonly referred to as "test tube experiments".

Why use the blood since only a small number of CTC's and CSC's are found in the blood?

It is well known and widely accepted by the scientific community that the primary tumour consists of stroma (fibroblast, monocytes, lymphocytes, vessels, etc.) and the malignant cells which are heterogeneous (not all cells are the same) since they are composed from different subgroups and subclones with different features and abilities. These are known as circulating tumour cells (CTC's). A portion of these CTC's are actually cancer stem cells (CSC's).

Only very few of this population will develop metastatic ability which will allow them to invade the surrounding tissue, pass into the circulation and perform the epithelial to mesenchymal transition (EMT) creating the CSC's. These cells now have all the information and ability to form micro colonization and micrometastases and often will later develop into potent micrometastases, this is why cancer returns. This is also why we see these CSC's still in the blood long (I know for certain a minimum of 34 years) after the patients primary tumour is not detectable by PET/CT, MRI, CT, standard blood markers (CA 125, CA 19-9 etc.) or any other standard traditional measurements. As we have said many times before, the CSC's are the real trouble makers.

Remember these CSC's are immortal (they have no Hayflick limit for cell division) and can divide as long as they live. They are circulating in your blood stream 24/7/365 days a year just waiting for the right opportunity (when your internal microenvironment is not "up to par") to start another tumour or they may go dormant for up to 30+ years only to raise their ugly heads again. For this reason RGCC has selected blood samples as the most appropriate form for analysis since it includes the circulating cancer cells with the most relevant information for calculating the risk for both a potent metastasis and/or a reoccurrence from a few months to many years later. This is why we feel this is the best way to test, since metastasis is really what will kill most every cancer patient even 30+ years later. That is also why we use this test for regular follow-up testing to see if CTC's/CSC's remain, how many and any sudden changes in the number of CTC's/CSC's that may occur in the circulation over the months or years. This gives us a good idea, with other standard medical measurements, to find reoccurrences (if any) much earlier. We do not limit our test to any one test. The standard medical test and biopsies are still recommended and used as well. This gives us the best and most comprehensive way to evaluate the patient individually.

How does RGCC assure the purity of the CTC's harvested from your blood?

RGCC-Ltd uses powerful sorters and flow cytometers as well as negative selection based interrogation. They are able to actually isolate the relevant CTC's/CSC's and not enrich them (not change your cancer cells in any way). Hence, they manage to have a pure sample of CTC's/CSC's and simultaneously harvest from a single blood sample.

How do we analyse the harvested cells and use them to show risk of cancer relapse in clinical reality?

This is done by appropriate expansion of the CTC's. The CTC's will expand as the cancer stem cell like cell and then enter into exponential phase of growth which will generate a respectful number of CSC's in a very short period of time. At the same time we manage to keep intact both the genotype and phenotype of the cells and avoid any changes to the primary CSC's.

Therefore, after the final expansion we have maintained the identical genotype. The key to this exponential (rapid) growth phase is the cell culture.

Dr. Papasotiriou has an international (world) patent on this cell culture which does not change the genetics or epigenetics of the original CTC's/CSC's as shown by his patent. This is what allows RGCC-Ltd to verify the actual agents as well as the genetics. Most other labs only show results on the genetics of the tumour and what they should be sensitive to. A respectable percentage of the time they do not actually respond. Plus, RGCC results give us an actual percentage of response or resistance.

How does RGCC analyse the gene expression profile for the sensitivity/resistivity of the CSC's?

The expanded cells will be analysed for one expression profile in a hall genome micro-array analysis. Hence, we now have all the information concerning epigenetic screening of the CTC's/CSC's. This profile will indicate to us which therapy (chemo therapeutics as well as nutritional therapeutics) your CTC's/CSC's are sensitive or resistant to and is personalized for each patient.

We have tested 600+ patients with a wide variety of cancers and have never had the same protocol on any 2, even if the cancers were the same. The difference between protocols is 30-60% different even in siblings with the same cancer.

How does RGCC verify that these chemosensitivity/chemoresistance results are really valid?

The information obtained from the gene expression analysis will be validated in a micro culture where the effect of each substance (chemo drugs) will be tested for 6 (six) days and plotting a graph of the total number of your cells that were killed, while maintaining proper osmolality of the cell culture. The natural substances are tested by leaving the herb, vitamin, etc…in contact with your cancer cells for 48 hours, because it takes this long for the natural compound to activate the caspase 3/9 and cytochrome-c pathway to induce apoptosis (cell death) of your cancer cells. These assays overcome the problem of linearity between gene expression and protein expression levels.

The RGCC lab does not rely on the genetic assay only, as most other labs do, they actually test each individual chemo and natural substance to see if it really works and how well it works, no one else in the world has this capability, or accuracy, in my opinion. The reason, remember, RGCC lab has the international, world patent, of the cell culture which allows them to grow (expand) the 5-200 CTC's/CSC's found in your blood and expand (grow) them to100's of billions and in some cases trillions. And RGCC does this without changing the genetics or epigenetic expression of your cancer cells. This is remarkable and what no one, I know of, can do at this time.

What All Is Tested with the TU-Profile Plus Test?

The absolute beauty of this test is the ability to test what your cancer responds to without having any idea of where the primary tumour is located or even the type cancer it is. The lab will test 52 common chemo drugs and 49 natural substances and are not limited because of location or the type cancer which most other labs are limited to the approved drug list.

RGCC, Ltd. will test [49] natural substances covering cytotoxic agents; immunostimulants/ immunomodulators, cytokines; and increase of PBMC & NK, growth factor inhibitors of EGFr, IGFr, VEGFr, PDGFr, FGFr, and signal transduction pathways. The results show in %, the effectiveness of each individual agent to induce apoptosis (cell death) of your cancer cells, i.e. ex-vivo.

The test also includes [52] chemotherapeutic agents. These include [21] alkylating agents, [1] epothilone, [3] inhibitors topoisomerase 1, [9] inhibitors topoisomerase 2, [5] nucleus spindle stabilizer 1, [3] nucleus spindle stabilizer 2, and [10] nucleoside analogues. You will also receive [4] resistance factors (MDR1, MRP, LRP, GST). The results shows, in %, the effectiveness of each individual agent tested. In other words which chemotherapy works best for your cancer cells.

You also receive results on [62] tumour related genes (this is very important): [22] genes related to growth factors and proliferation stimuli; [11] genes related to self repair and stimuli; [5] genes for angiogenesis; [6] genes for cell cycle regulation and immortalization/apoptosis; [4] genes covering angiogenesis-metastasis; [11] genes concerned with drug metabolism and targets; and [2] for markers. You receive these results in % of over or under control, very exact. This will give your health care provider more detailed information about your cancer and their ability to grow and metastasize faster, their ability to resist certain drugs, the tendency to become immortal and much more.

How Do We Use All This To Personalize Support For Those With Cancer?

Our first goal is to decrease the tumour burden and the CTC's/CSC's. This severely stresses your immune system everyday all day. There are several ways of doing this. First, surgery early on will work 50% of the time for the primary tumour (not necessarily the CTC's/CSC's). Second, is chemo therapy which can be used in conjunction with surgery. Third is radiation and this can be used in combination with any of the previous. However, this is up to your oncologist to decide. In regard to the natural substances, our goal is to help support the immune system, help improve lifestyle, and support the physiological and biochemical processes of the human body by offering the integration of various nutritional support systems. This is also done using the test results to develop a personalized program for each individual patient. Generally we will re-test (onco-count or onco-trail, not the entire test as in the beginning) every 3-4 months to measure the actual circulating tumour and/or stem cell like cell numbers. We also recommend you follow your oncologist schedule of ongoing PET/CT, MRI etc. testing. Since cancer is a systemic, chronic disease our goal is to help you live a quality and productive life, for as long as you should, and then leave this earth with the cancer NOT from the cancer.

I have included a number of quotes and publications below that may be helpful for you to in making a truly informed decision of which lab you want testing your circulating cancer tumour and stem cells to see which chemotherapeutics and natural substances can cause the decrease of your tumour burden. While keeping in mind, the circulating CTC's/CSC's are the real ongoing, long term trouble makers for over 90% of cancer patients.

REPORT TIPS

The first parameter to detect the risk of relapse is the concentration of CTCs. The cut-off point varies between the types of malignancies (above that level considered as high risk of relapse):

- Breast carcinoma: **5cells/7.5ml**
- Lung carcinoma: **10cells/ml**
- Prostate: **20cells/ml**
- Colon: **5cells/ml**
- Sarcoma: **15cells/6.5ml**
- Others: **5cells/ml**

The second but most important parameter is the phenotype of the CTCs. The more stem markers are expressed in CTCs, the worse the prognosis.

- Nanog
- Sox-2
- Oct ¾
- CD133
- CD44

The expression of EMT-MET markers expressed, the worse the prognosis. (c-MET)

NOTE: CTCs phenotype prevails to the number of CTCs as risk factor.

**This test will NOT DETECT cancers of the brain or other cancers that have been 'encapsulated' by the body, not releasing circulating tumour or stem cells (CTC, CSC) into the blood stream or if any of these cells are dormant. We still recommend the use of biopsy, blood markers and/or various scans with this test when cancer is suspected or known to exist. No test is 100% accurate.

Ask Customer Service for copies of sample reports.

WHO IS R.G.C.C. LTD?

R.G.C.C. Limited was officially established in 2004 by Dr. Ioannis Papasotiriou. This means that the company is relatively new but the establisher and most of our members are active and have participated to previous companies and universities. Specifically, Dr Papasotiriou was working in the department of Experimental Physiology and Biochemistry in the Medical School of Thessaloniki in multiple projects such as Magnobrain, Mermaid, and EPET I etc.

R.G.C.C. International GmbH is a growing innovative and pioneer company in the area of chemosensitivity/ chemo-resistance testing with many branches and representatives all over the world. Specifically, we have branch offices in the U.S.A., in Central Europe, in the United Kingdom, in Cyprus and Hungary.

The company's headquarters are in Switzerland but the laboratories are in Northern Greece.

The lab is composed with the most advanced and innovating equipment as well as all the new technologies concerning assays and services in molecular medicine and especially in molecular oncology. Techniques such as micro array analysis of gene expression or proteins can become applicable and customized in our facilities. Quantification of genes through real time PCR is also a routine process.

The company apart from the services that offers in the clinical field has now expanded in the field of Research and Development in the pharmaceutical industry by creating the R.G.C.C. Pharma Ltd.

Accreditation

R.G.C.C. is pleased to announce they have passed the assessment and validation from the National Organism of Accreditation and they have accredited our lab with the ISO 17025:2008 for the following methods:

- CTC/CSC isolation and immunophenotyping
- Cancer cell culture viability/cytotoxicity assays after exposure to substances
- Gene expression assays (mainly related with cancer stemness)

They trust this will help physicians and their patients understand our level of commitment to quality and remove any doubts that may have existed concerning the validity of the assays since this accreditation includes inter-laboratory validation (performance of the assays from different labs which we have no interest or relation). We will utilize such tools as often as needed to promote the quality and sincerity of our work. ISO 17025 identifies the high technical competence and management system requirements that guarantee your test results and calibrations are consistently accurate. This accreditation is recognized and accepted throughout the international scientific and laboratory communities as the standard of excellence.

http://www.rgcc-genlab.com