

**R.G.C.C. - RESEARCH GENETIC CANCER CENTRE S.A.**

Florina, \_\_/\_\_/\_\_\_\_

Dear colleague,

Below are the results from the analysis on a patient Mr./Ms. \_\_\_\_\_ suffering from \_\_\_\_\_ carcinoma stage \_\_\_\_\_. The sample that was sent to us for analysis was a sample of \_\_\_\_\_ ml of whole blood that contained EDTA-Ca as anti-coagulant, and packed with an ice pack.

Our laboratory processed with the following:

1. White Blood Cell (WBC) isolation with Biocoll solution.
2. mRNA isolation using TRIzol reagent.
3. cDNA synthesis from mRNA with First strand cDNA synthesis kit.
4. Specific gene markers were quantified with Real Time PCR assay.
5. Flow cytometry run for the specific markers directly to whole blood.

The data arising from the two assays were then analyzed and compared to the normal ones.

During the test the environmental temperature was:  $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$ .

The results are summarized in the following table:

<u>Metastasis location</u>	<u>Marker</u>	<u>Sample levels</u>	<u>Normal levels</u>	<u>Results</u>
<b>General</b>	TGF-β R2	18.14	15.41	↓
	ITGB-4 R	20.58	21.09	-
	ITGB-5 R	16.37	14.84	↓
	ITGB-6 R	19.31	21.47	↑
<b>Pleura</b>	CCR6	23.19	19.69	↓
	Mesothelin	21.48	18.95	↓
<b>Skin</b>	CCR7	20.08	18.67	↓
<b>Lung</b>	IGF-R2	16.89	16.59	-
	Phospho-ERK1/2	19.54	18.60	↓
<b>Bone</b>	BMPR 1a/1b/2	22.14	16.39	↓
	CXCR4	18.05	20.08	↑
	RANK	21.52	18.15	↓
	BST-2	13.52	13.06	↓
<b>Liver</b>	CXCR4	20.08	20.08	-
	TRAIL-R2	10.08	14.99	↑
	FAS R	18.54	17.24	↓
	HGFR	19.9	16.93	↓
<b>Brain</b>	Phospho-STAT-3	16.13	15.60	-
	CX3CR1	21.03	17.61	↓
	DSC-2	16.58	17.61	↑

### **Results discussion**

Two methods were used in order to increase the accuracy of the results. Flow cytometry was carried out with the use of the above specific antibodies and Real Time PCR was held using primers designed to recognize specifically the genes of these proteins. The genes that are in up regulation are the ITGB-4 R, ITGB-6 R, CXCR4, TRAIL-R2 and DSC-2.

According to the results, there is a major trend of metastasis of the primary tumor mainly towards the Bone, Liver and Brain and less to the rest organs.

This Test Report is issued based on testing the sample / specimen examined by the Laboratory. Modification of data, selective breeding and using portions of this test report is forbidden. The laboratory assumes no liability for improper use or improper interpretation of the results.

Mr./Ms. \_\_\_\_\_

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