# METASTAT RGCC ™

Results

Analysis on a patient test patient 1 suffering from Breast carcinoma stage II.

The sample that was sent to us for analysis was a sample of 20ml Blood that contains anti-coagulant, and packed with an ice pack.

## Laboratory Process

1. CTCs are isolation from whole blood through negative selection.
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: 23o C ± 1o C.

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| METASTASIS LOCATION | MARKER | SAMPLE LEVELS | NORMAL LEVELS | RESULTS |
|---|---|---|---|---|
| General | TGF-b R2 | 7.27 | 7.27 | - |
| | ITGB-4 R | 15 | 7.98 | - |
| | ITGB-5 R | 10.05 | 11.11 | UP REGULATED |
| | ITGB-6 R | 15 | 11.59 | - |
| Pleura | CCR6 | 9.58 | 9.58 | - |
| | Mesothelin | 15 | 10.36 | - |
| Skin | CCR7 | 15 | 6.78 | - |
| Lung | IGF-R2 | 9.65 | 9.65 | - |
| | Phospho-ERK1 | 15 | 11.07 | - |
| | Phospho-ERK2 | 8.14 | 9.44 | UP REGULATED |
| Bone | BMPR 1a | 15 | 11.66 | - |
| | BMPR 1b | 10.62 | 12.25 | UP REGULATED |
| | BMPR 2 | 15 | 11.58 | - |
| | CXCR4 | 15 | 9.51 | - |
| | RANK | 15 | 13.49 | - |
| | BST-2 | 5.87 | 5.87 | - |

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| METASTASIS LOCATION | MARKER | SAMPLE LEVELS | NORMAL LEVELS | RESULTS |
|---|---|---|---|---|
| Liver | CXCR4 | 15 | 9.51 | - |
| | TRAIL-R2 | 15 | 9.03 | - |
| | FAS R | 15 | 9.18 | - |
| | HGFR | 8.75 | 8.75 | - |
| Brain | Phospho-STAT-3 | 15 | 7.30 | - |
| | CX3CR1 | 15 | 5.93 | - |
| | DSC-2 | 11.96 | 11.96 | - |

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.

Genes in up regulation are ITGB-5 R,Phosho-ERK2,BMPR 1b

According to the results, there is a major trend of metastasis of the primary tumor mainly towards the Lung,Bone



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