



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

Florina, 28/04/2017

Dear Colleague,

We send you the results from the analysis on a patient Ms xxxxxxxxxxxx suffering from **sarcoma stage IV**. The sample that was sent to us for analysis was a sample of 25ml of whole blood that contained EDTA-Ca as anti-coagulant, and packed with an ice pack.

In our laboratory we made the following:

- We isolated the malignant cells using Oncoquick with a membrane that isolates malignant cells from normal cells after centrifugation and positive selection using epithelial membrane marker and anti-CD99 and negative selection using anti-CD45 particles

The results during the isolation procedure are presented below:

Table of markers:

CD45 positive cells (Hematologic origin cells)		CD45 negative cells (non Hematologic origin)	
Nanog	NEGATIVE	Nanog	POSITIVE
OKT-4	NEGATIVE	OKT-4	Dim_POSITIVE
Sox-2	NEGATIVE	Sox-2	Dim_POSITIVE
		CD99	POSITIVE
		EpCam	NEGATIVE
		CD133	POSITIVE
		c-MET	POSITIVE
		CD31	NEGATIVE
		PanCK	NEGATIVE
		NSE	NEGATIVE
		Vimentin	POSITIVE
		Synaptophysin	NEGATIVE

Index of marker: CD45: Hematologic origin cell marker, **CD133, Sox-2, OKT-4, Nanog:** tumor stem cell marker, **CD31:** endothelial cell membrane antigen, **CD99, Vimentin, Synaptophysin, NSE:** sarcoma membrane marker, **EpCam:** Epithelial origin marker, **PanCK:** epithelial origin marker, **c-MET:** membrane antigen that regulates the mesenchymal to epithelial transition.

The final results after the isolation procedure are presented below: We notice that after isolation procedure there are remaining malignant cells. The concentration of these cells was isolated **7.8cells/6.5ml, SD +/- 0.3cells.**

Sincerely,

Ioannis Papisotiriou M.D., PhD
Head of molecular medicine dpt of
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Index of circulating cells number: (If Over Limit: Advanced or Progression of Disease, If Less than limit: Early disease or disease is responding to a treatment plan).

Breast cancer: < 5cells /7.5ml , **Prostate cancer** < 20cells/ml , **Sarcoma:** <15cells/6.5ml, **Colon cancer:** <5cells/ml, **Lung cancer (Lc=0, r=0.99):** <10cell/ml. **All cancer types other than those listed above should be <5 cells/ml.**

*This test will NOT DETECT cancers of the brain or other cancers that have been "encapsulated" by the body, not releasing circulating tumor 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.

Ms xxxxxxxxxxxxxxxx

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