R.G.C.C.-RESEARCH GENETIC CANCER CENTRE LTD

Florina, 21/01/2013

Dear colleague,

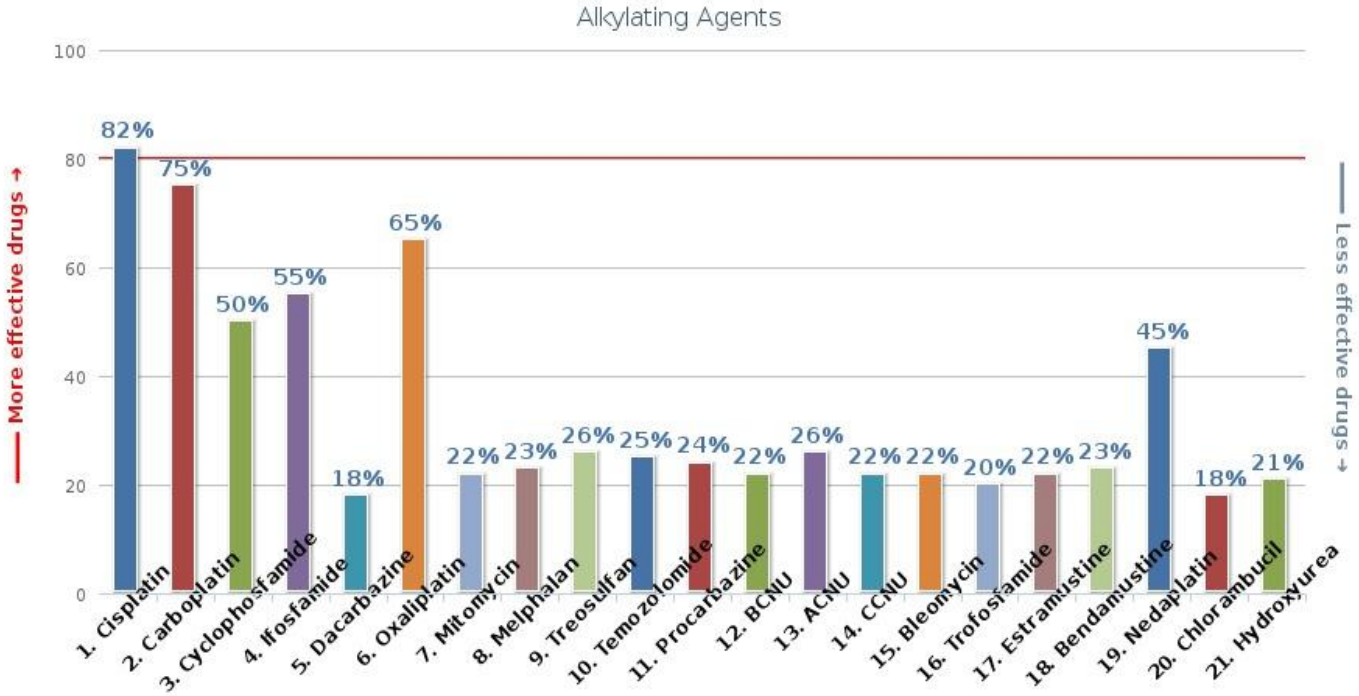
We send you the results from the analysis on a patient **(test)** suffering from Unknown carcinoma stage N/A. The sample that was sent to us for analysis was a sample of 20ml of whole blood that contained EDTA-Ca as anti-coagulant, and packed with an ice pack.

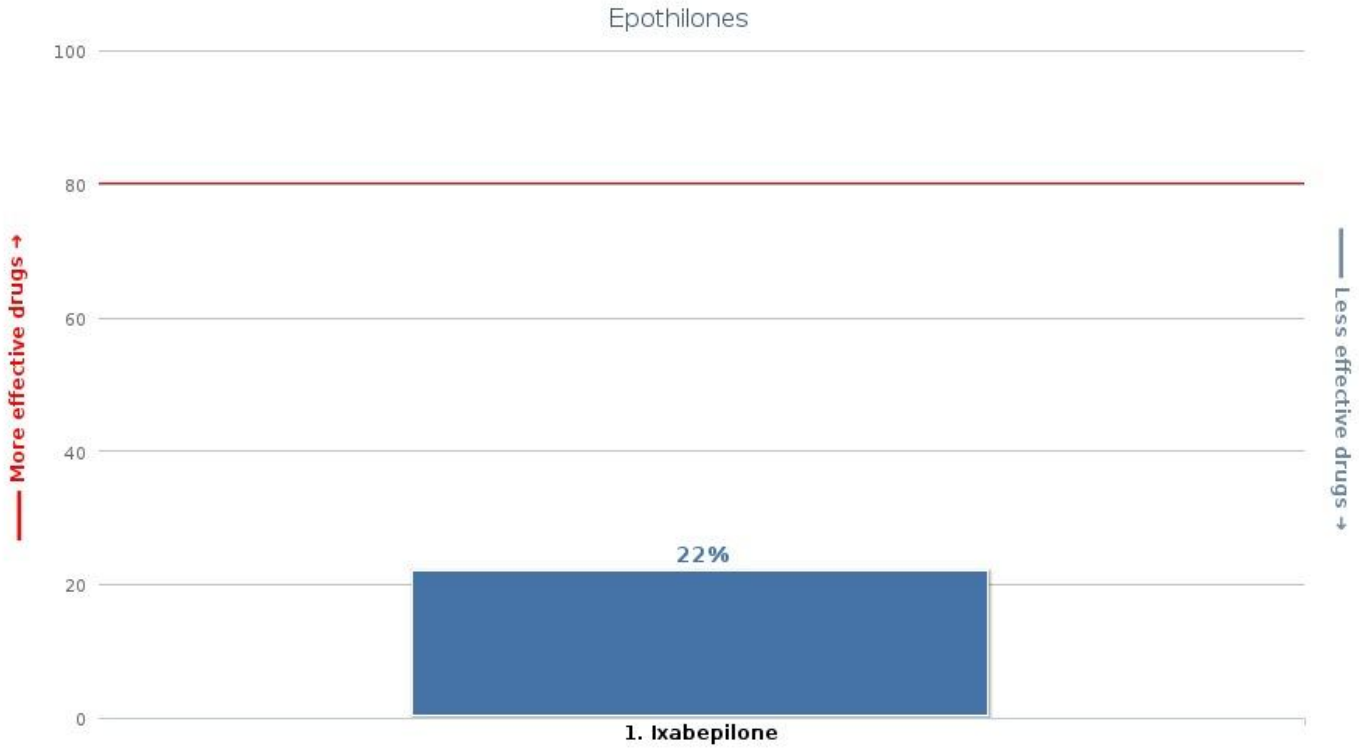
In our laboratory we made the following:

- We isolated the malignant cells using Oncoquick with a membrane that isolates malignant cells from normal cells after centrifugation and positive selection using anti-EpCam and negative selection using anti-CD45 particles (isolated 12.4cells/ml, SD +/- 0.3cells).
- Then we developed cell cultures in a fetal calf serum media and at the same time we developed colony cultures in soft agar. In each culture of the well plate we added a chemotherapeutic substance that is used in clinical application. Then we developed those cultures and we harvested a sample every 24 hours for 6 days and made the following assays.
- There was made an isolation of the genomic DNA using the kit Invisorb of INVITEK.
- We isolated mRNA using the mRNA Magprep blood isolation kit of NOVAGEN.
- We traced the mRNA and the genes of MDR1 (multi drug resistant 1), MRP and LRP using the technique of Northern Blot (resistance in drugs used in chemotherapies).
- We tracked the mRNA and the gene of topoisomerase I and II a & b using the technique of Northern Blot (sensitivity in cytostatic inhibitors of topoisomerase).
- We tracked the quantity of the mRNA of the tubulin using the RT-PCR (sensitivity in cytostatics of the kind of taxanes and the products of the alkaloids of Vinca).
- We defined the activity of the enzyme complex of the glutathione-S-transferases (GST kit of NOVAGEN) (resistance in drugs used in chemotherapies-especially in platinum compounds).
- We defined the DNA methyl transferase which is a target of the alkylating factors (products of platinum, cyclophosphamide and the products of it).
- We defined the mRNA of the Thymidylatesynthetase (TS) and the DHFR (sensitivity in 5-FU, capecitabine and methotrexate).
- We defined the mRNA ofthe reductase of 5-CMP (sensitivity in gemcitabine).
- We defined the receptors of the MMP and the receptors of laminin (invasive ability of the tumor).
- We defined the expression of protein p27 that is responsible for cell arrest in G0 stage.
- We defined the VEGF (neoangiogenetic factor) and the induction of the apoptotic pathway using ONCOGENE kit from NOVAGEN.
- We defined the ability of acting of the nucleus protein kinases which are a target of the Carbazinecompounds.
- We defined the overexpression of TGFa and TGFb factors as targets for Suraminsulfate.
- We defined the overexpression of somatostatin receptor (SS-R) , of COX-2 and 5-LOX , of c-erb-B2 (Her/Neu2) , c-erb-B1, and androgen, estrogen and progesterone receptors.

The above conclusions were confirmed by the cell cultures of the tumor (or circulating tumor cells and the results are displayed in the bar graph on the next pages.

INTERPRETATION: The numbers above the bars indicate % of cancer cell **DEATH** caused by the drug tested. This equates the % **SENSITIVITY** to that drug. Therefore, the drugs with the highest numbers are the most effective drugs at inducing cancer cell death for the patient tested. The numbers below or beside the bars refer to the drugs tested, as indicated in the diagrams in pages 2 to 7.





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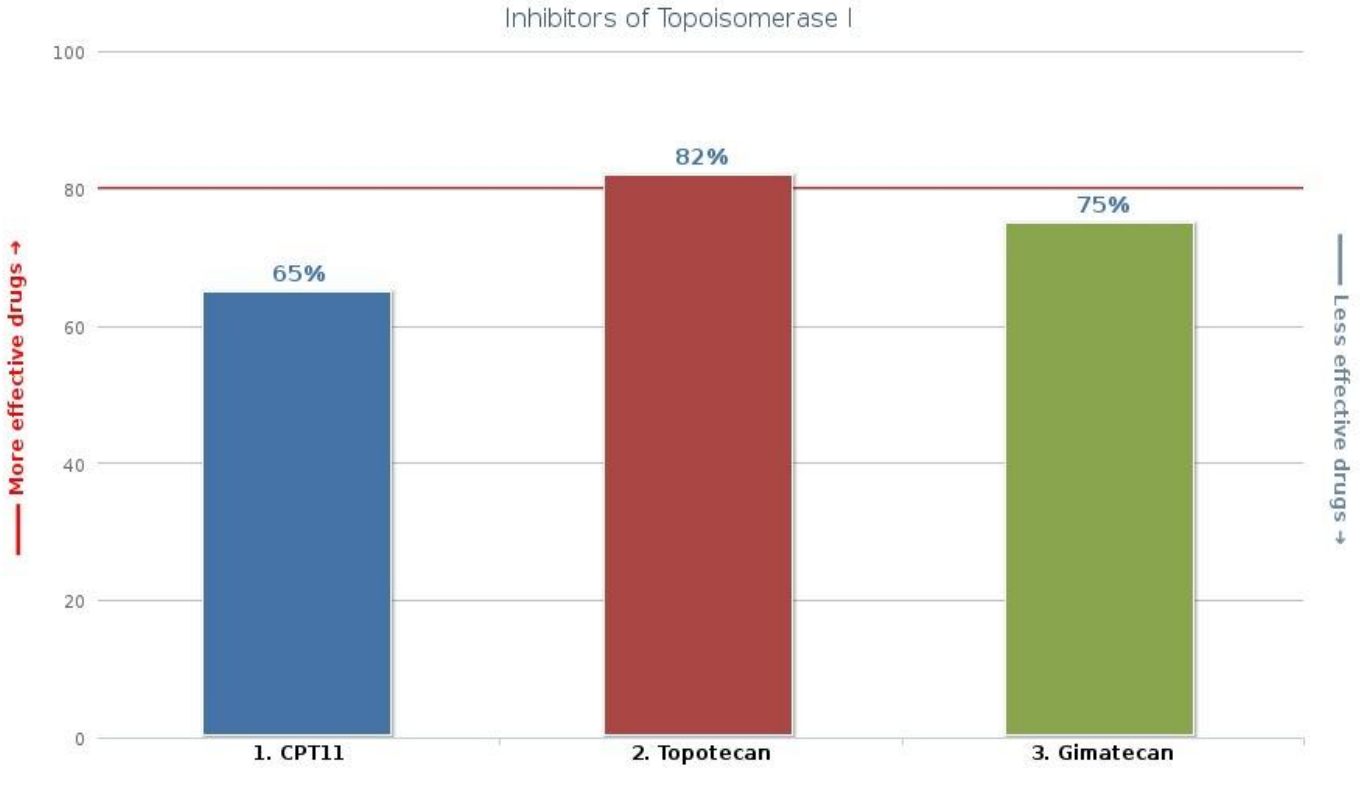

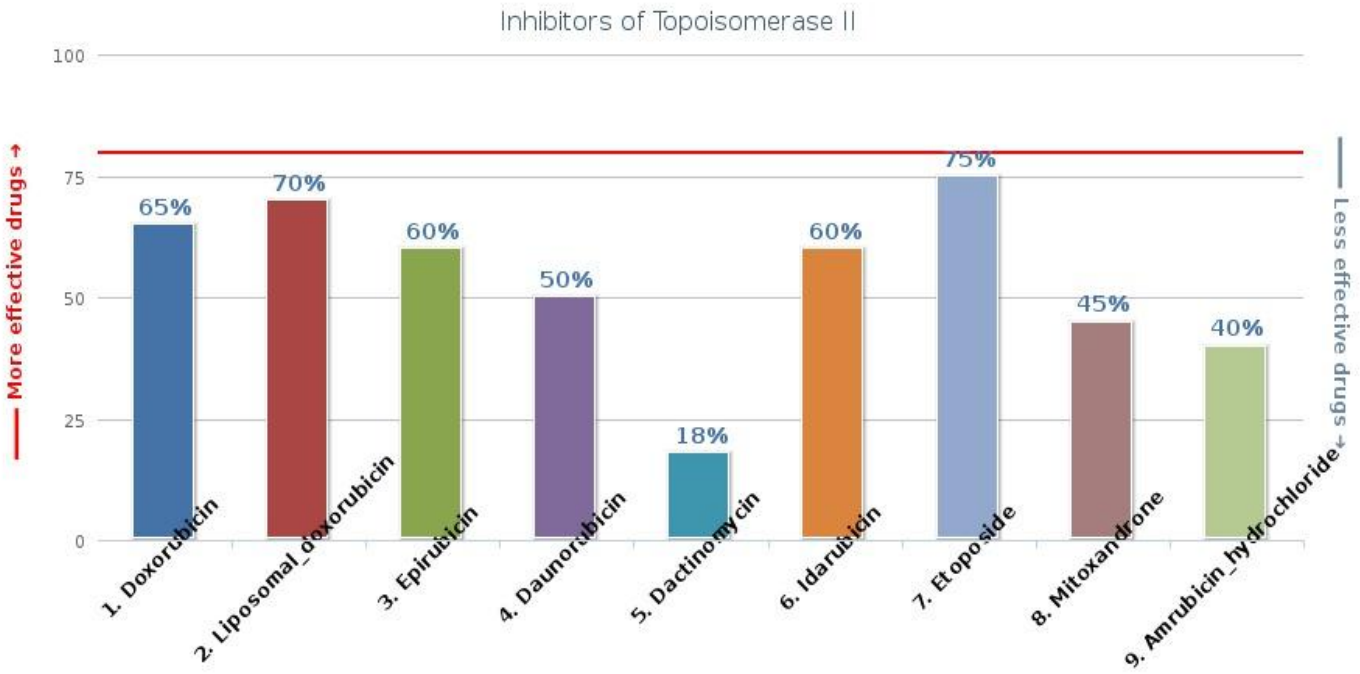

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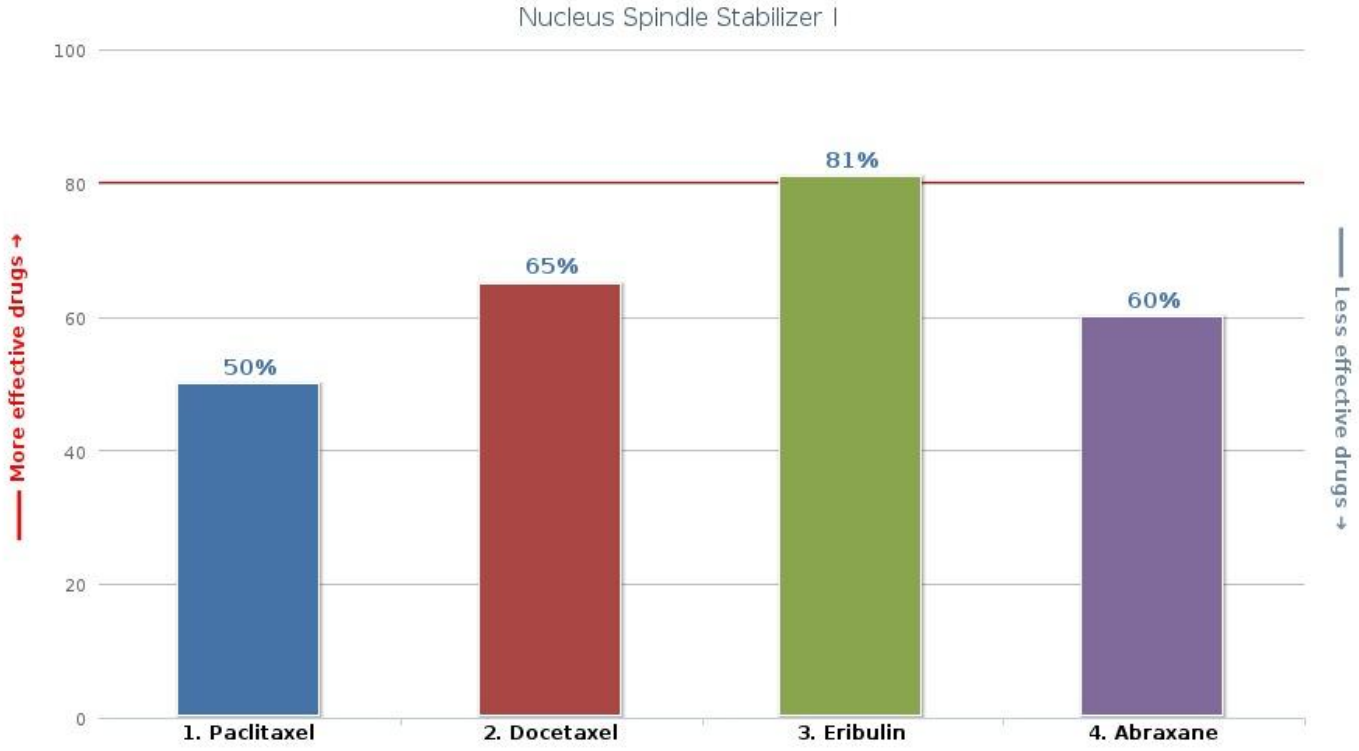

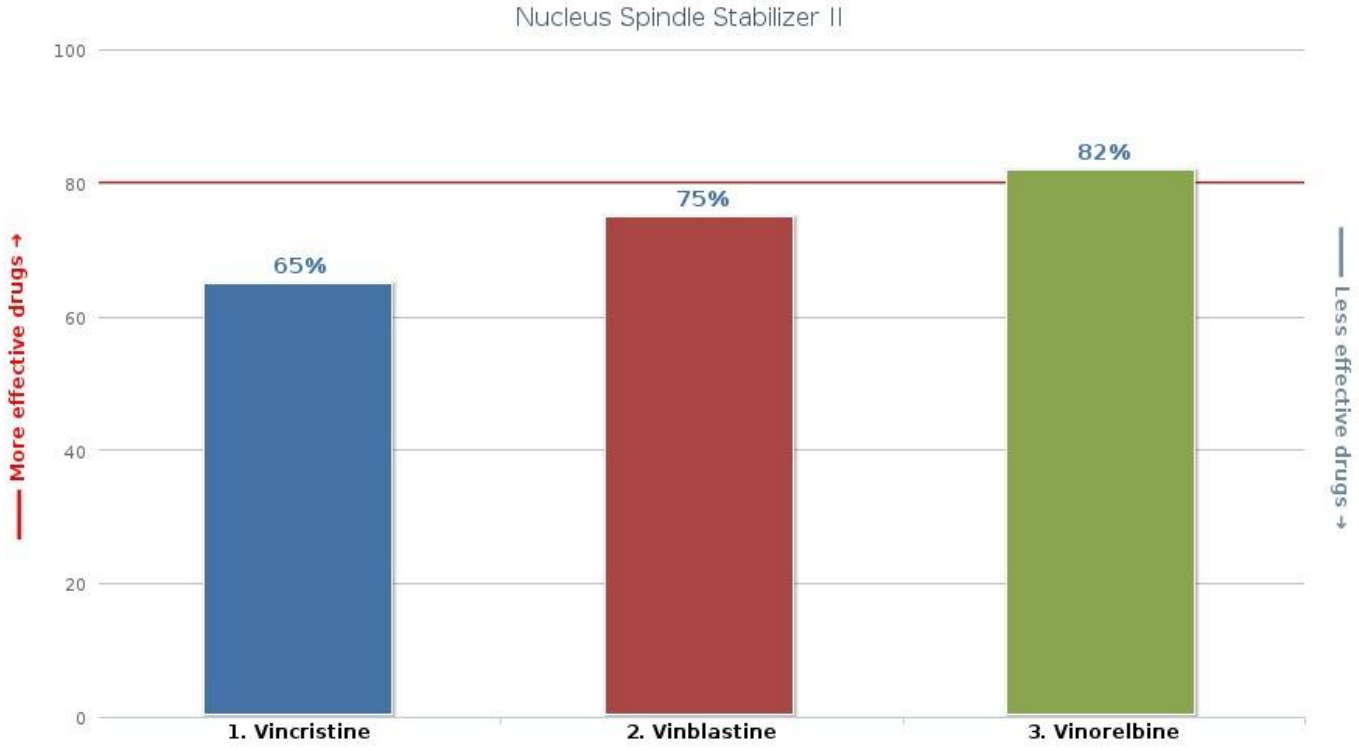

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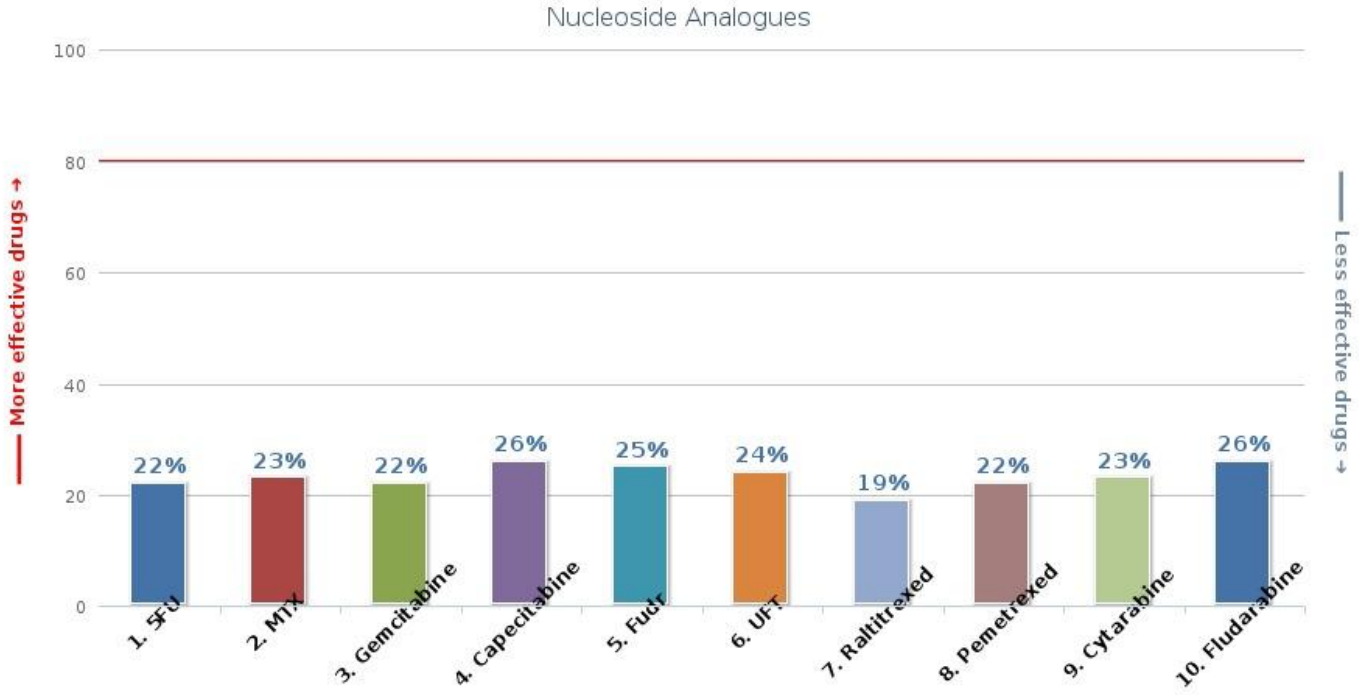

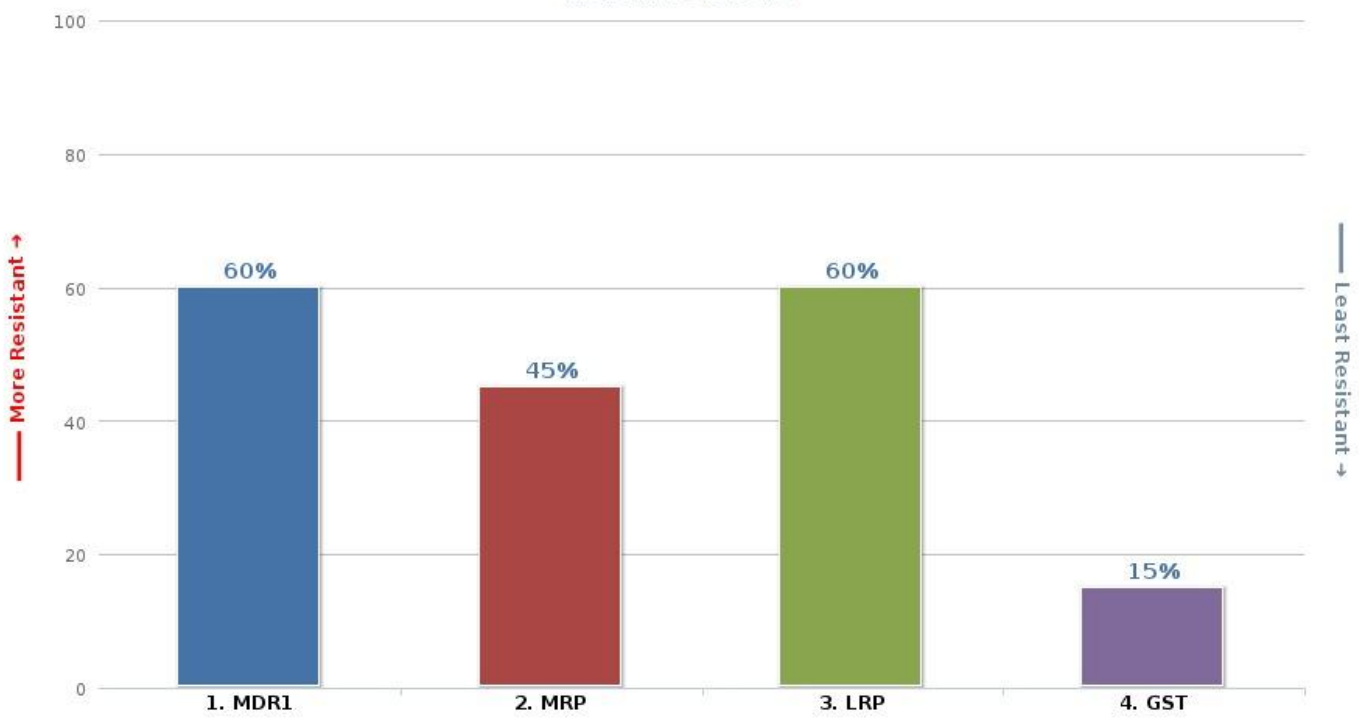

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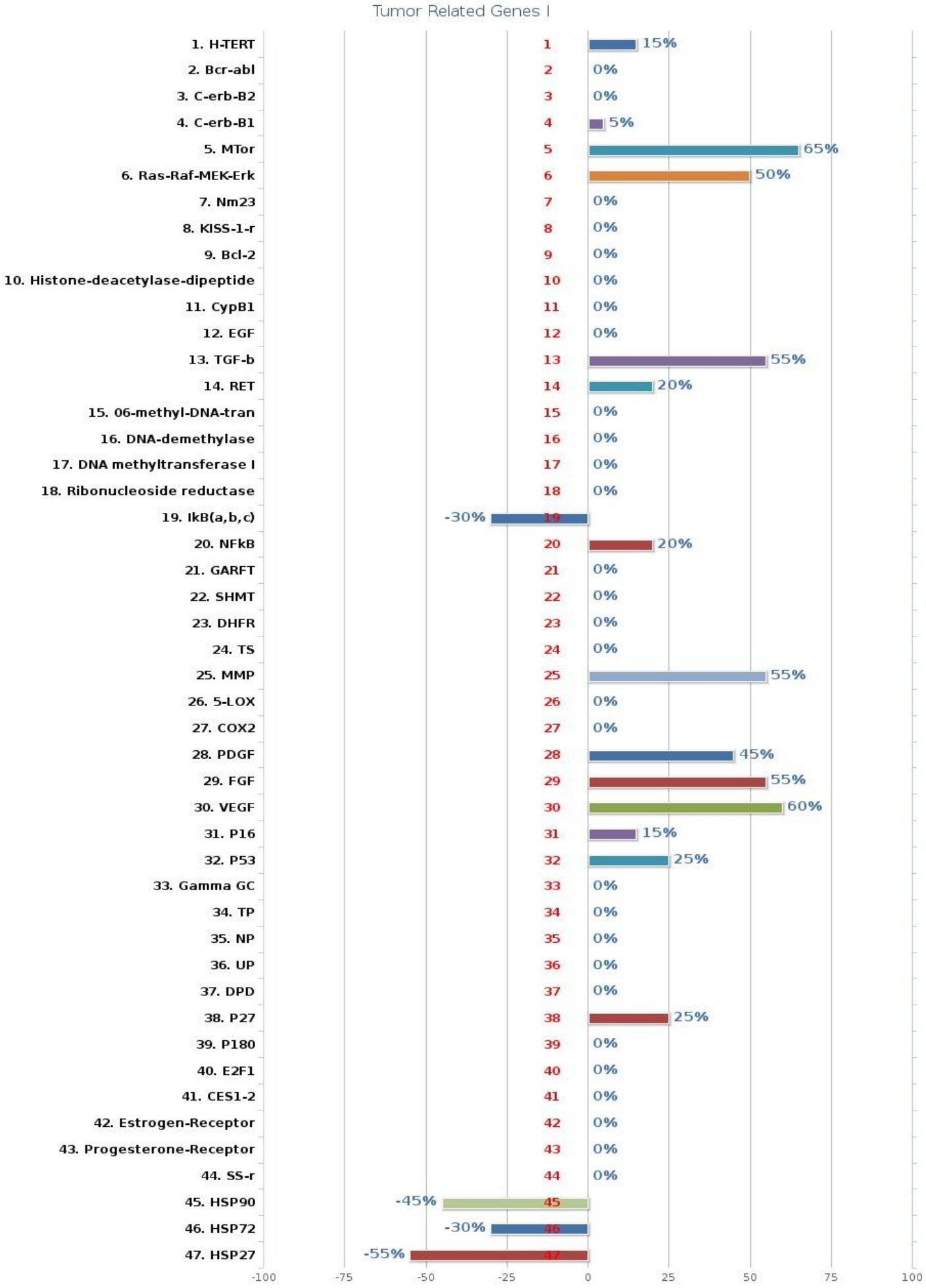

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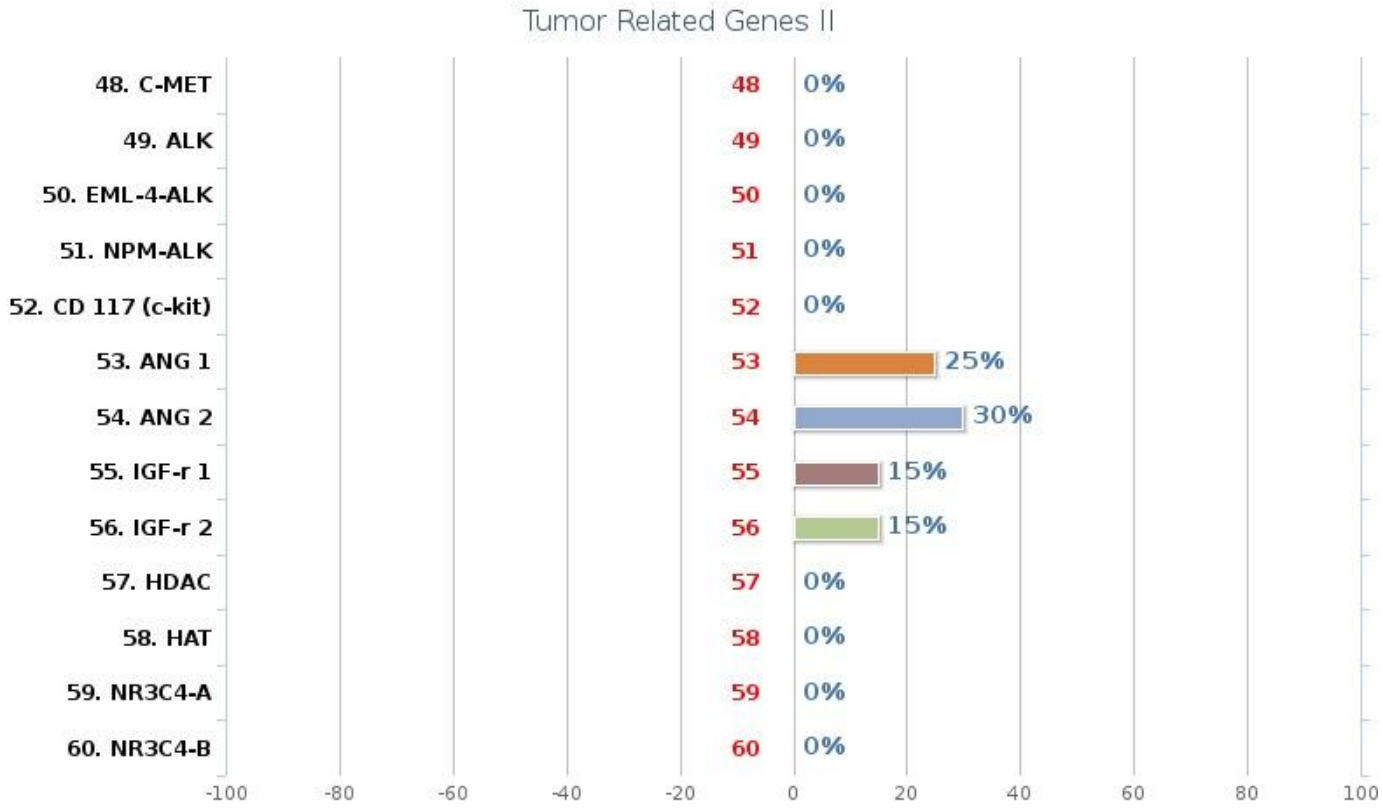

Tumor Related Genes

Growth factors proliferation stimuli		
NAME	RELATED	RESULTS
SS-r	Somatostatin receptor	**normal**
Progesterone Receptor	Growth Factor receptor	**normal**
Estrogen Receptor	Growth Factor receptor	**normal**
p180	Tyrosin kinase growth f.	**normal**
COX2	Tumour Growth	**normal**
5-LOX	Tumour Growth	**normal**
NFkB	Transcription fact	**20% over control**
IkB(a,b,c)	Inhibitor of NFκB	**30% below control**
EGF	Tumour Growth	**normal**
Ras/Raf/MEK/Erk	Transduction pathway	**50% over control**
mTOR	Transduction pathway	**65% over control**
c-erb-B1	Her1	**normal**
c-erb-B2	Her/neu2	**normal**
Bcr-abl	Resist phenotype	**normal**
ALK	Acute Leukemia kinase	**normal**
EML-4-ALK	Fusion EML with ALK	**normal**
NPM-ALK	Fusion NPM with ALK	**normal**
CD 117(c-kit)	Proliferate growth factor receptor 1	**normal**
RET	proto-oncogene	**20% over control**
IGF-r 1	Insulin like growth factor receptor I	**15% over control**
IGF-r-2	Insulin like growth factor receptor II	**15% over control**
NR3C4-A	Nucleous receptor group III Class 4 (androgen receptor A)	**normal**
NR3C4-B	Nucleous receptor group III Class 4 (androgen receptor B)	**normal**

SELF REPAIR - RESISTANCE		
NAME	RELATED	RESULTS
HSP27	Heat Shock Protein	**55% below control**
HSP72	Heat Shock Protein	**30% below control**
HSP90	Heat Shock Protein	**45% below control**
Gamma GC	Resist to alkylating drug	**normal**
DNA methyltransferase I	DNA methylation	**normal**
DNA demethylase	DNA methylation	**normal**
06-methyl-DNA-tran.	DNA methylation	**normal**
TGF-b	Tumour Growth	**55% over control**
Histone deacetylase-dipeptide	DNA coiling(nucleosome)	**normal**
HDAC	Histone deacetylase	**normal**
HAT	Histone acetyl transferase	**normal**

ANGIOGENESIS		
NAME	RELATED	RESULTS
VEGF	Angiogenesis	**60% over control**
FGF	Angiogenesis	**55% over control**
PDGF	Angiogenesis	**45% over control**
ANG 1	Angiogenin I	**25% over control**
ANG 2	Angiogenin II	**30% over control**

CELL CYCLE REGULATION & IMMORTALIZATION / APOPTOSIS		
NAME	RELATED	RESULTS
E2F1	Transcr. Fact of TS &topoI	**normal**
p27	Cell arrest (G0)	**25% over control**
p53	Cell cycle regulator	**25% over control**
p16	Apoptosis	**15% over control**
Bcl-2	Apoptosis	**normal**
h-TERT	M2 crisis-aggressive phen.	**15% over control**

ANGIOGENESIS-METASTASES		
NAME	RELATED	RESULTS
KISS-1-r	Metastases regulator	**normal**
Nm23	Metastases regulator	**normal**
MMP	Metastases	**55% over control**
c-MET	Mesenchymal to epithelial transition	**normal**

DRUG METABOLISMS & TARGETS		
NAME	RELATED	RESULTS
CES1&2 (carboxyesterase)	Resist to camptothecin	**normal**
DPD	Resist to 5FU	**normal**
UP	Resist to 5FU	**normal**
NP	Resist topyrim. Antagonist	**normal**
TP	Resist to 5FU	**normal**
TS	Rapid cell cycle (THFA)	**normal**
DHFR	Rapid cell cycle (THFA)	**normal**
SHMT	Rapid cell cycle (THFA)	**normal**
GARFT	Rapid cell cycle(THFA)	**normal**
Ribonucleosidereductase	DNA synthesis	**normal**
CypB1	Xenobiotic metabolism	**normal**

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MARKERS		
NAME	RELATED	RESULTS
CD33	Myeloid cell origin	**15% over control**
CD52	Leukaemia marker	**15% over control**
CD20	Lymphoma related antigen	**20% over control**
EpCAM	Epithelial marker	**normal**

From the investigation above we concluded to the following:

1. From the whole neoplasmic population we have an expression of LRP in a percentage of 60% over control sample (positive in the check of resistance).
2. The activity of GST is stable in the low limits (no resistance to platinum compounds).
3. The activity of GammaGC is in normal range (no resistance to platinum compounds).
4. The activity of CES1 and CES2 is in normal range (no resistance to camptothecin compounds).
5. The concentration of p180 is in normal range.
6. Increased activity of the Laminin and the MMP (increased invasive ability).
7. There is partial sensitivity in taxanes (Paclitaxel ,Docetaxel).
8. There is great sensitivity in alkaloids of vinca (Vinorelbine).
9. There is great sensitivity in Eribulin.
10. No sensitivity noticed in 5FC, in 5-FU, in UFT, IN FUdr, in Capecitabine, in Raltitrexed, in methotrexate, in Cytarabine, in Flutarabine, in Gemcitabine and in Pemetrexed.
11. There is no sensitivity in Epothilones.
12. Increased sensitivity in alkylating factors (Cisplatin).
13. There is great overexpression of NFkB (20% over control), TGF-b (55% over control), there is normal expression of EGF, but there is suppression of expression of IkB(a,b,c) (30% below control).
14. It appears to have partial sensitivity in the inhibitors of topoisomerase II a and II b.
15. There is great sensitivity in the inhibitors of Topoisomerase I (Topotecan).
16. There is normal expression of 5-LOX, SS-r, COX2, C-erb-B1, C-erb-B2, Estrogen-Receptor and Progesterone-Receptor.
17. We notice great neoangiogenetic ability (overexpression of VEGF-R 60% over control sample).
18. Finally, there is no sensitivity in Dacarbazine.
19. We notice that taurolidine cannot induce the apoptosis to the malignant cells (in IV route dosage).
20. We notice that taurolidine can induce the apoptosis to the malignant cells (in intraperitoneal route dosage).
21. We notice down-regulation of HSP27 (Heat Shock Protein) at 55% below control, HSP72 (Heat Shock Protein) at 30% below control and HSP90 (Heat Shock Protein) at 45% below control.
22. There is over-expression of ANG 1 at 25% over control, ANG 2 at 30% over control, IGF-r 1 at 15% over control, IGF-r 2 at 15% over control, but we notice no down-regulation of ALK, EML-4-ALK, C-MET, NPM-ALK, CD 117 (c-kit), HDAC, HAT, NR3C4-A and NR3C4-B.

Conclusion :

- The specific tumor appears to have resisting populations because of the LRP overexpression that can be reversed by the use of verapamil combined with imidazole compounds (ketoconazole).
- The neoplasmatic cells have the greatest sensitivity in the alkylating agent (**Cisplatin**), in the inhibitors of Topoisomerase I (**Topotecan**), in the nucleous spindle stabilizer (**Eribulin**) and in the tubulin dimmer polymerization inhibitors (**Vinorelbine**).
- Also can be used Bevacizumab as inhibitor of neo-angiogenesis, Sorafenib as inhibitor of Ras/Raf/MEK/Erk transaction pathway, Tositumomab (Bexxar) as inhibitor of CD20, Transtuzumab as inhibitor of c-erb-B2 (Her/neu2) and Vandetanib as inhibitor of EGF-r, VEGF-r and RET.

Sincerely,

Ioannis Papasotiriou MD., PhD
Head of molecular medicine dpt. of
R.G.C.C.-RESEARCH GENETIC CANCER CENTRE LTD

INDEX: M0: Abnormal p16, normal p53 and hTERT ,
M1: Normal hTERT , abnormal p53 , p16 ,
M2 crisis: over-expression of hTERT , p53 , p16
Sample viability: <35% no sensitivity, 35%-80% partial sensitivity, >80% great sensitivity

*Be advised that any nutritional program suggested is not intended as a treatment for any disease. The intent of any nutritional recommendation is to support the physiological and biochemical processes of the human body, and not to diagnose, treat, cure, prevent any disease or condition. Always work with a qualified healthcare provider before making changes to your diet, prescription medication, lifestyle or exercise activities

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