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

Florina, __/__/____

Dear colleague,

We send you 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.

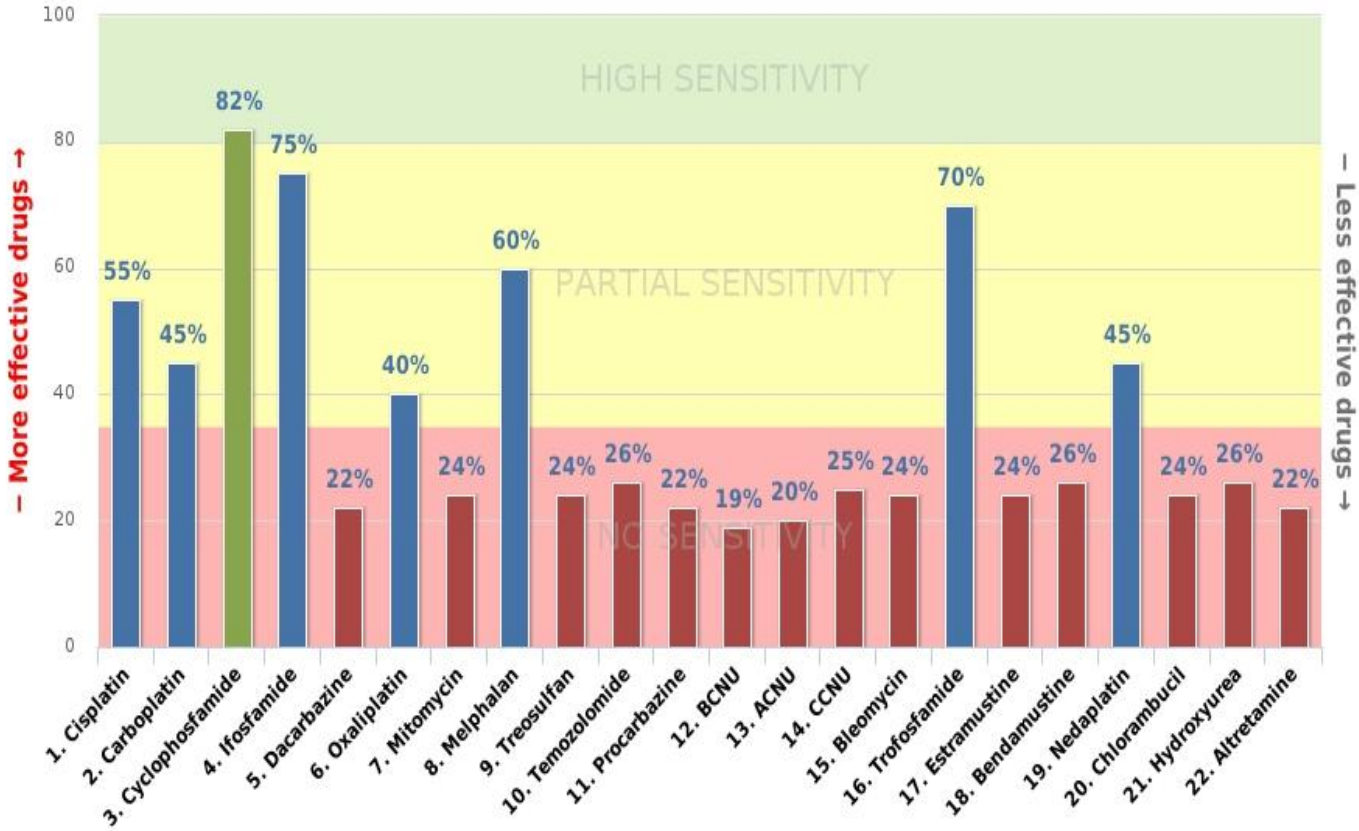
In our laboratory we made the following:

- 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 ___cells/___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 Thymidylate synthetase (TS) and the DHFR (sensitivity in 5-FU, capecitabine and methotrexate).
- We defined the mRNA of the 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 Carbazine compounds.
- We defined the over expression of TGFa and TGFb factors as targets for Suramin sulfate.
- We defined the over expression of somatostatin receptor (SS-R), of COX-2 and 5-LOX, of c-erb-B2 (Her/Neu2), c-erb-B1, 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.

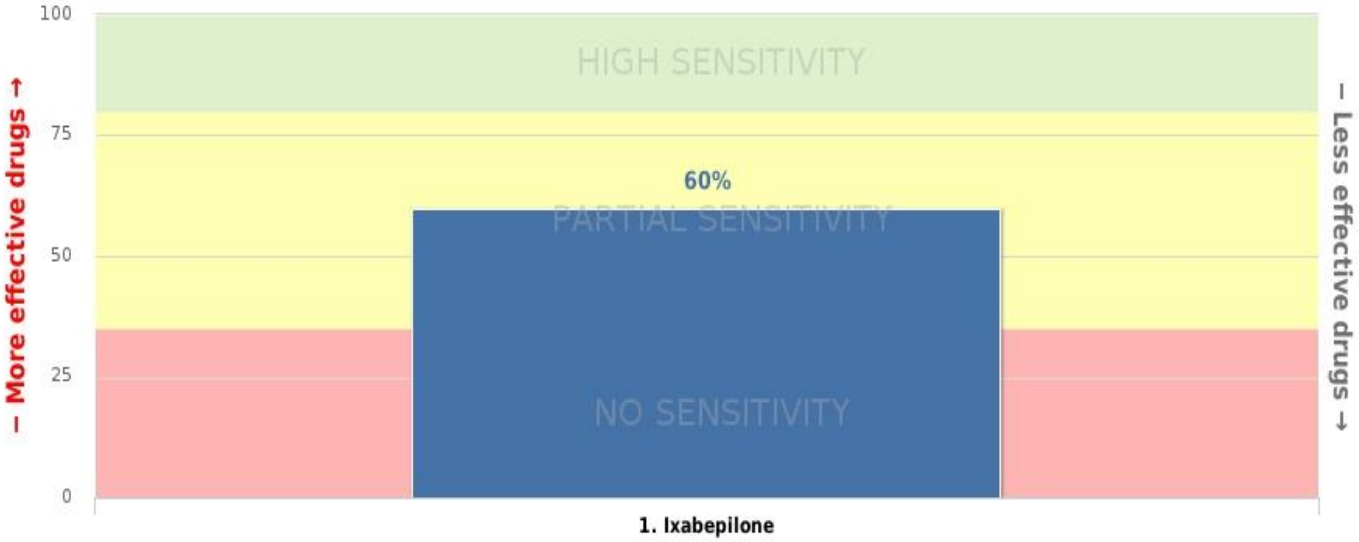
INTERPRETATION: The numbers above the bars indicate % of cancer cell **DEATH** caused by the drug tested. This equates the % **SENSITIVITY** to that drug. Therefore, the drugs with the highest numbers are the most effective drugs at inducing cancer cell death for the patient tested. The numbers below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

Alkylating Agents



High Sensitivity: Cyclophosfamide
Partial Sensitivity: Cisplatin, Carboplatin, Ifosfamide, Oxaliplatin, Melphalan, Trofosfamide, Nedaplatin
No Sensitivity: Dacarbazine, Mitomycin, Treosulfan, Temozolomide, Procarbazine, BCNU, ACNU, CCNU, Bleomycin, Estramustine,
↳ Bendamustine, Chlorambucil, Hydroxyurea, Altretamine

Epothilones

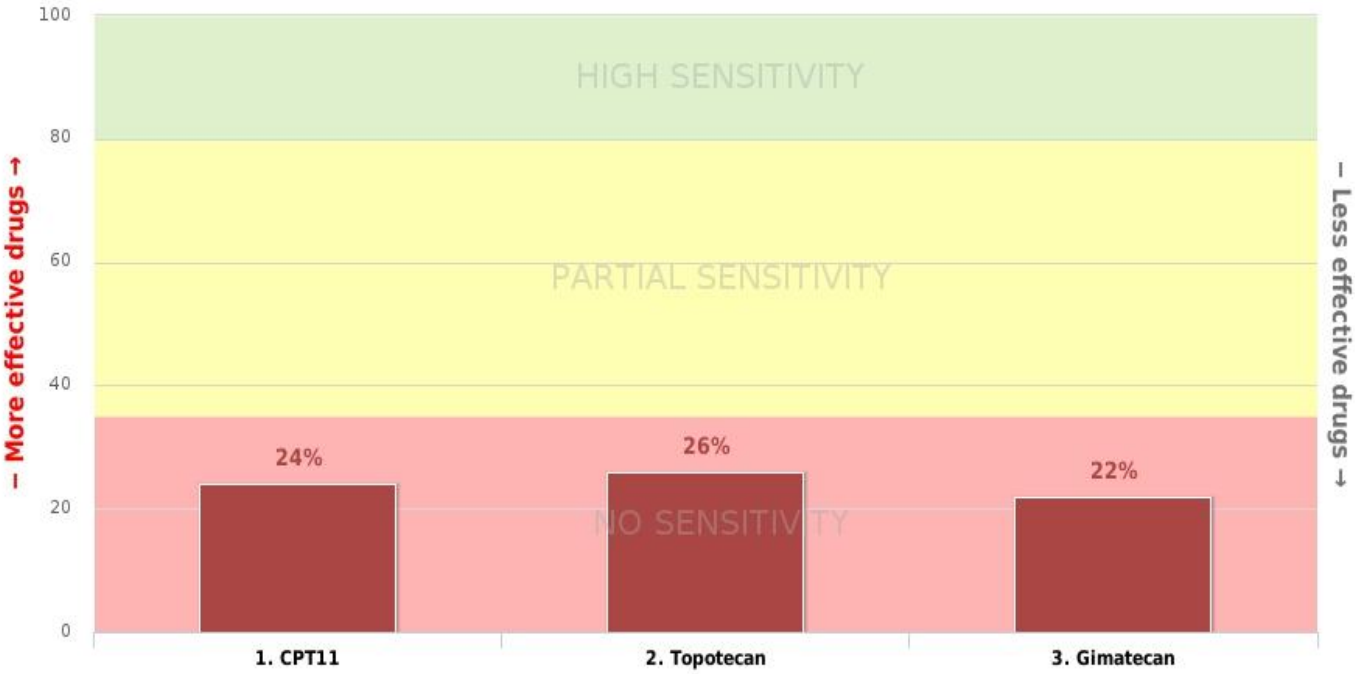


Partial Sensitivity: Ixabepilone

Mr./Ms. _____
Industrial Area of Florina, GR 53100 – Florina, Greece
Tel.: +30 23850 41950, 41951, 41960, 41961, Fax.: +30 23850 41931
Website: www.rgcc-group.com E-mail:papasotiriou.ioannis@rgcc-genlab.com

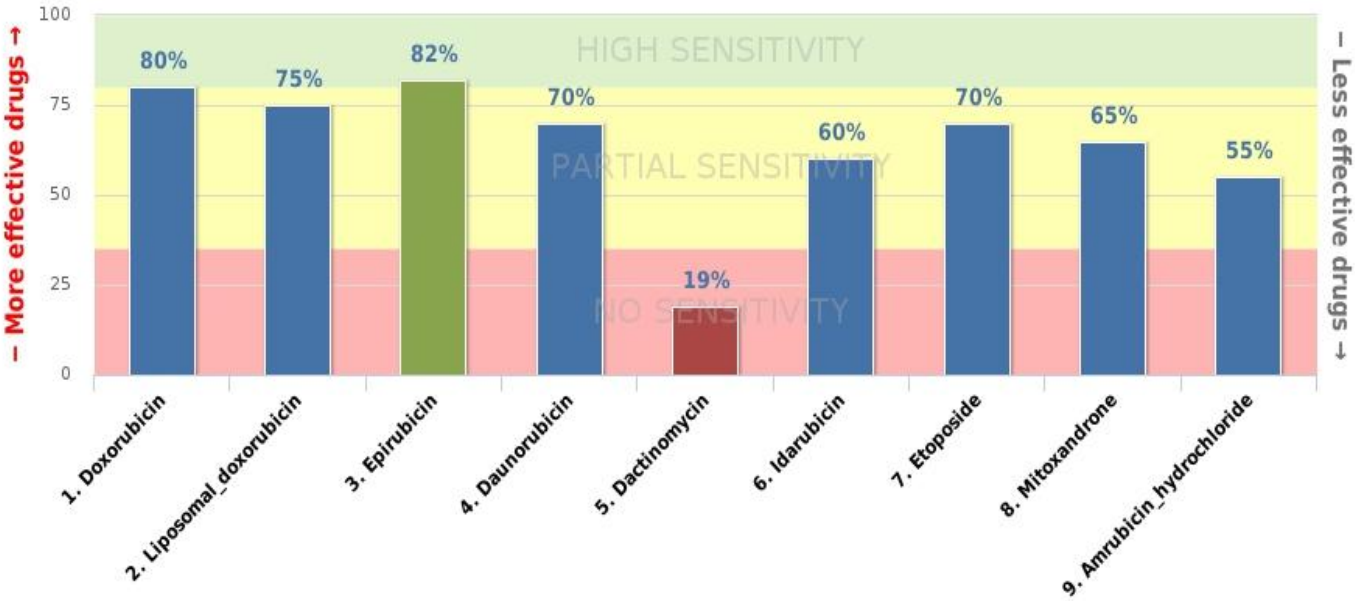
Day, ___/___/_____

Inhibitors of Topoisomerase I



No Sensitivity: CPT11, Topotecan, Gimatecan

Inhibitors of Topoisomerase II



High Sensitivity: Epirubicin
Partial Sensitivity: Doxorubicin, Liposomal_doxorubicin, Daunorubicin, Idarubicin, Etoposide, Mitoxandrone, Amrubicin_hydrochloride
No Sensitivity: Dactinomycin

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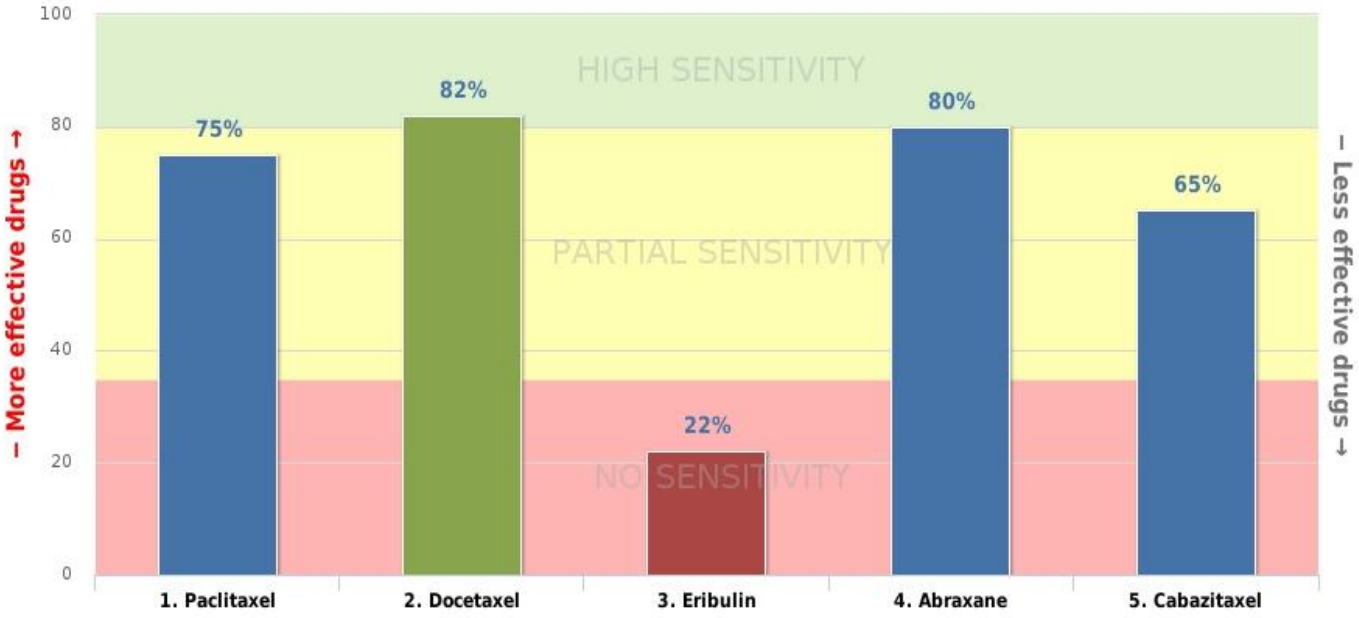
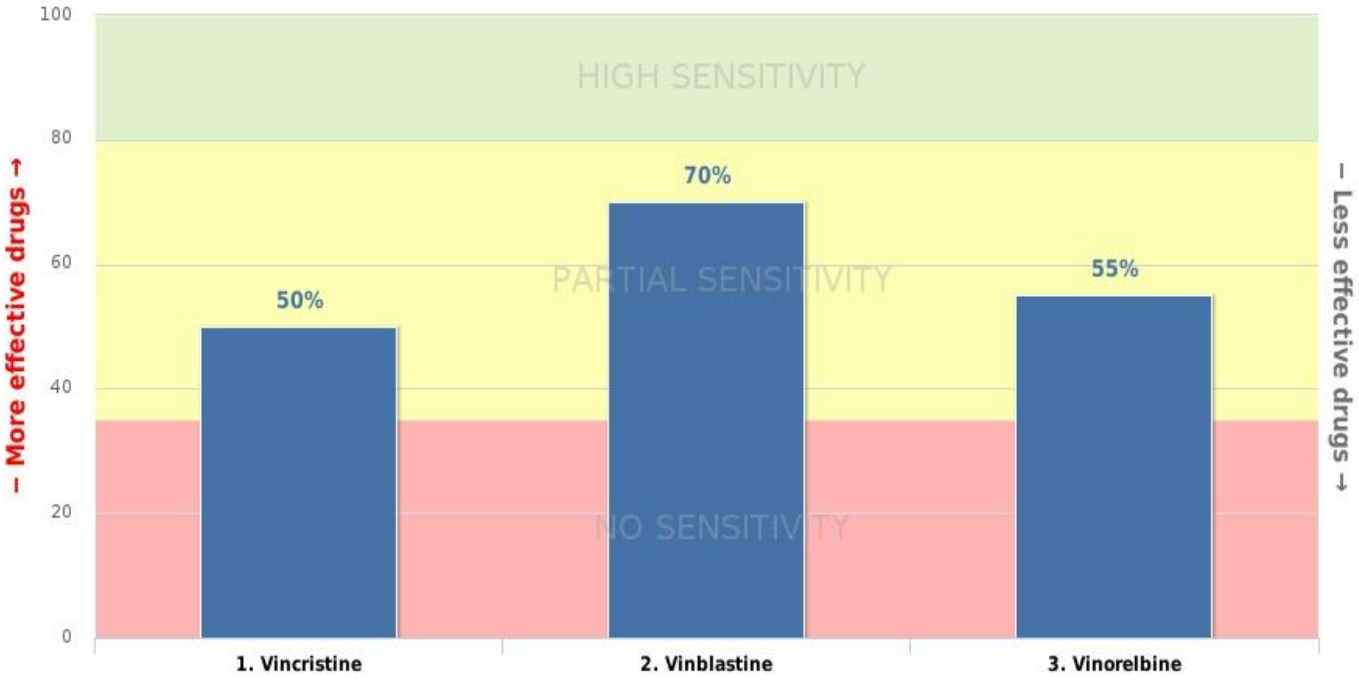
Day, ___/___/_____

Nucleus Spindle Stabilizer I



High Sensitivity: Docetaxel
Partial Sensitivity: Paclitaxel, Abraxane, Cabazitaxel
No Sensitivity: Eribulin

Nucleus Spindle Stabilizer II



Partial Sensitivity: Vincristine, Vinblastine, Vinorelbine

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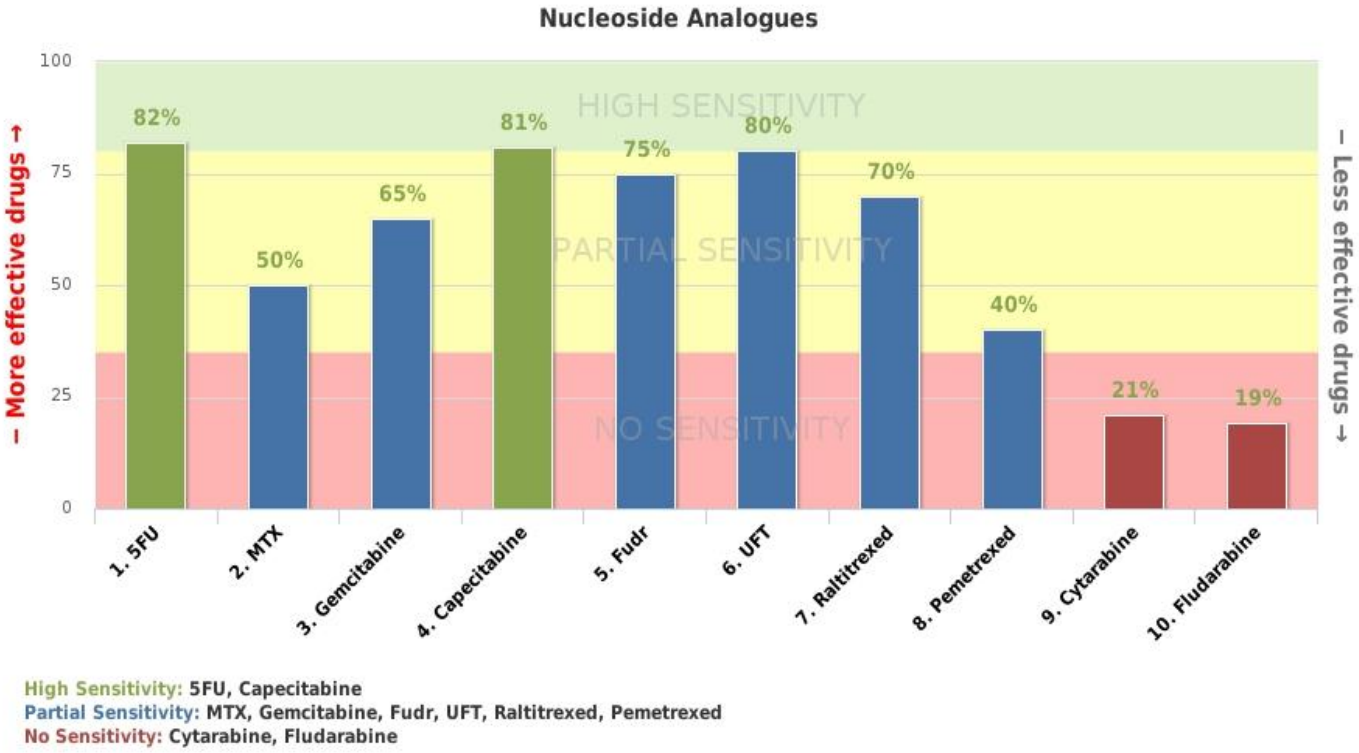
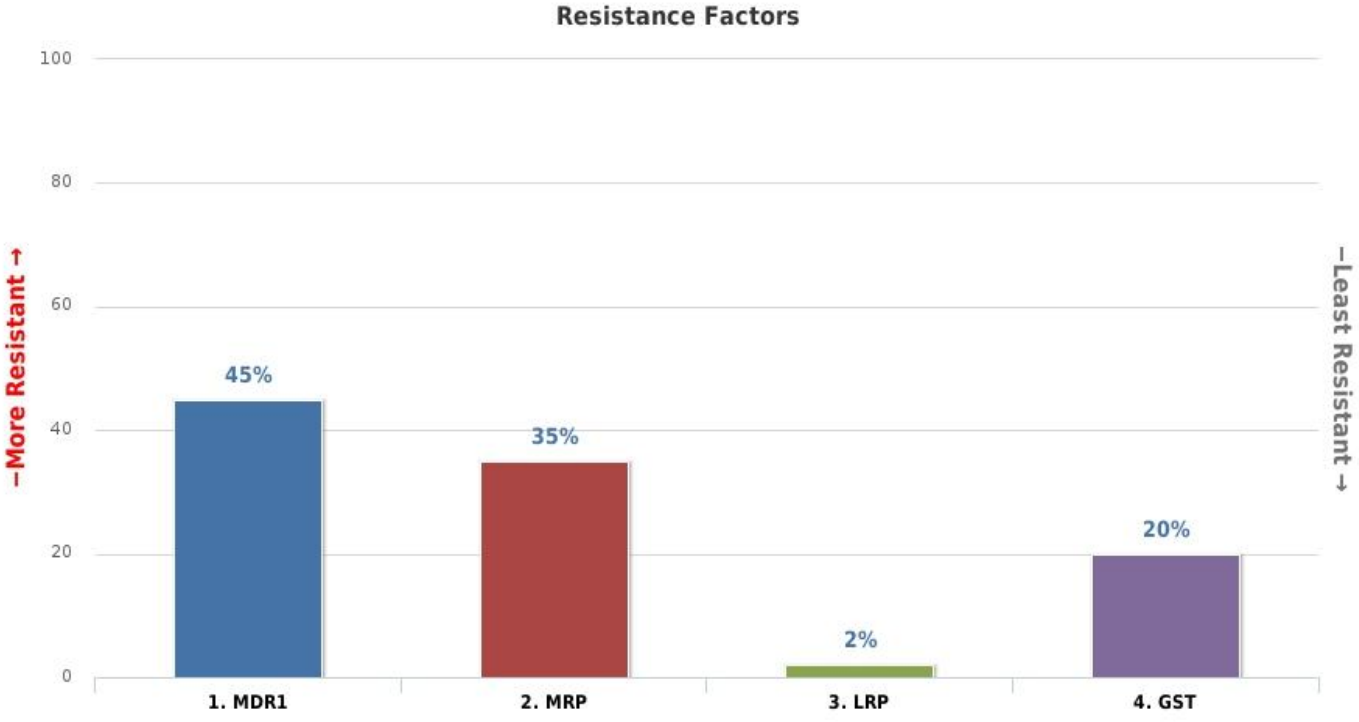


High Sensitivity: 5FU, Capecitabine
Partial Sensitivity: MTX, Gemcitabine, Fudr, UFT, Raltitrexed, Pemetrexed
No Sensitivity: Cytarabine, Fludarabine



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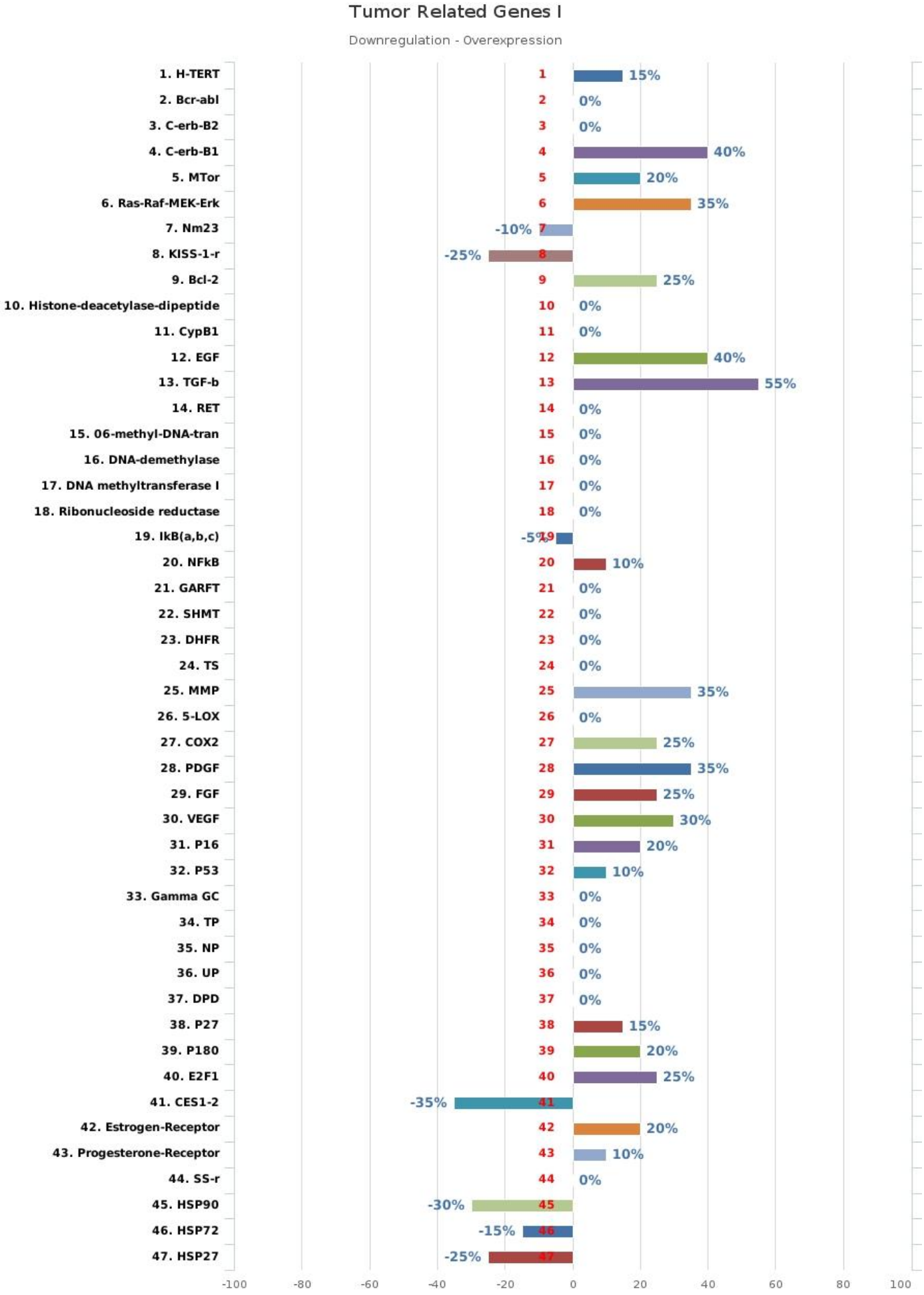

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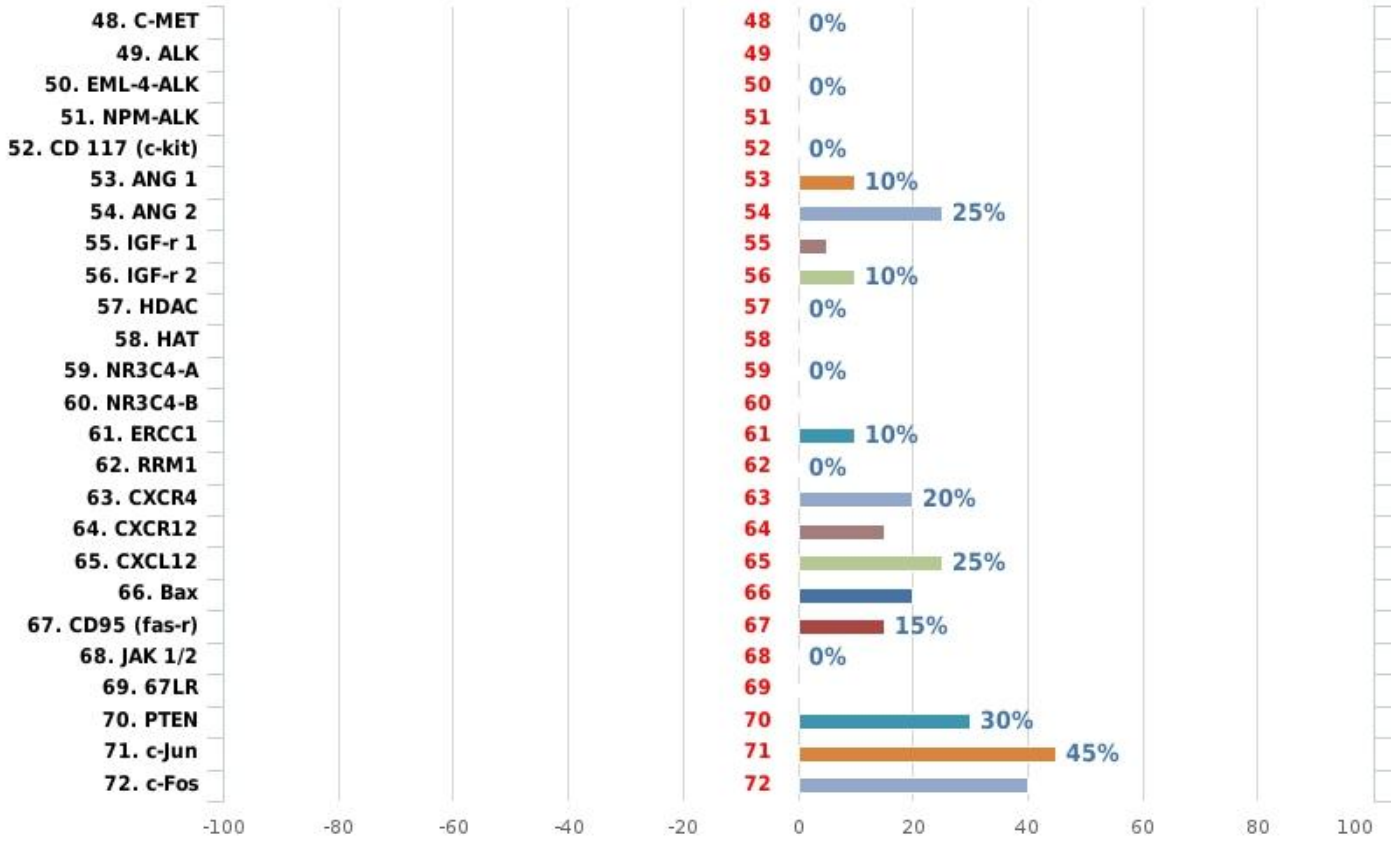
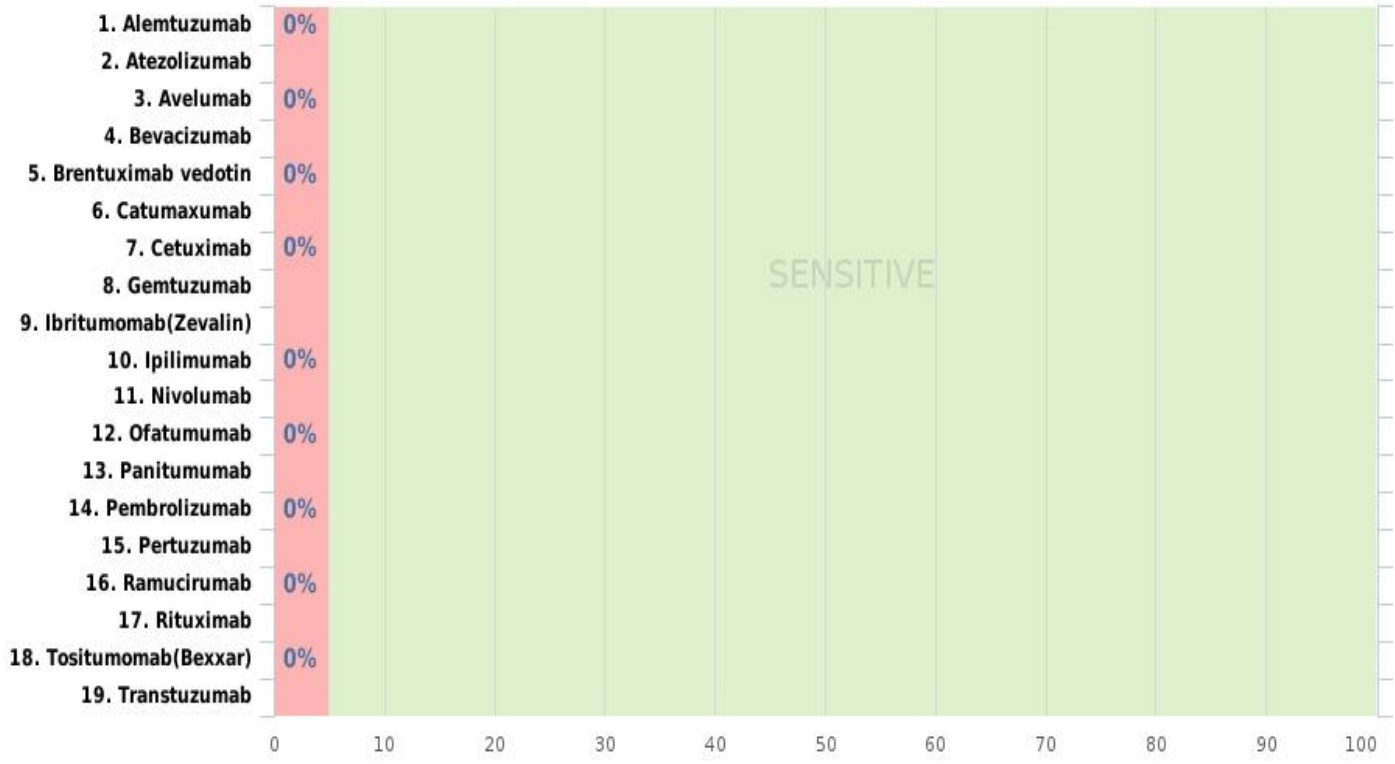
Day, ___/___/_____

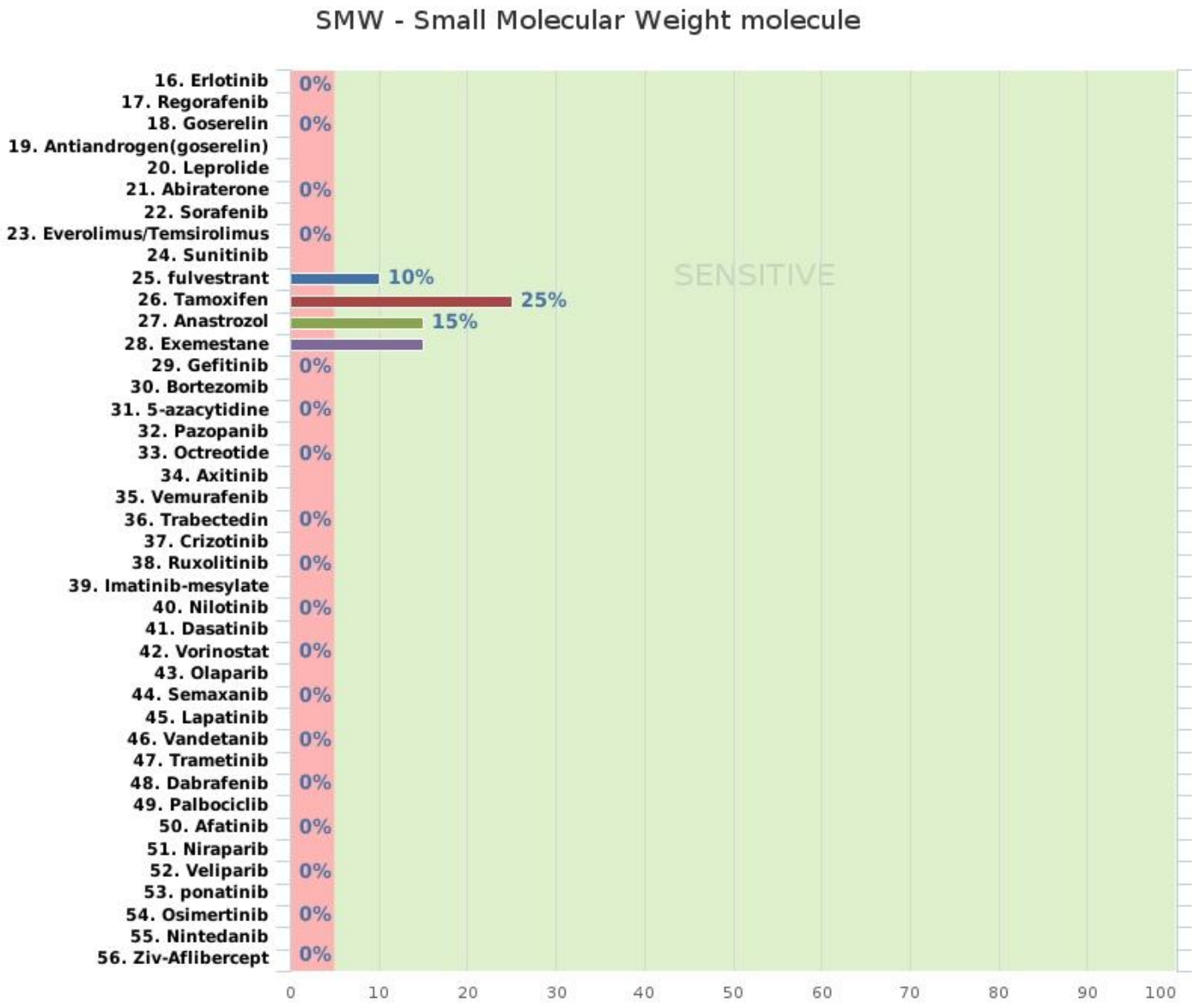
Tumor Related Genes II

Downregulation - Overexpression



Moab - Monoclonal Antibodies





Tumor Related Genes

GROWTH FACTORS PROLIFERATION STIMULI

NAME	RELATED	RESULTS	OUTCOME	FUNCTION	CLINICAL RISK
p180	Tyrosin kinase growth f.	**20%**	HIGH RISK	Preprotein for Cellular stress	HIGH RISK
Bcr-abl	Resist phenotype	normal	LOW RISK	Fusion Protein	LOW RISK
PTEN	Tumor Suppressor Gene	**30%**	HIGH RISK	Repair Related Gene	HIGH RISK

COX2	Tumour Growth	**25%**	HIGH RISK	Eicosanoid related protein	HIGH RISK
5-LOX	Tumour Growth	normal	LOW RISK		

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Day, ___/___/_____

NFkB	Transcription fact	**10%**	HIGH RISK	Proteasome inhibitors	HIGH RISK
IkB(a,b,c)	Inhibitor of NFκB	normal	LOW RISK		

ALK	Acute Leukemia kinase	normal	LOW RISK	Proto-Oncogene	LOW RISK
EML-4-ALK	Fusion EML with ALK	normal	LOW RISK		
NPM-ALK	Fusion NPM with ALK	normal	LOW RISK		
RET	proto-oncogene	normal	LOW RISK		

SS-r	Somatostatin receptor	normal	LOW RISK	Growth Factor Receptor	HIGH RISK
CD 117(c-kit)	Proliferate growth factor receptor 1	normal	LOW RISK		
IGF-r 1	Insulin like growth factor receptor I	normal	LOW RISK		
IGF-r-2	Insulin like growth factor receptor II	**10%**	HIGH RISK		
EGF	Tumour Growth	**40%**	HIGH RISK		
c-erb-B1	Her1	**40%**	HIGH RISK		
c-erb-B2	Her/neu2	normal	LOW RISK		

JAK 1/2	Single transduction pathway	normal	LOW RISK	Signal transduction pathway	HIGH PROLIFERATIVE SIGNAL
c-Jun	Proto-Oncogene	**45%**	HIGH RISK		
c-Fos	Proto-Oncogene	**40%**	HIGH RISK		
Ras/Raf/MEK/Erk	Transduction pathway	**35%**	HIGH RISK		
mTOR	Transduction pathway	**20%**	HIGH RISK		

Progesterone Receptor	Growth Factor receptor	**10%**	HIGH RISK	Hormone Receptors	HORMONE DEPENDENT
Estrogene Receptor	Growth Factor receptor	**20%**	HIGH RISK		
NR3C4-A	Nucleous receptor group III Class 4 (androgen receptor A)	normal	LOW RISK		
NR3C4-B	Nucleous receptor group III Class 4 (androgen receptor B)	normal	LOW RISK		

SELF REPAIR - RESISTANCE

NAME	RELATED	RESULTS	OUTCOME	FUNCTION	CLINICAL RISK
TGF-b	Tumour Growth	**55%**	HIGH RISK	Signal transduction pathways	HIGH RISK

HSP27	Heat Shock Protein	**-25%**	SENSITIVE	Radiotherapy/ Hyperthermia sensitivity	SENSITIVE
HSP72	Heat Shock Protein	**-15%**	SENSITIVE		
HSP90	Heat Shock Protein	**-30%**	SENSITIVE		

DNA methyltransferase I	DNA methylation	normal	LOW RISK	Resistant Phenotype Markers	RESISTANT
DNA demethylase	DNA methylation	normal	LOW RISK		
06-methyl-DNA-tran.	DNA methylation	normal	LOW RISK		
Histonedeacetylase-dipeptide	DNA coiling (nucleosome)	normal	LOW RISK		
HAT	Histone acetyl transferase	normal	LOW RISK		
CXCR4	Resistant Phenotype	**20%**	HIGH RISK		
CXCR12	Resistant Phenotype	**15%**	HIGH RISK		
CXCL12	Resistant Phenotype	**25%**	HIGH RISK		
Gamma GC	Resist to alkylating drug	normal	LOW RISK		
HDAC	Histone deacetylase	normal	LOW RISK		

ANGIOGENESIS

NAME	RELATED	RESULTS	OUTCOME	FUNCTION	CLINICAL RISK
VEGF	Angiogenesis	**30%**	HIGH RISK	Angiogenesis	HIGH RISK
FGF	Angiogenesis	**25%**	HIGH RISK		
PDGF	Angiogenesis	**35%**	HIGH RISK		
ANG 1	Angiogenin I	**10%**	HIGH RISK		
ANG 2	Angiogenin II	**25%**	HIGH RISK		

CELL CYCLE REGULATION & IMMORTALIZATION / APOPTOSIS

NAME	RELATED	RESULTS	OUTCOME	FUNCTION	CLINICAL RISK
E2F1	Transcr. Fact of TS & topo I	**25%**	HIGH RISK	Increase protein Synthesis	HIGH RISK
CDC6	Initiation of DNA replication	normal	LOW RISK	Rapid Cell Cycle	LOW RISK
h-TERT	M2 crisis-aggressive phen.	**15%**	HIGH RISK	Immortalization	HIGH RISK

Bcl-2	Apoptosis	**25%**	HIGH RISK	Regulation of apoptosis	HIGH RISK
Bax	Apoptosis	**20%**	HIGH RISK		
CD95 (fas-r)	Apoptosis related receptor	**15%**	HIGH RISK		

p27	Cell arrest (G0)	**15%**	LOW RISK	Cell cycle Rate	RAPID
p53	Cell cycle regulator	**10%**	HIGH RISK		
p16	Apoptosis	**20%**	HIGH RISK		

ANGIOGENESIS - METASTASES

NAME	RELATED	RESULTS	OUTCOME	FUNCTION	CLINICAL RISK
c-MET	Mesenchymal to epithelial transition	normal	LOW RISK	Migration invasion	HIGH RISK
67LR	67 Laminin receptor	normal	LOW RISK		
KISS-1-r	Metastases regulator	**-25%**	HIGH RISK		
Nm23	Metastases regulator	**-10%**	HIGH RISK		
MMP	Metastases	**35%**	HIGH RISK		

DRUG METABOLISMS & TARGETS

NAME	RELATED	RESULTS	OUTCOME	FUNCTION	CLINICAL RISK
CES1&2 (carboxyesterase)	Resist to camptothecin	**-35%**	LOW RISK	Activation of camptothecin	LOW RISK
DPD	Resist to 5FU	normal	LOW RISK	Nucleoside Import transformation	LOW RISK
UP	Resist to 5FU	normal	LOW RISK		
NP	Resist topyrim. Antagonist	normal	LOW RISK		
TP	Resist to 5FU	normal	LOW RISK		
TS	Rapid cell cycle (THFA)	normal	LOW RISK		
DHFR	Rapid cell cycle (THFA)	normal	LOW RISK		
SHMT	Rapid cell cycle (THFA)	normal	LOW RISK		
GARFT	Rapid cell cycle(THFA)	normal	LOW RISK		
Ribonucleosidereductase	DNA synthesis	normal	LOW RISK		
CypB1	Xenobiotic metabolism	normal	LOW RISK	Xenobiotic	LOW RISK
ERCC1	DNA repair mechanism	**10%**	HIGH RISK	DNA repair related gene	HIGH RISK
RRM1	Nucleotide polymerizationss	normal	LOW RISK		

MARKERS

NAME	RELATED	RESULTS	OUTCOME	CLINICAL RISK
CD33	Myeloid cellorigin	normal	LOW RISK	LOW RISK
CD52	Leukaemia marker	normal	LOW RISK	LOW RISK
CD20	Lymphoma related antigen	normal	LOW RISK	LOW RISK
EpCAM	Epithelial marker	**20%**	HIGH RISK	HIGH RISK
PD-L1	Immunoregulatory factor	normal	LOW RISK	LOW RISK

PD 1		normal	LOW RISK	LOW RISK
PD-L2		normal	LOW RISK	LOW RISK

From the investigation above we concluded to the following:

1. From the whole neoplasmic population we have an expression of MDR1 in a percentage of 45% 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 low limits (no resistance to camptothecin compounds).
5. The concentration of p180 is in high range.
6. Increased activity of the Laminin and the MMP (increased invasive ability).
7. There is great sensitivity in taxanes (Docetaxel).
8. There is partial sensitivity in alkaloids of vinca.
9. There is no sensitivity in Eribulin.
10. Partial sensitivity noticed in MTX, in Gemcitabine, in Fudr, in UFT, in Raltitrexed, in Pemetrexed, no sensitivity noticed in Cytarabine, in Fludarabine but there is great sensitivity in (5FU, Capecitabine).
11. There is partial sensitivity in Epothilones.
12. Increased sensitivity in alkylating factors (Cyclophosfamide).
13. There is great overexpression of NFkB (10% over control), EGF (40% over control), TGF-b (55% over control) but there is normal expression of IkB(a, b, c).
14. It appears to have great sensitivity in the inhibitors of topoisomerase II (Epirubicin).
15. There is no sensitivity in the inhibitors of Topoisomerase I.
16. There is great over-expression of COX2 (25% over control), C-erb-B1 (40% over control), Estrogen-Receptor (20% over control), Progesterone-Receptor (10% over control) but there is normal expression of 5-LOX, SS-r, C-erb-B2.
17. We notice great neoangiogenetic ability (overexpression of VEGF-R 30% 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 25% below control, HSP72 (Heat Shock Protein) at 15% below control and HSP90 (Heat Shock Protein) at 30% below control.
22. There is over-expression of ANG 1 at 10% over control, ANG 2 at 25% over control, IGF-r 2 at 10% over control, but we notice no down-regulation of ALK, EML-4-ALK, C-MET, NPM-ALK, CD 117 (c-kit), IGF-r 1, HDAC, HAT, NR3C4-A and NR3C4-B.

Conclusion:

- The specific tumor appears to have resisting populations because of the MDR1 overexpression that can be reversed by the use of inhibitors of ABCG2 pumps.
- The neoplasmatic cells have the greatest sensitivity in the alkylating agent (**Cyclophosfamide**), in the inhibitors of Topoisomerase II (**Epirubicin**), in the nucleous spindle stabilizer (**Docetaxel**) and in the antagonist (**5FU, Capecitabine**)
- Also can be used **Fulvestrant** as inhibitor of estrogen positive proliferative signal, **Tamoxifen** as inhibitor of estrogen positive feedback, **Anastrozol** as inhibitor of estrogen synthesis and **Exemestane** as inhibitor of aromatase enzyme.

Sincerely,

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

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