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

**Florina, 03/10/2017**

Dear colleague,

We send you the results from the analysis on a patient Mr xxxxxxxxxxxxx suffering from cholangiocarcinoma 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.

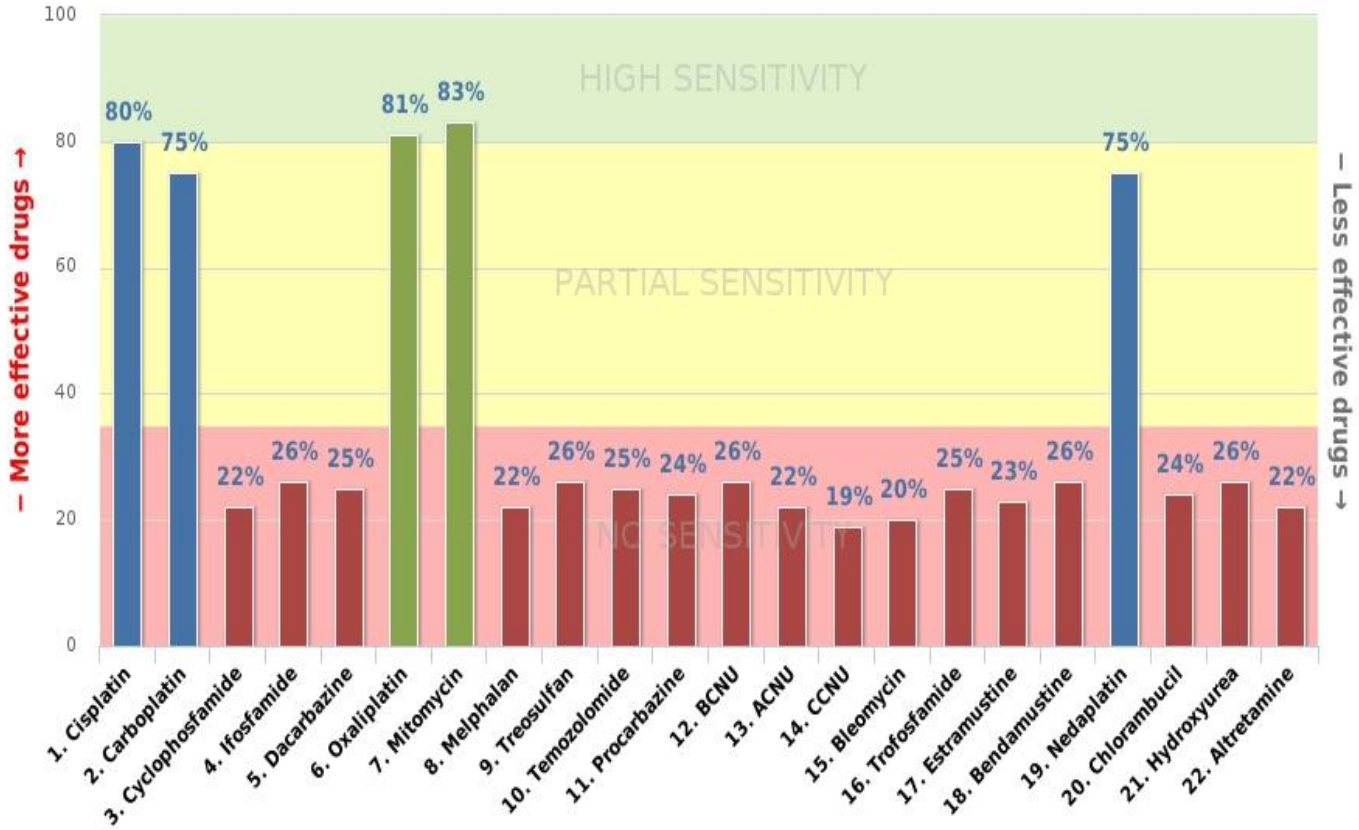
In our laboratory we made the following:

- 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 6.6cells/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.

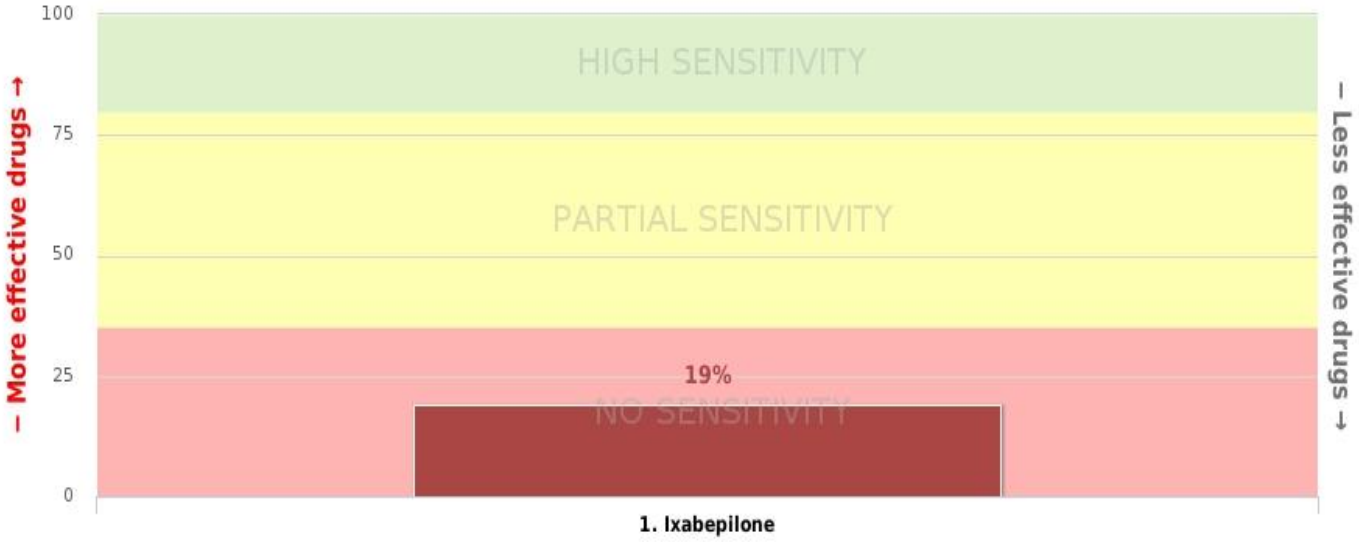
**INTERPRETATION:** The numbers above the bars indicate % of cancer cell **DEATH** caused by the drug tested. This equates the % **SENSITIVITY** to that drug. Therefore, the drugs with the highest numbers are the most effective drugs at inducing cancer cell death for the patient tested. The numbers below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

## Alkylating Agents




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

## Epothilones




**No Sensitivity:** Ixabepilone

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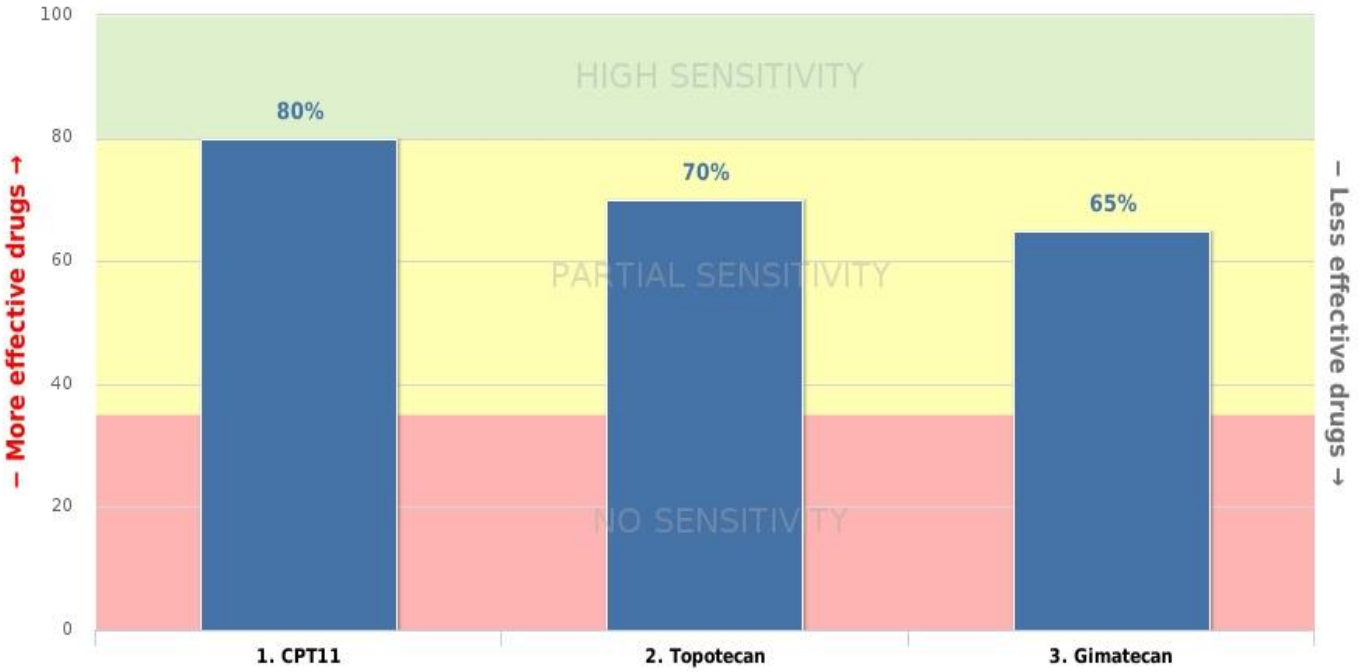
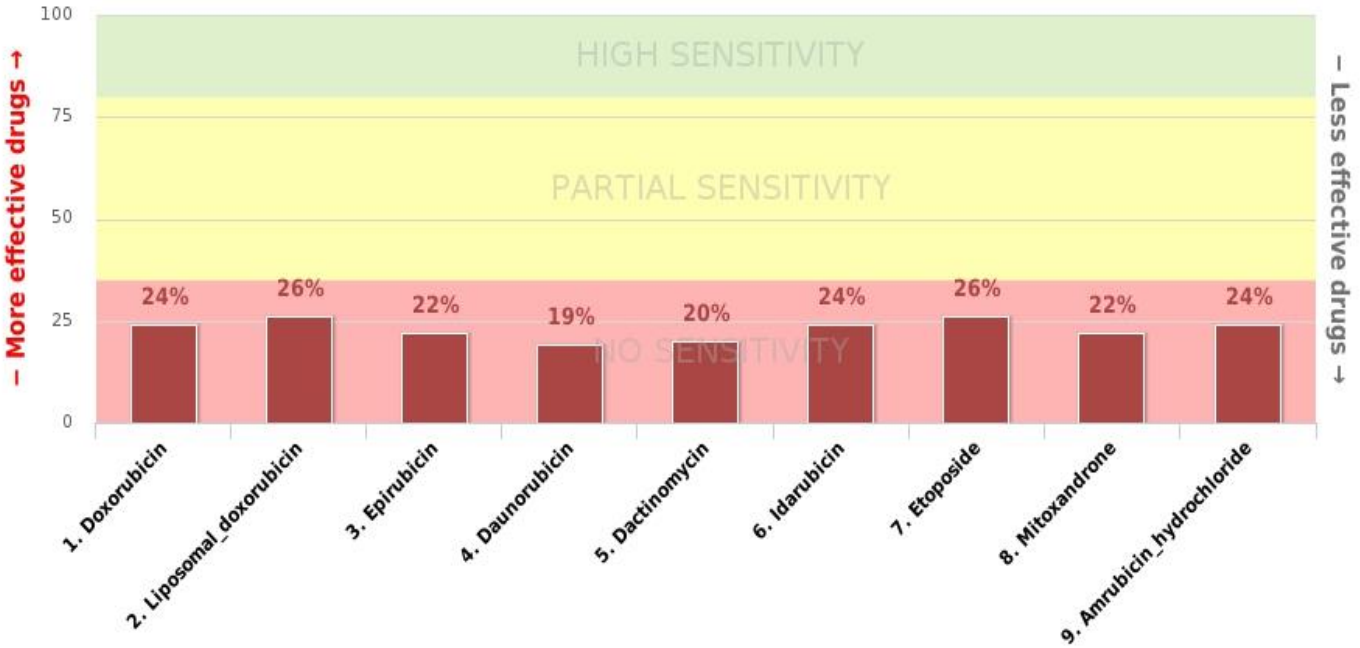
## Inhibitors of Topoisomerase I



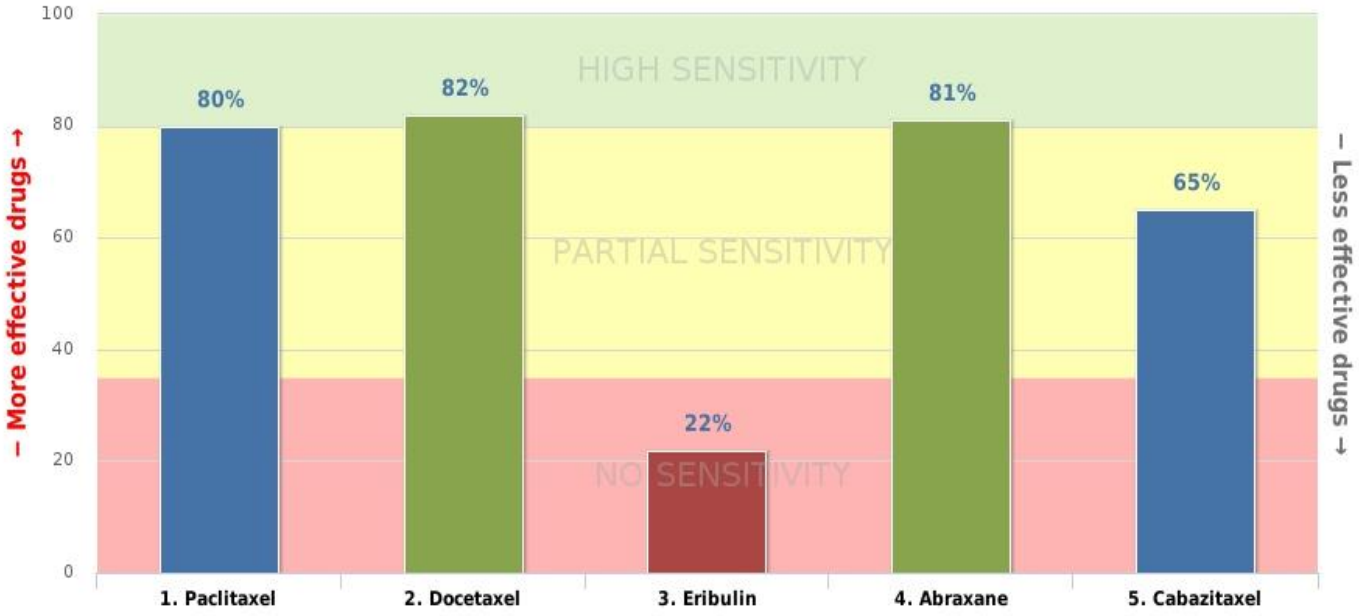

**Partial Sensitivity:** CPT11, Topotecan, Gimatecan

## Inhibitors of Topoisomerase II



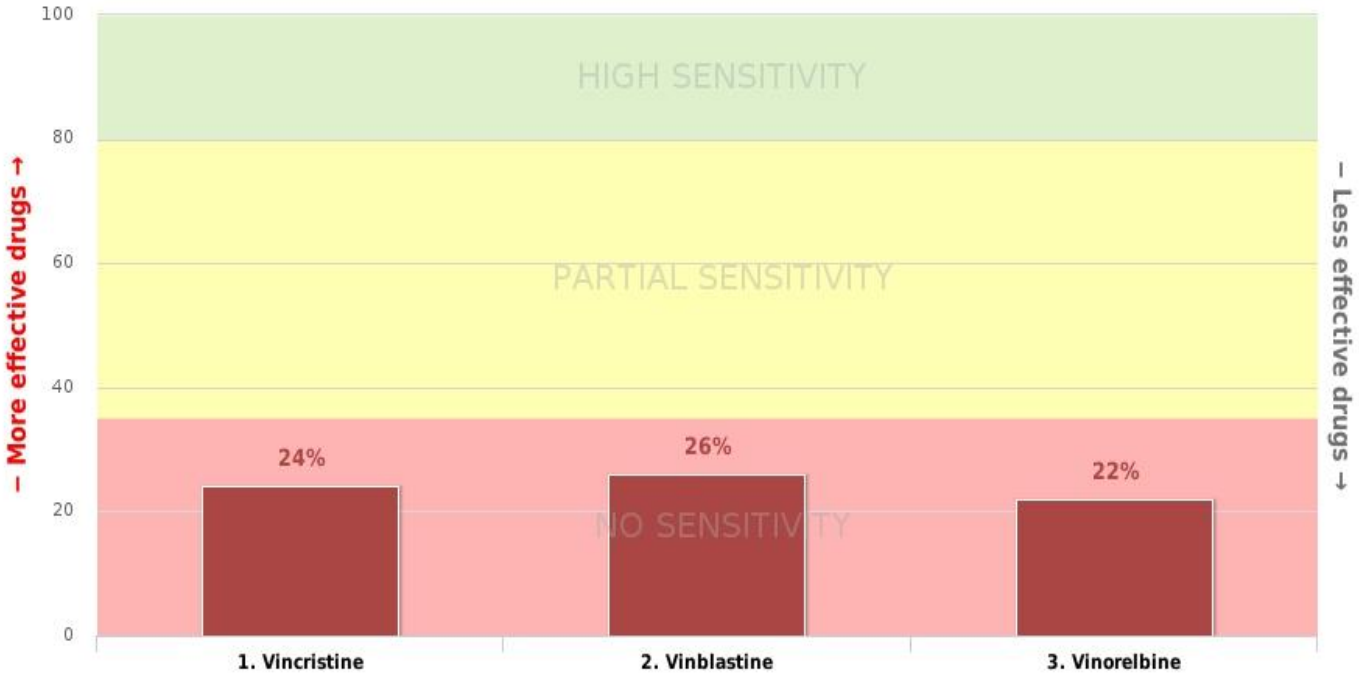

**No Sensitivity:** Doxorubicin, Liposomal_doxorubicin, Epirubicin, Daunorubicin, Dactinomycin, Idarubicin, Etoposide,
↳ Mitoxandrone, Amrubicin_hydrochloride

## Nucleus Spindle Stabilizer I




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

## Nucleus Spindle Stabilizer II




No Sensitivity: Vincristine, Vinblastine, Vinorelbine

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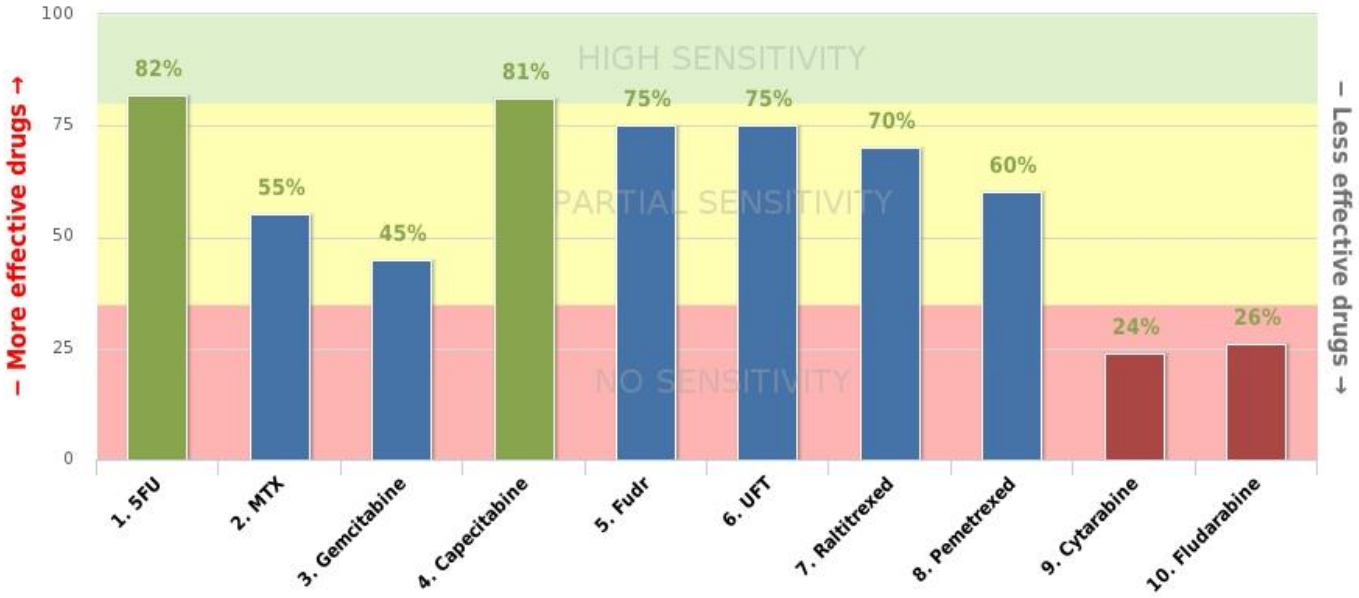
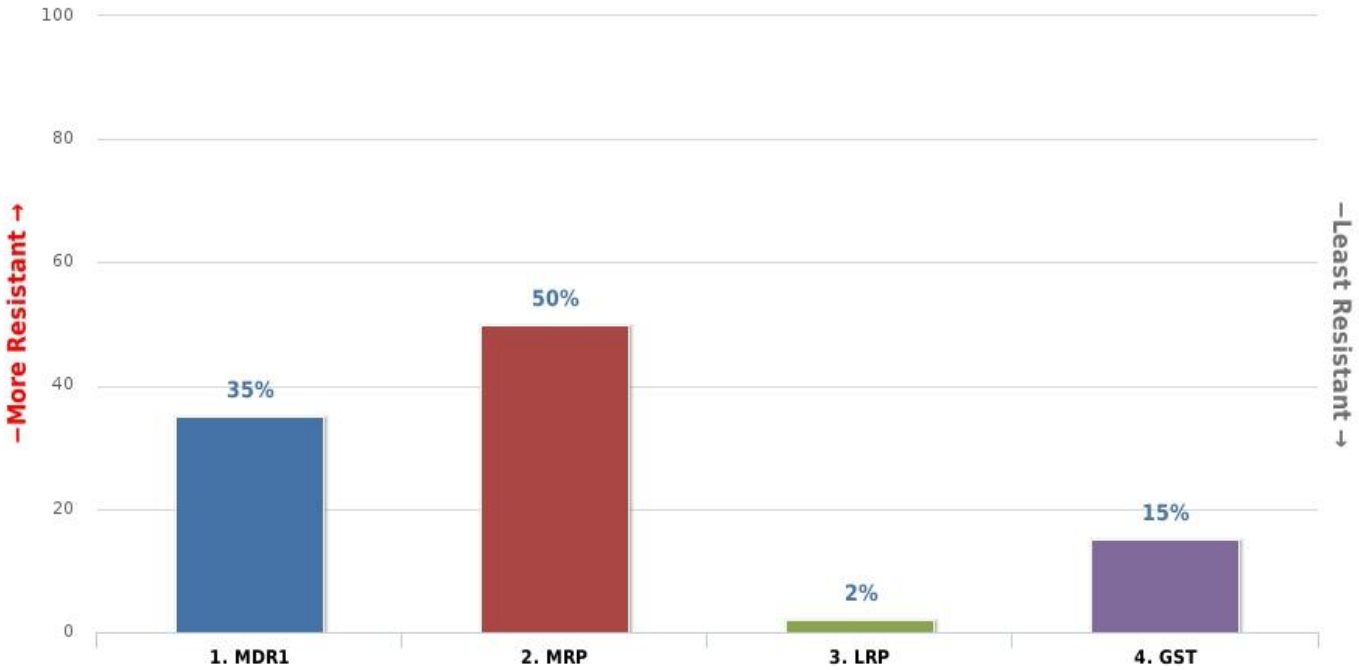
## Nucleoside Analogues




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

## Resistance Factors

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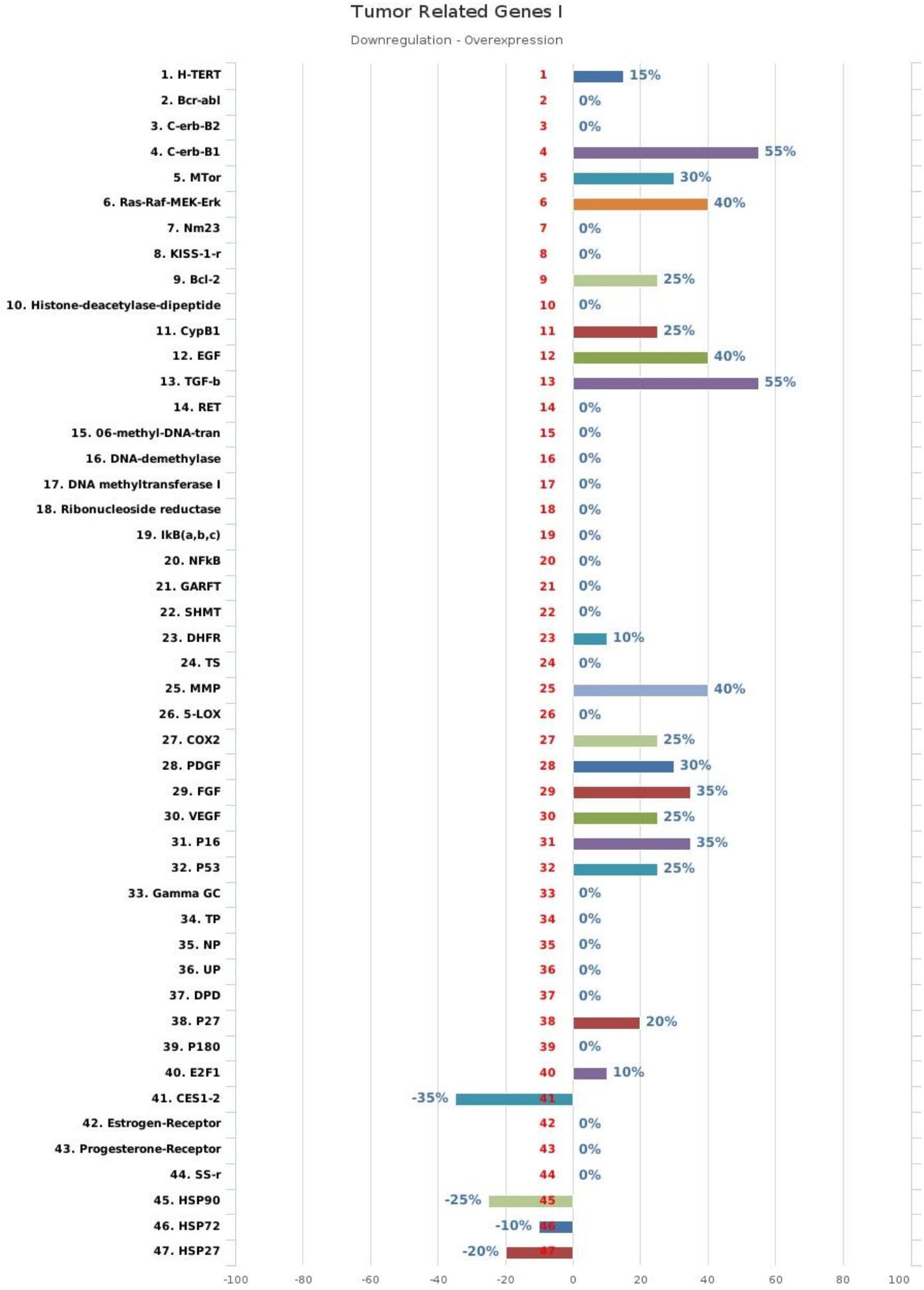


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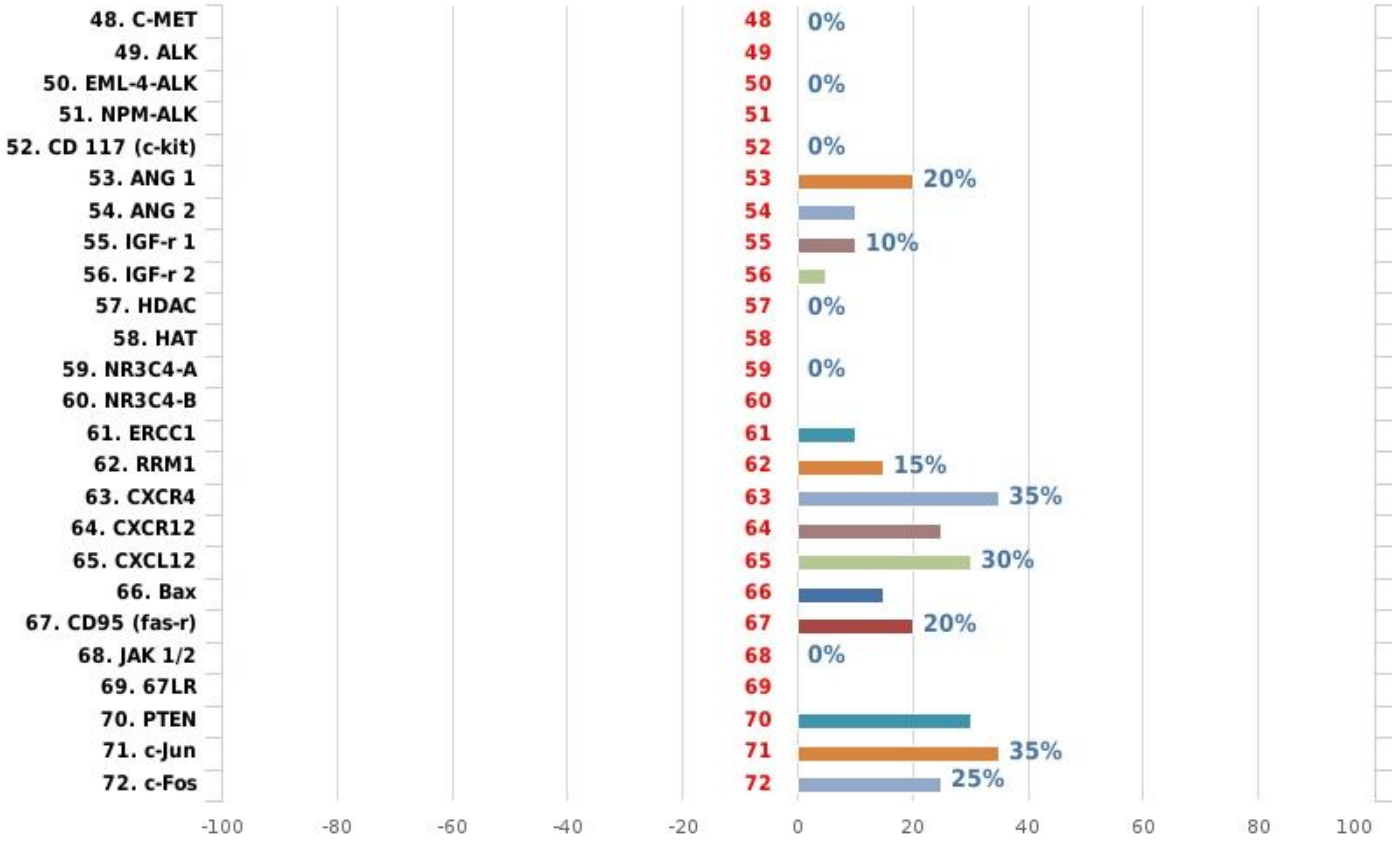
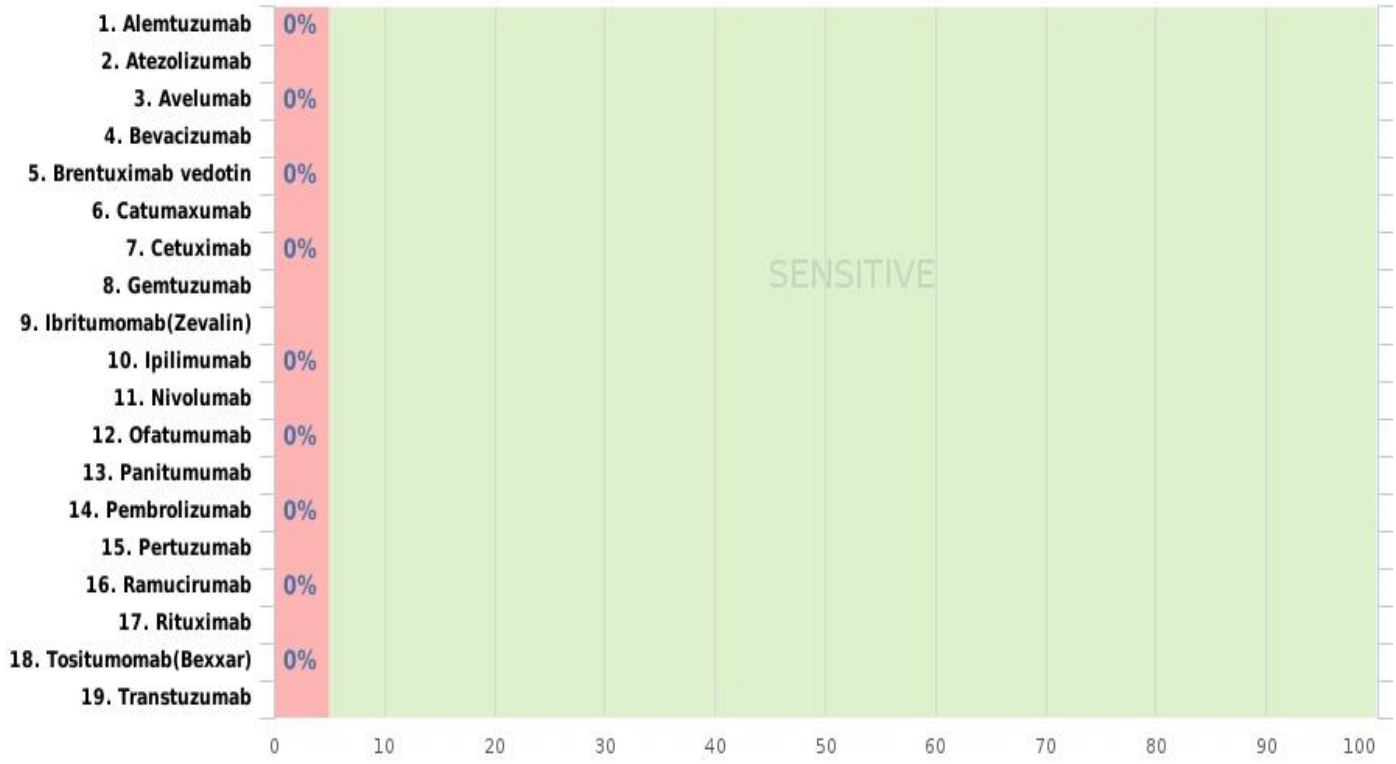
## Tumor Related Genes II

Downregulation - Overexpression




## Moab - Monoclonal Antibodies

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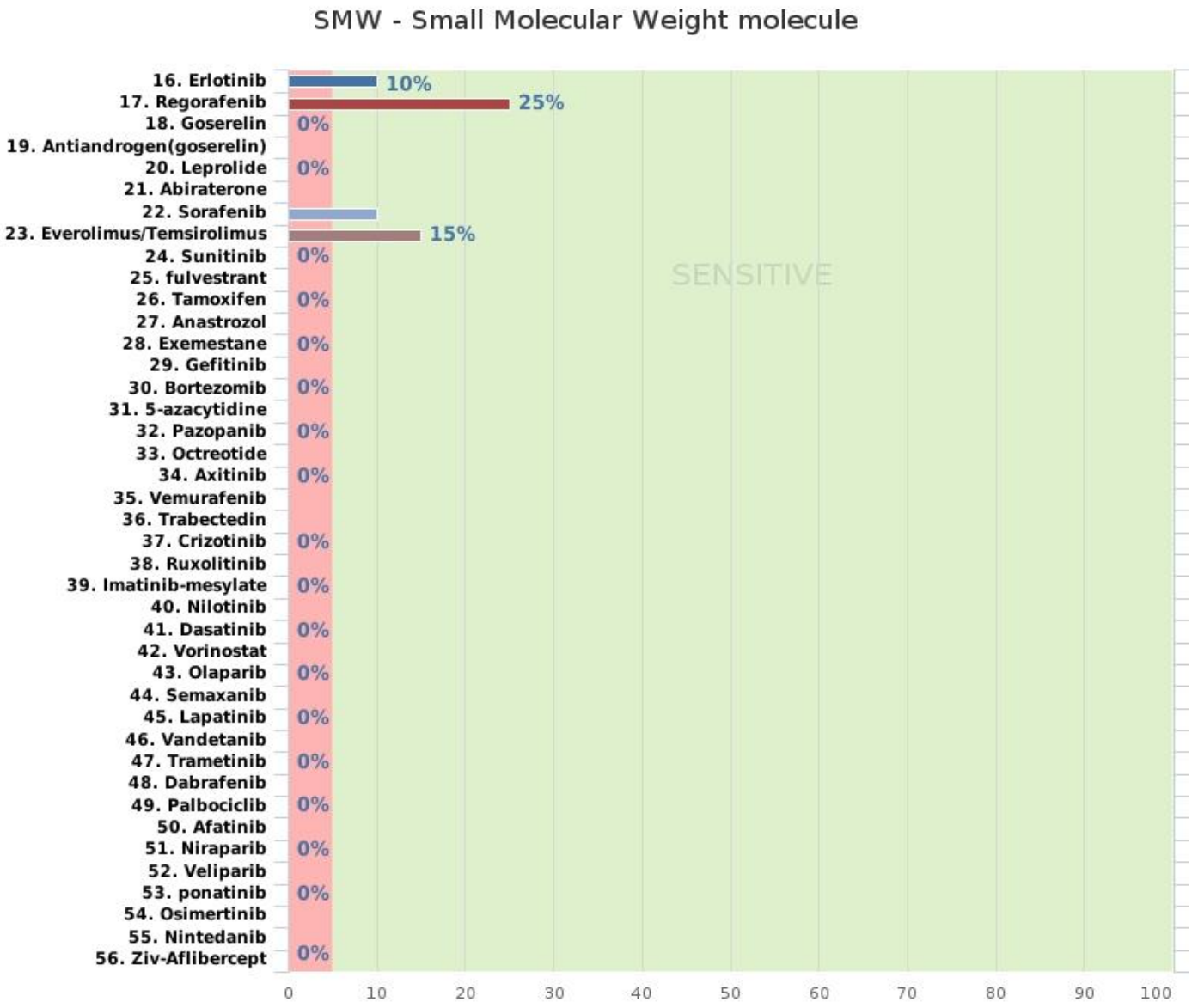


## Tumor Related Genes

## GROWTH FACTORS PROLIFERATION STIMULI

| NAME | RELATED | RESULTS | OUTCOME | FUNCTION | CLINICAL RISK |
|---|---|---|---|---|---|
| p180 | Tyrosin kinase growth f. | normal | LOW RISK | Preprotein for Cellular stress | LOW 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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| | | | | | |
|---|---|---|---|---|---|
| NFkB | Transcription fact | normal | LOW RISK | Proteasome inhibitors | LOW 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 | **10%** | HIGH RISK | | |
| IGF-r-2 | Insulin like growth factor receptor II | normal | LOW RISK | | |
| EGF | Tumour Growth | **40%** | HIGH RISK | | |
| c-erb-B1 | Her1 | **55%** | 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 | **35%** | HIGH RISK | | |
| c-Fos | Proto-Oncogene | **25%** | HIGH RISK | | |
| Ras/Raf/MEK/Erk | Transduction pathway | **40%** | HIGH RISK | | |
| mTOR | Transduction pathway | **30%** | HIGH RISK | | |

| | | | | | |
|---|---|---|---|---|---|
| Progesterone Receptor | Growth Factor receptor | normal | LOW RISK | Hormone Receptors | HORMONE INDEPENDENT |
| Estrogene Receptor | Growth Factor receptor | normal | LOW 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 | **-20%** | SENSITIVE | Radiotherapy/ Hyperthermia sensitivity | SENSITIVE |
| HSP72 | Heat Shock Protein | **-10%** | SENSITIVE | | |
| HSP90 | Heat Shock Protein | **-25%** | 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 | **35%** | HIGH RISK | | |
| CXCR12 | Resistant Phenotype | **25%** | HIGH RISK | | |
| CXCL12 | Resistant Phenotype | **30%** | 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 | **25%** | HIGH RISK | Angiogenesis | HIGH RISK |
| FGF | Angiogenesis | **35%** | HIGH RISK | | |
| PDGF | Angiogenesis | **30%** | HIGH RISK | | |
| ANG 1 | Angiogenin I | **20%** | HIGH RISK | | |
| ANG 2 | Angiogenin II | **10%** | HIGH RISK | | |

## CELL CYCLE REGULATION & IMMORTALIZATION / APOPTOSIS

| NAME | RELATED | RESULTS | OUTCOME | FUNCTION | CLINICAL RISK |
|---|---|---|---|---|---|
| E2F1 | Transcr. Fact of TS & topo I | **10%** | 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 | **15%** | HIGH RISK | | |
| CD95 (fas-r) | Apoptosis related receptor | **20%** | HIGH RISK | | |

| | | | | | |
|---|---|---|---|---|---|
| p27 | Cell arrest (G0) | **20%** | LOW RISK | Cell cycle Rate | RAPID |
| p53 | Cell cycle regulator | **25%** | HIGH RISK | | |
| p16 | Apoptosis | **35%** | 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 | normal | LOW RISK | | |
| Nm23 | Metastases regulator | normal | LOW RISK | | |
| MMP | Metastases | **40%** | 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 | HIGH 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) | **10%** | HIGH 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 | **25%** | HIGH RISK | Xenobiotic | HIGH RISK |
| ERCC1 | DNA repair mechanism | **10%** | HIGH RISK | DNA repair related gene | HIGH RISK |
| RRM1 | Nucleotide polymerizationss | **15%** | HIGH 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 MRP in a percentage of 50% 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 normal range.
6. Increased activity of the Laminin and the MMP (increased invasive ability).
7. There is great sensitivity in taxanes (Docetaxel, Abraxane).
8. There is no 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, but no sensitivity noticed in Cytarabine, in Fludarabine but there is great sensitivity in (5FU, Capecitabine).
11. There is no sensitivity in Epothilones.
12. Increased sensitivity in alkylating factors (Oxaliplatin, Mitomycin).
13. There is great overexpression of EGF (40% over control), TGF-b (55% over control), there is normal expression of IkB(a, b, c), NFkB.
14. It appears to have no sensitivity in the inhibitors of topoisomerase II a and II b.
15. There is partial sensitivity in the inhibitors of Topoisomerase I.
16. There is great over-expression of COX2 (25% over control), C-erb-B1 (55% over control), there is normal expression of 5-LOX, SS-r, C-erb-B2, Estrogen-Receptor, Progesterone-Receptor.
17. We notice great neoangiogenetic ability (overexpression of VEGF-R 25% 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 20% below control, HSP72 (Heat Shock Protein) at 10% below control, HSP90 (Heat Shock Protein) at 25% below control.
22. There is over-expression of ANG 1 at 20% over control, ANG 2 at 10% over control, IGF-r 1 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 2, HDAC, HAT, NR3C4-A, NR3C4-B.

**Conclusion:**

- The specific tumor appears to have resisting populations because of the MRP overexpression that can be reversed by the use of inhibitors of ABCG2 pumps.
- The neoplasmatic cells have the greatest sensitivity in the alkylating agent (**Oxaliplatin, Mitomycin**), in the nucleous spindle stabilizer (**Docetaxel**, **Abraxane**), in the antagonist (**5FU, Capecitabine**)
- Also can be used **Erlotinib** as inhibitor of EGFr, **Regorafenib** as inhibitor of angiopoietin 1, PDGF r and RET, **Sorafenib** as inhibitor of Ras/Raf/MEK/Erk transaction pathway, **Everolimus/Temsirolimus** as inhibitor of Akt/mTOR pathway.

Sincerely,

Ioannis Papasotiriou MD., PhD
Head of molecular medicine dpt. of
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**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