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

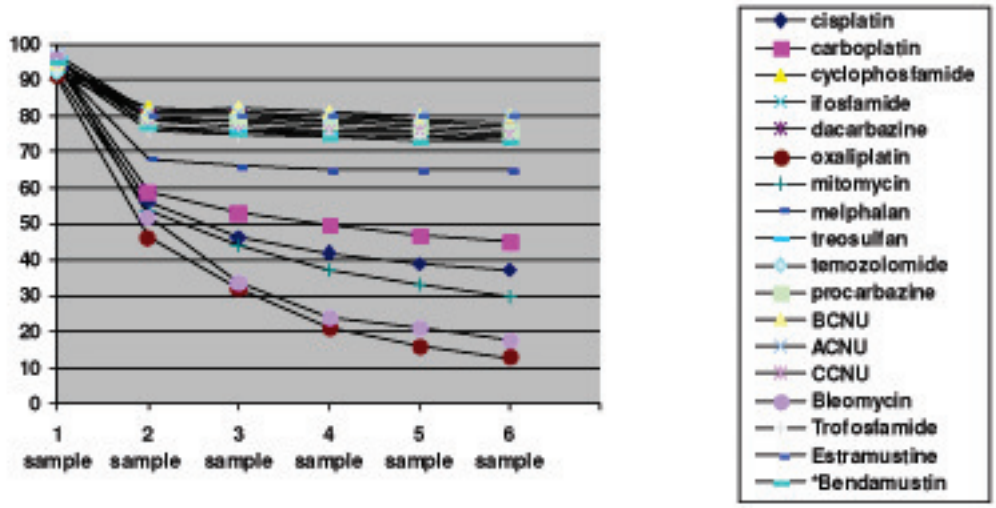
Florina , 15 / 12 / 2009

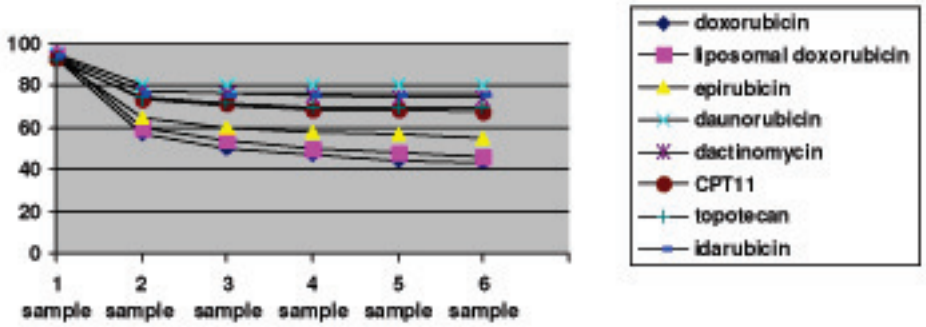
we send you the results from the analysis made about a patient suffering from squamous cell carcinoma stage ? The sample that was sent to us for analysis was a sample of 40ml of whole blood that contained EDTA-Ca as anti-coangulant , all packed with water ice .

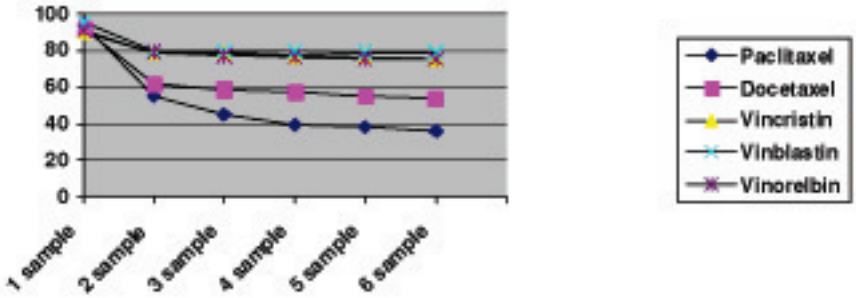
In our laboratory we made the following :

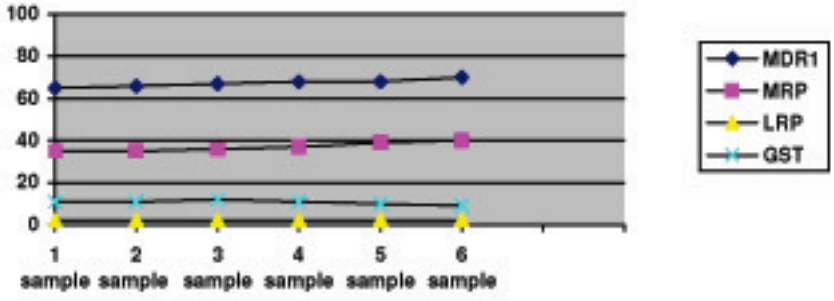
- We isolated the malignant cells using Oncoquick with a membrane that isolates malignant cells from normal cells after centrifugation and positive selection using epithelial membrane marker and negative selection using anti-CD45 particles.
- Then we developed cell cultures in a fetal calf serum media 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 gloutathion-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 alkyliating 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 gemcitabin)
- 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 nucleous protein kinases which are a target of the carbazin compounds .
- We defined the overexpression of TGFa and TGFb factors as targets for suramin sulfate.
- We defined the overexression 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.

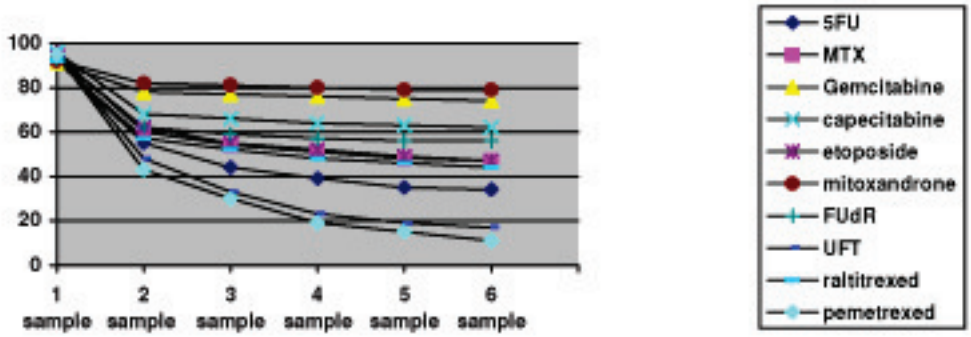
The above conclusions were also confirmed by the cell cultures of the tumor and in the diagrams there is a development curve for each category of cytostatics.











NAME	RELATED	RESULTS
CES1 &2 (carboxyesterase)	Resist to camptothecin	Normal
E2F1	Transcr. Fact of TS & topoI	Normal
p180	Tyrosin kinase growth f.	Normal
p27	Cell arrest (G_0)	65% over control
DPD	Resist to 5FU	Normal
UP	Resist to 5FU	Normal
NP	Resist to pyrim. antagonist	Normal
TP	Resist to 5FU	Normal
Gamma GC	Resist to alkyliating drug	Normal
p53	Cell cycle regulator	35% over control
p16	Apoptosis	50% over control
VEGF	Angiogenesis	80% over control
FGF	Angiogenesis	50% over control
PDGF	Angiogenesis	75% over control
COX2	Tumour Growth	Normal
5-LOX	Tumour Growth	Normal
MMP	Metastases	70% over control
TS	Rapid cell cycle (THFA)	Normal
DHFR	Rapid cell cycle (THFA)	Normal
SHMT	Rapid cell cycle (THFA)	Normal
GARFT	Rapid cell cycle(THFA)	Normal
NFκB	Transcription fact	20% over control
IκB (a,d,e)	Inhibitor of NFκB	10% below control
Ribonucleoside reductase	DNA synthesis	Normal
DNA methyltransferase I	DNA methylation	Normal
DNA demethylase	DNA methylation	Normal
O6-methylguanin-DNA-tran.	DNA methylation	Normal
TGF-b	Tumour Growth	45% over control
EGF	Tumour Growth	65% over control
IGF	Tumour Growth	Normal
CypB1	Xenobiotic metabolism	Normal
Histone deacylase -dispeptide	DNA coiling(nucleosome)	Normal
c-erb-B2	Her/neu2	Normal
c-erb-B1	Her1	Normal
Bcr-abl	Resist phenotype	Normal
h-TERT (Human telomerase)	M2 crisis-aggresive phen.	10% over control

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 70% 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 stable in the low limits (no resistance to platinum compounds)
4. The activity of CES1 and CES2 is 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 no sensitivity in taxanes (paclitaxel, docetaxel) and partial sensitivity noticed in alkaloids of Vinca .
8. Minimal sensitivity noticed in 5-FU, in 5FC, in FUdR in capecitabine, in raltitrexed , in methotrexate, in gemcitabine but there is great sensitivity in UFT and in pemetrexed.
9. Increased sensitivity in alkyliating factors (especially in oxaliplatin and bleomycin).
10. There is great overexpression of TGF b (45% over control) , of NFkBeta (20% over control) and EGF-r (65%,<70%) growth factors and supression of expression of isoforms of IκB (a, d, e) (10% below control) .
11. It appears to have no sensitivity in the inhibitors of topoisomerase II a and II b.
12. There is minimal sensitivity in the inhibitors of topoisomerase I .
13. There is no overexpression of SS-r receptor and there is no overexpression of COX-2 , of c-erb-B2, of estrogen receptor mRNA, progesterone receptor mRNA , of 5-LOX mRNA, and of c-erb-B1.
14. We notice great neoangiogenetic ability (overexpression of VEGF-R – 80% over control sample).
15. Finally , there is no sensitivity in dacarbazine .
16. We notice that taurolidin cannot induce the apoptosis to the malignant cells (in IV route dosage).
17. We notice that taurolidin can induce the apoptosis to the malignant cells (in intraperitoneal route dosage).
18. We notice down-regulation of HSP 27 (Heat shock proteins) (by 45% below control) , HSP 90 (55% below control) and HSP 72 (15% below control).

Conclusion :

- The specific tumor appears to have resisting populations because of the MDR1 overexpression that can be reversed by the use of verapamil combined with disulfiram.
- The neoplasmatic cells have the greatest sensitivity in the alkyliating agents **oxaliplatin** and **bleomycin**, and in the antagonist **uracil-tegafur (UFT)** and **pemetrexed**.
- **Also you can use:** cetuximab (C225) as inhibitor of EGF-r , bortezomib as inhibitor of proteasome over-activity and indirectly the transcriptional activity of NFκB and bevacizumab as inhibitor of angiogenesis.

Regardly,

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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
6th Sample viability : <20% greater sensitivity , 65%-20% partial sensitivty , >65% no sensitivity

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