

Patient NAME Sarah Williams	DATE OF BIRTH 1990-Aug-15	DISEASE Small Intestine	STAGE I	Physician NAME Dr. Mary Cavaggion
SPECIMEN 15ml Blood	VIAL IDs 133928			

REPORT SUMMARY

CTCs COUNT: Isolated 2.4 cells/ml , SD +/- 0.3 cells

SENSITIVITY - GENE EXPRESSION

Sensitivity

High sensitivity: alkylating factors, inhibitors of topoisomerase I, 5FU

Partial sensitivity: alkaloids of vinca, Capecitabin, Fudr, UFT, Raltitrexed

Expression

Over expression: C-erb-B1, EGF, COX2, IGF-r 1, IGF-r 2, TGF-b, VEGF, ANG 1, ANG 2, C-MET

Down regulation: HSP90, HSP72, HSP27

NATURAL SUBSTANCES SENSITIVITY

Class I

Cytotoxic Agents

Artecina, Amygdalin-(B17), Agaricus
Blazei Murill, Artesunate, Ascorbic acid,
Bio D Mulsion NuMedica D3, DCA
(dichloroacetate), Frankincense, Onkobel
Pro, Oxaloacetate (Cronaxal), Salicinium,
Super Artemisinin

Class II

Immunostimulants / Immunomodulators

Class III

PK Inhibitors

Angiostop, Apigenin, CoQ10, Curcumin
(turmeric), Genistein, Indol 3 Carbinol,
Naltrexone, Paw-Paw, Pure Quercetin,
Quercetin, VascuStatin

* Disclaimer! The natural substances that are tested in our lab facilities are not bonded from restriction for medical use.

Conclusion Results

- The neoplastic cells have the greatest sensitivity in Cisplatin, Carboplatin, Oxaliplatin, CPT11, 5FU
- Also can be used Bevacizumab, Ramucirumab, Regorafenib, Ziv-Aflibercept
- The specific tumor appears to have resisting populations because of the MDRI overexpression that can be reversed by the use of inhibitors of ABCG2 pumps

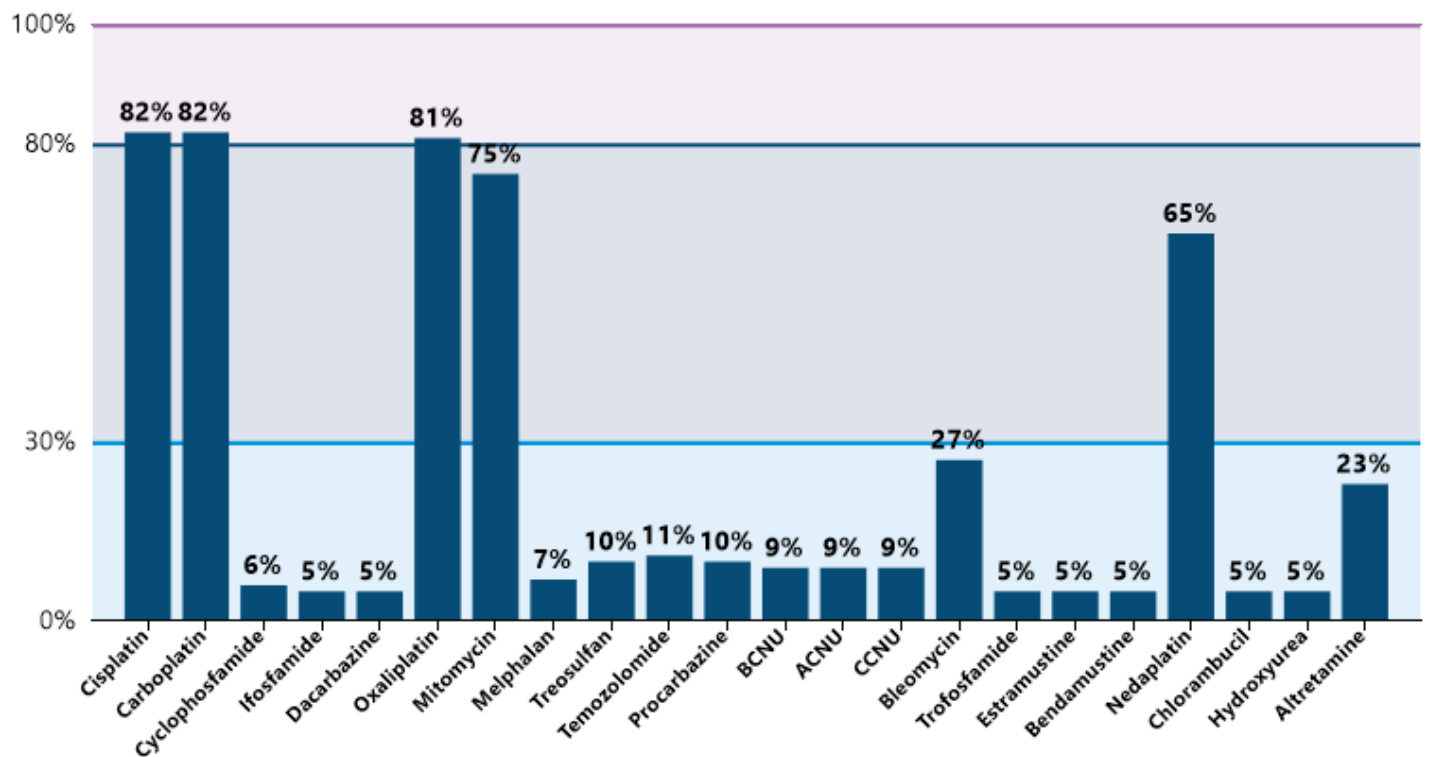
No sensitivity

Partial sensitivity

High sensitivity

Alkylating Agents

High sensitivity: **Cisplatin, Carboplatin, Oxaliplatin**

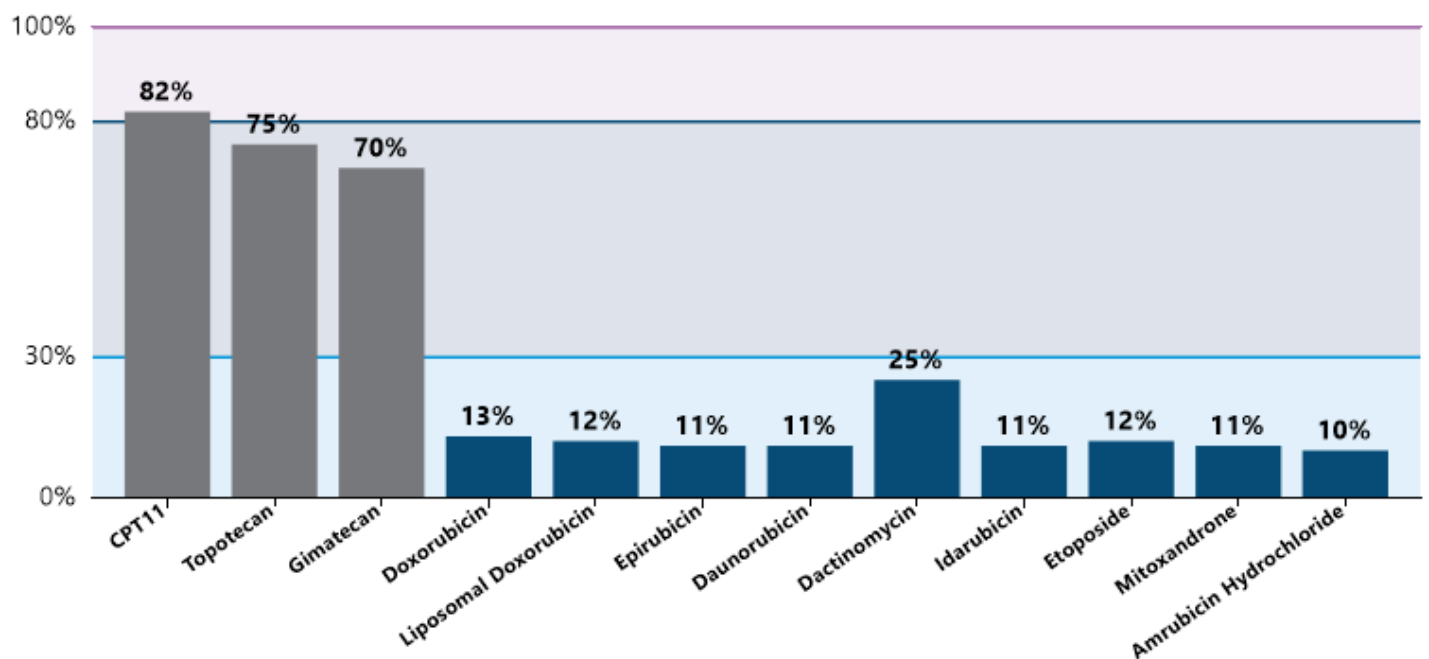


Inhibitors of Topoisomerase I & II

High sensitivity: **CPT11**

Inhibitors of Topoisomerase I

Inhibitors of Topoisomerase II



No sensitivity
 Partial sensitivity
 High sensitivity

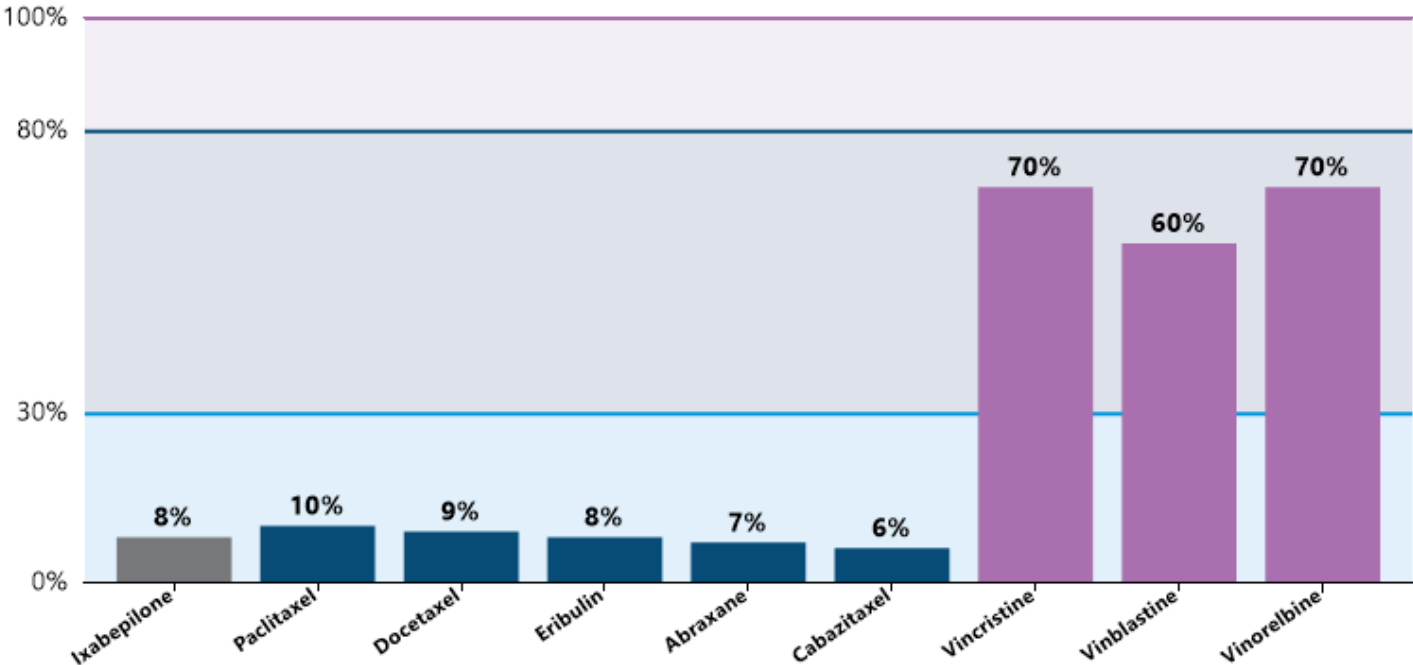
Epothilones & Nucleus Spindle Stabilizer I & II

High sensitivity:

Epothilones

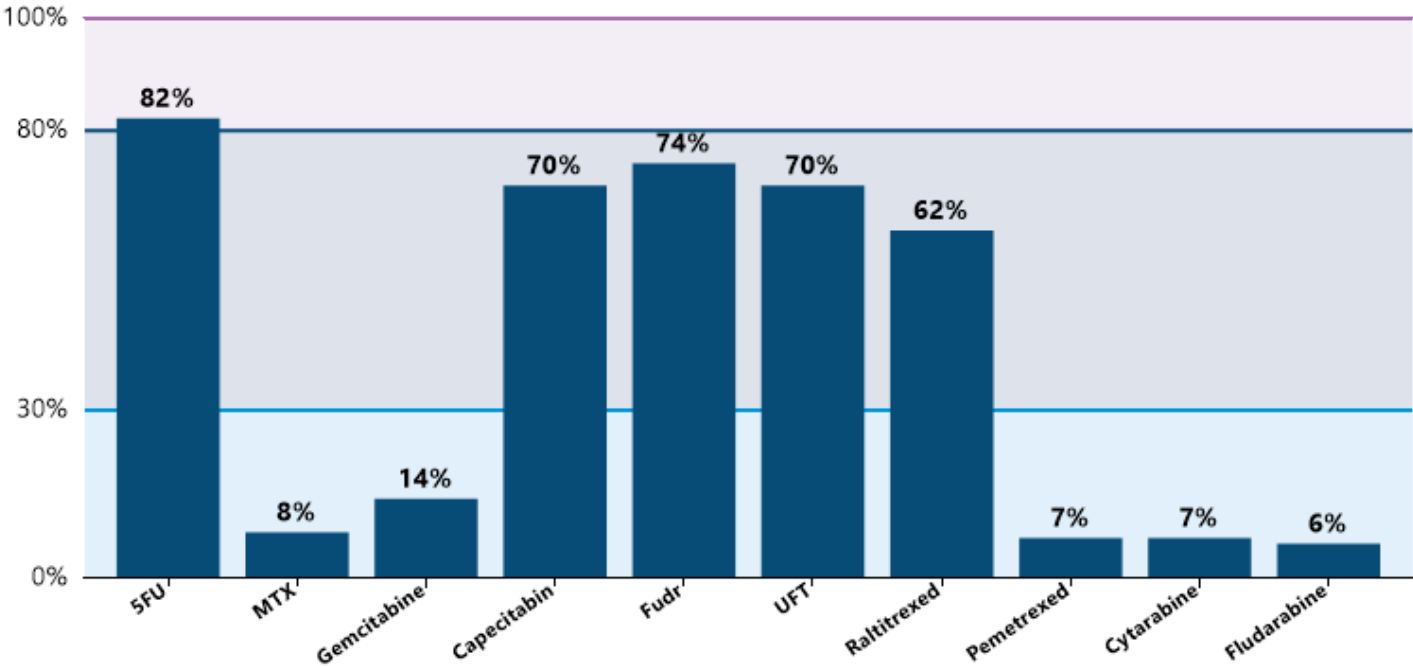
Nucleus Spindle Stabilizer I

Nucleus Spindle Stabilizer II

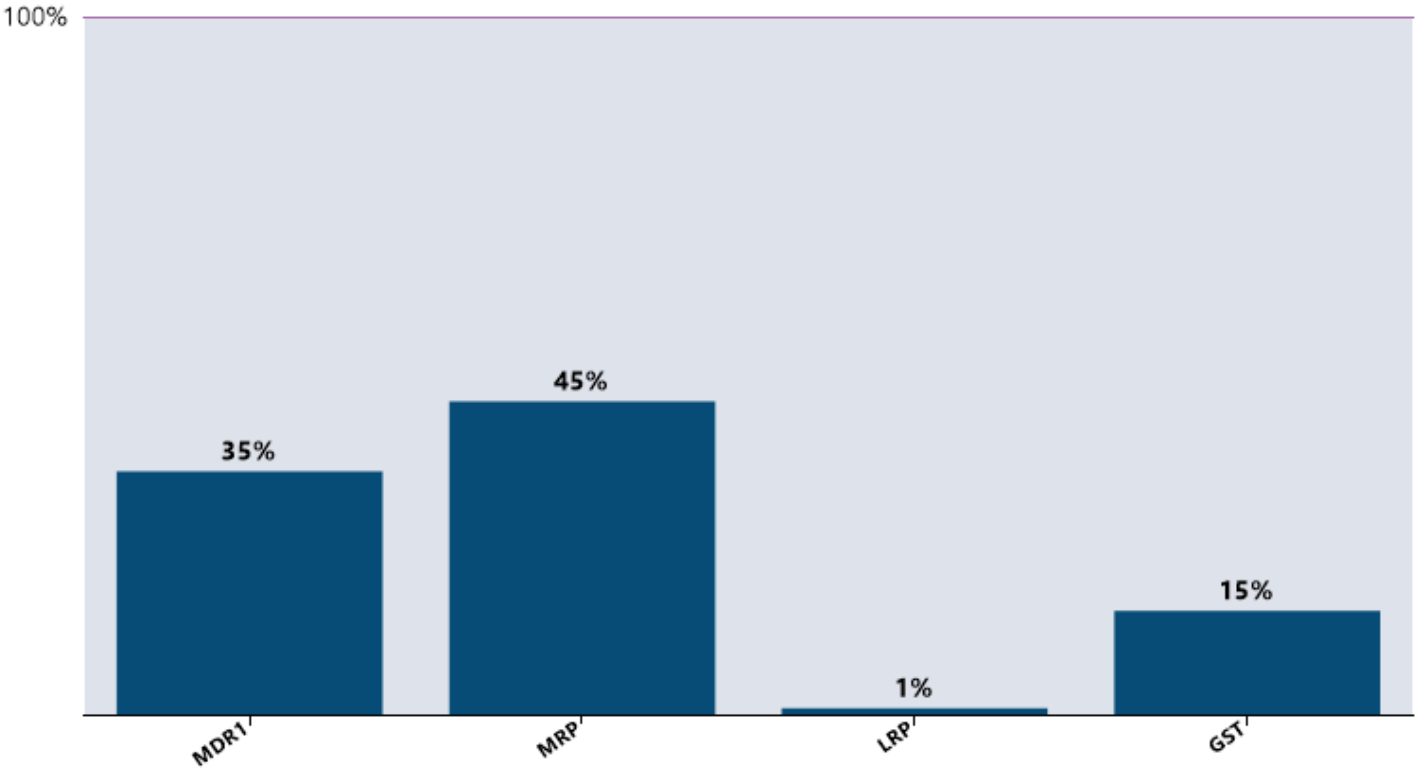


Nucleoside Analogues

High sensitivity: 5FU

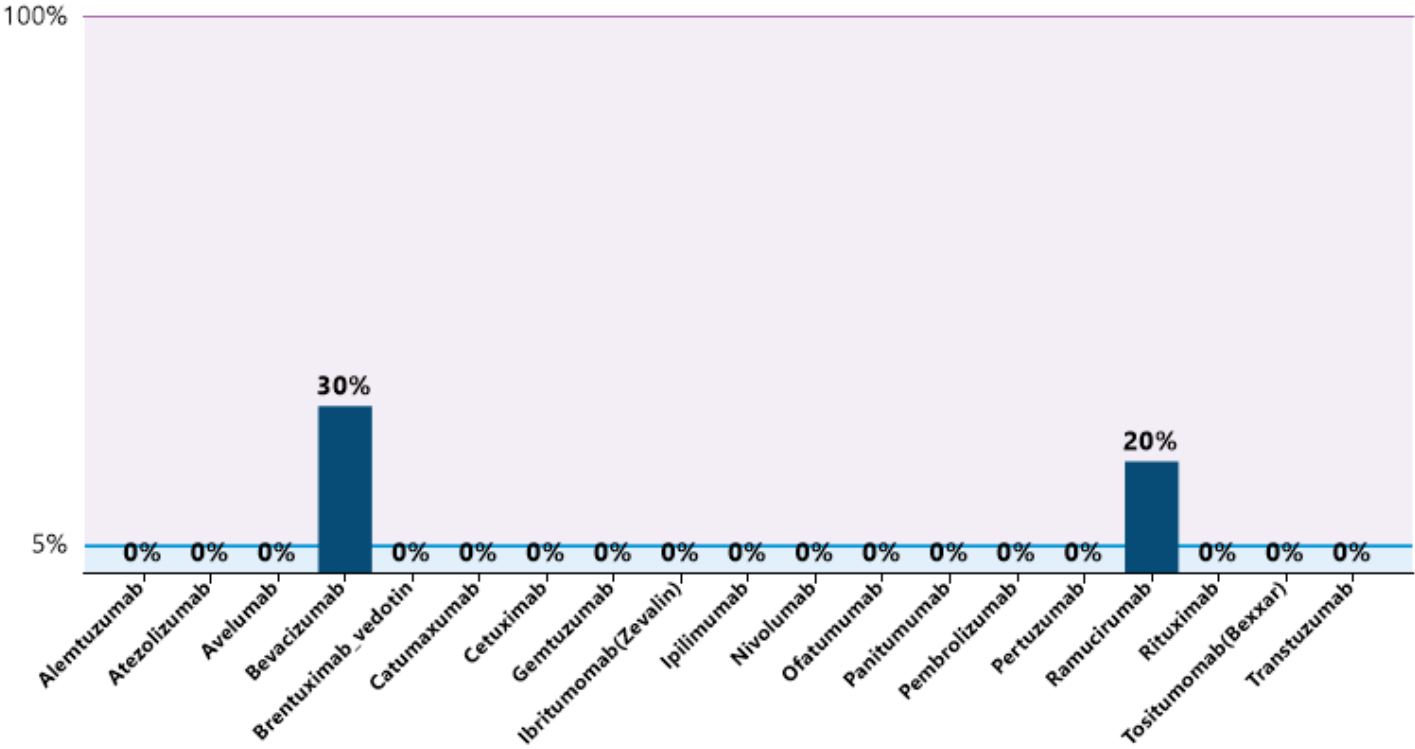


Resistance Factors



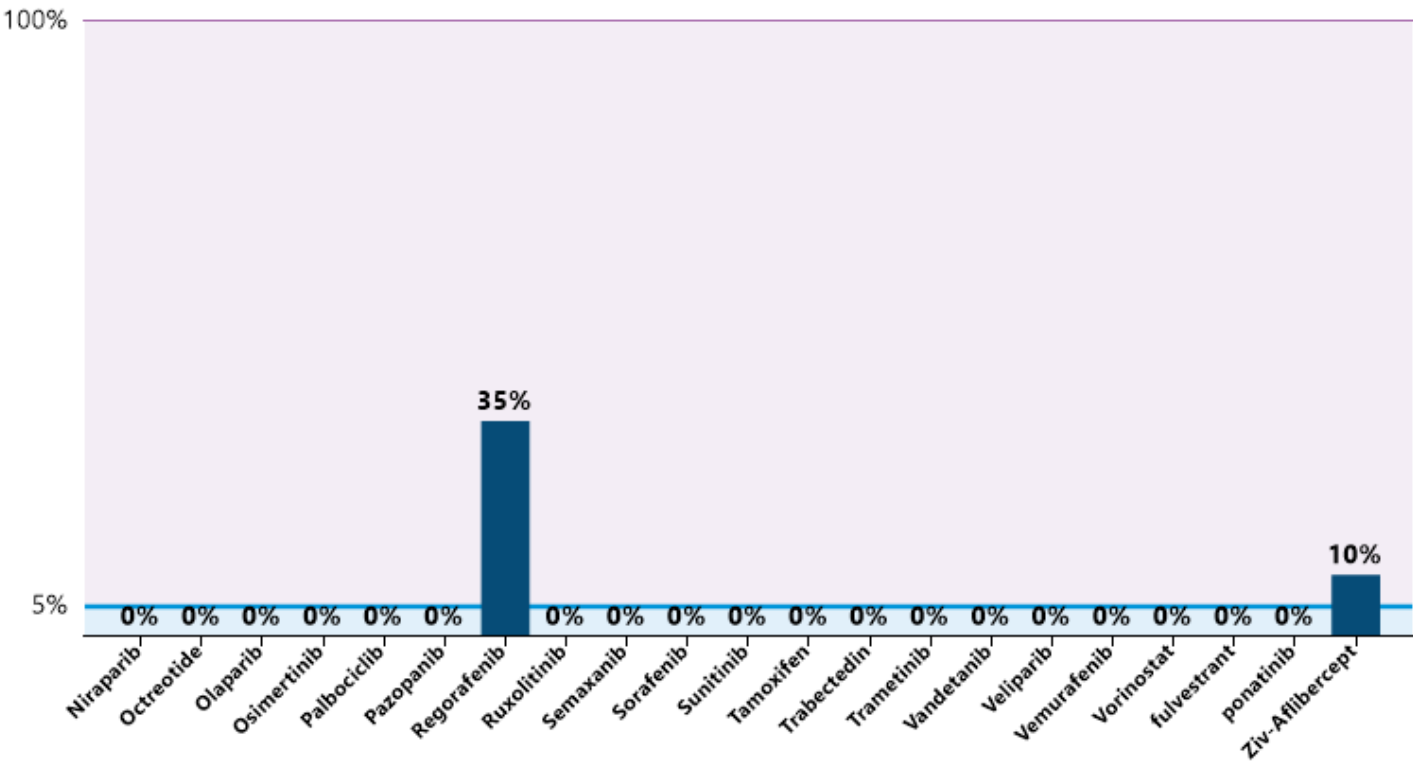
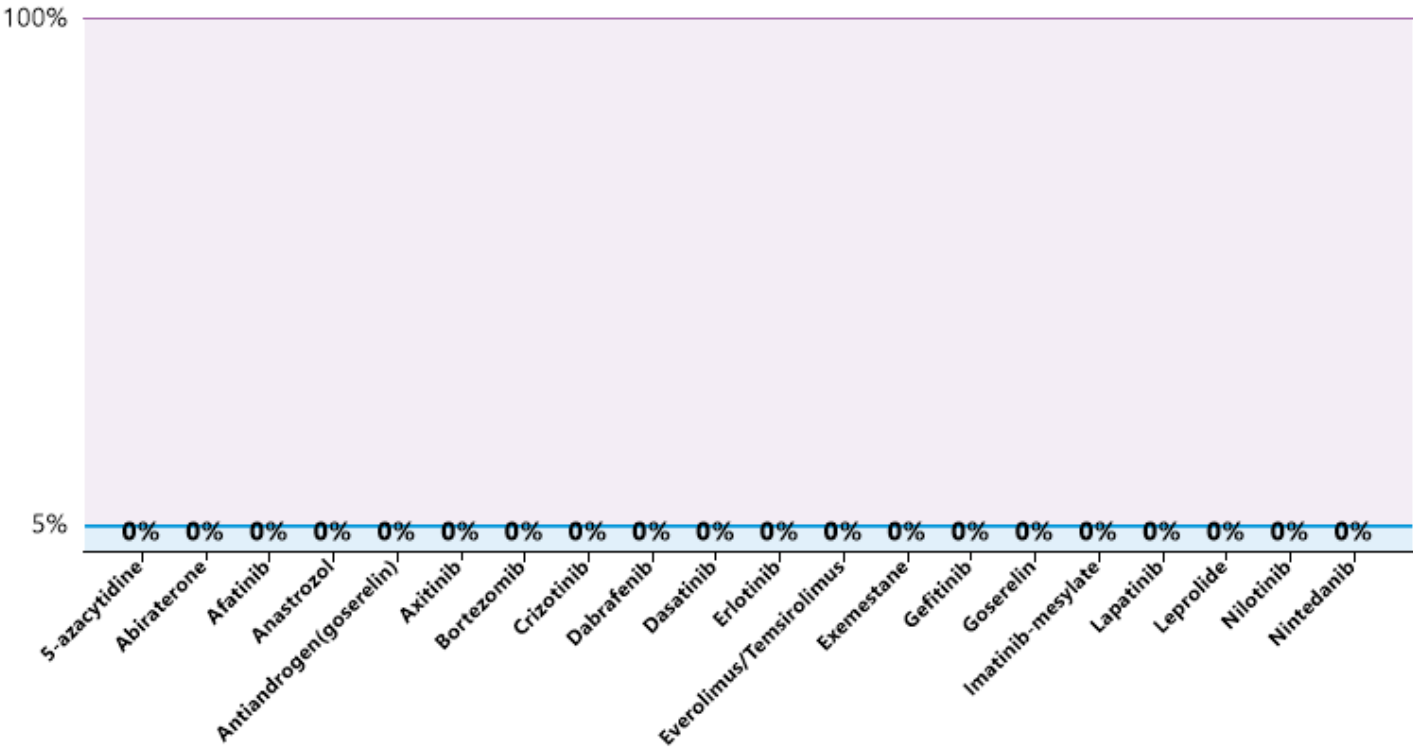
Moab –Monoclonal Antibodies

No sensitivity Sensitivity



No sensitivity
Sensitivity

SMW - Small Molecular weight molecule



Growth Factors Proliferation Stimuli

NAME	FUNCTION	CLINICAL RISK	RELATED	RESULTS %	OUTCOME
p180	Preprotein for Cellular stress	LOW RISK	Tyrosin kinase growth f.	0	LOW RISK
Bcr-abl	Fusion Protein	LOW RISK	Resist phenotype	0	LOW RISK
PTEN	Repair Related Gene	HIGH RISK	Tumor Suppressor Gene	20	HIGH RISK
COX2	Eicosanoid related protein	HIGH RISK	Tumour Growth	15	HIGH RISK
5-LOX			Tumour Growth	0	LOW RISK
NFkB	Proteasome inhibitors	LOW RISK	Transcription fact	0	LOW RISK
IkB(a,b,c)			Inhibitor of NFkB	0	LOW RISK
ALK	Proto-Oncogene	LOW RISK	Acute Leukemia kinase	0	LOW RISK
EML-4-ALK			Fusion EML with ALK	0	LOW RISK
NPM-ALK			Fusion NPM with ALK	0	LOW RISK
RET			Proto-Oncogene	0	LOW RISK
				0	LOW RISK

Preprotein for Cellular stress



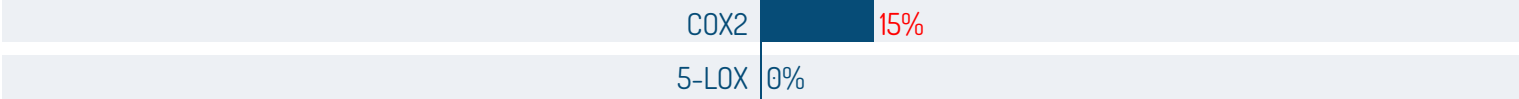
Fusion Protein



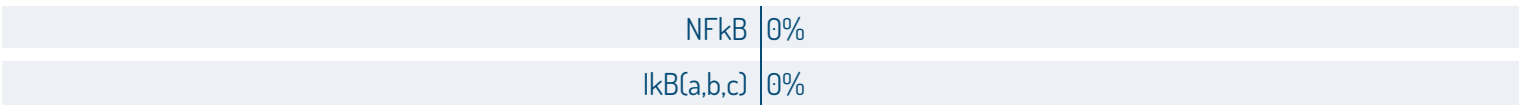
Repair Related Gene



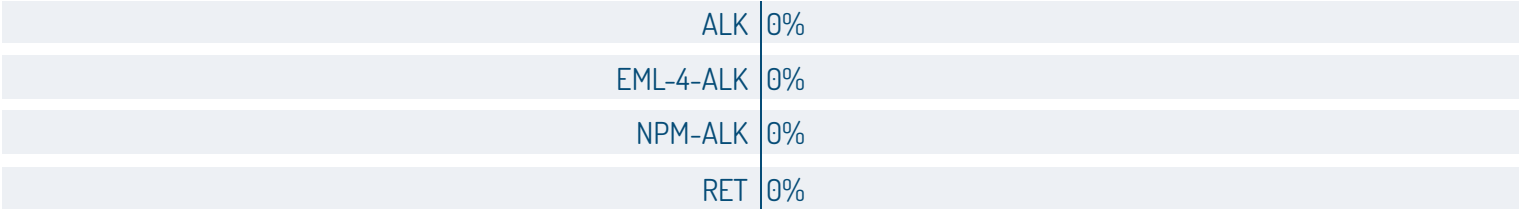
Eicosanoid related protein



Proteasome inhibitors



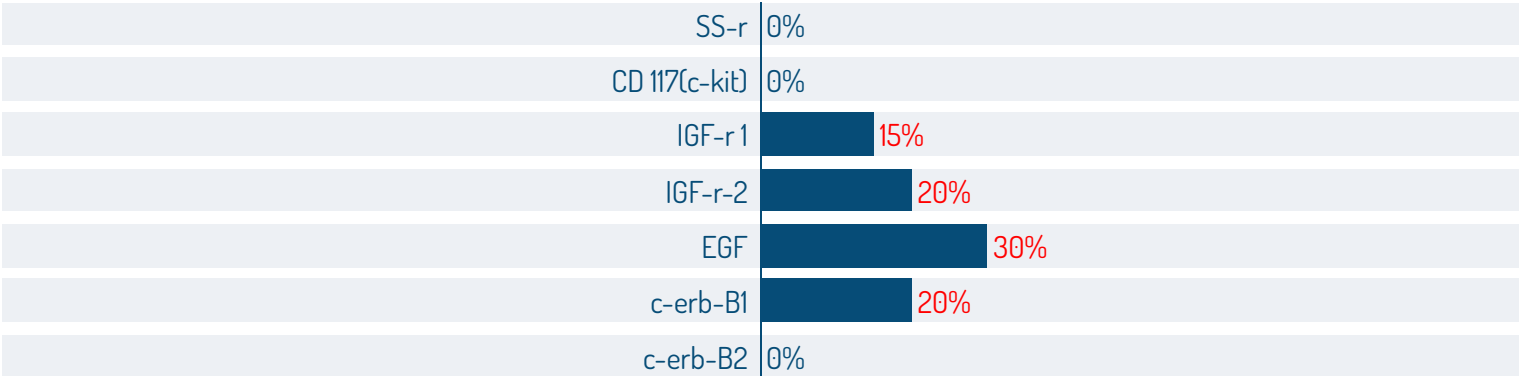
Proto-Oncogene



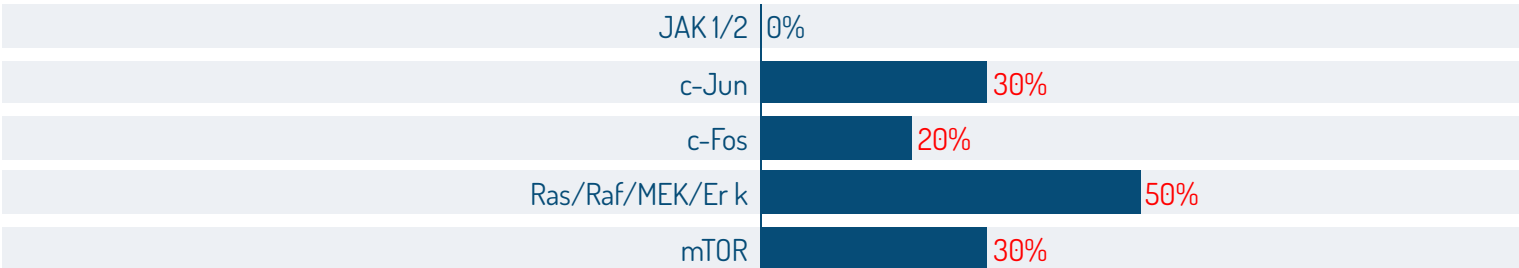
Growth Factors Proliferation Stimuli

NAME	FUNCTION	CLINICAL RISK	RELATED	RESULTS %	OUTCOME
SS-r	Growth Factor Receptor	HIGH RISK	Somatostatin receptor	0	LOW RISK
CD 117(c-kit)			Proliferate growth factor receptor	0	LOW RISK
IGF-r 1			Insulin like growth factor receptor	15	HIGH RISK
IGF-r-2			Insulin like growth factor receptor	20	HIGH RISK
EGF			Tumour Growth	30	HIGH RISK
c-erb-B1			Her1	20	HIGH RISK
c-erb-B2			Her/neu2	0	LOW RISK
JAK 1/2	Signal Transduction Pathway	HIGH RISK	Single transduction pathway	0	LOW RISK
c-Jun			Proto-Oncogene	30	HIGH RISK
c-Fos			Proto-Oncogene	20	HIGH RISK
Ras/Raf/MEK/Er k			Transduction pathway	50	HIGH RISK
mTOR			Transduction pathway	30	HIGH RISK
Progesterone Receptor	Hormone Receptors	LOW RISK	Growth Factor Receptor	0	LOW RISK
Estrogene Receptor			Growth Factor Receptor	0	LOW RISK
NR3C4-A			Nucleous receptor group III Class 4(androg...	0	LOW RISK
NR3C4-B			Nucleous receptor group III Class 4(androg...	0	LOW RISK

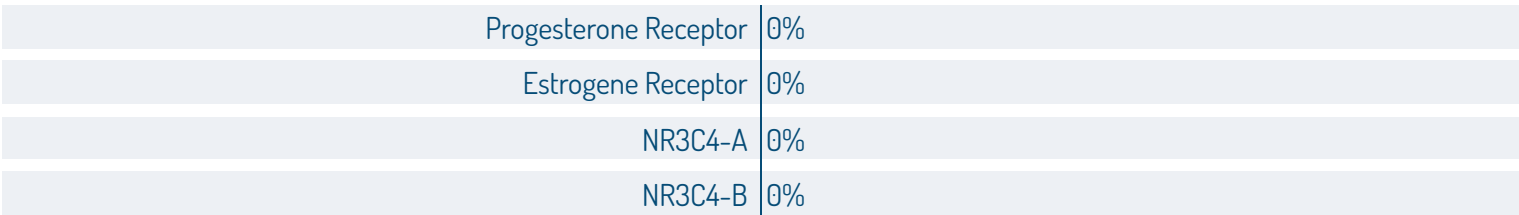
Growth Factor Receptor



Signal Transduction Pathway



Hormone Receptors



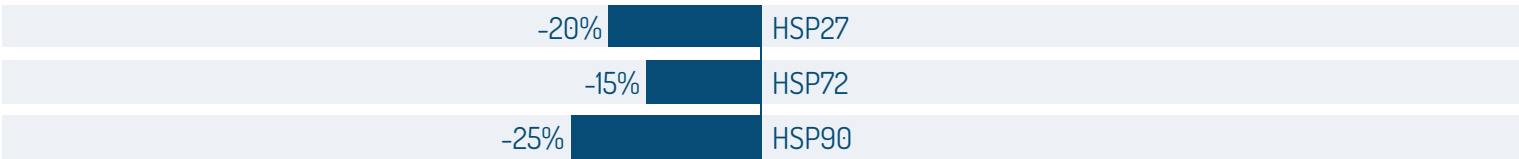
SELF REPAIR – RESISTANCE

NAME	FUNCTION	CLINICAL RISK	RELATED	RESULTS %	OUTCOME
TGF-b	Signal transduction	HIGH RISK	Tumour Growth	15	HIGH RISK
HSP27	Radiotherapy / Hyperthermia sensitivity	SENSITIVE	Heat Shock Protein	-20	SENSITIVE
HSP72			Heat Shock Protein	-15	SENSITIVE
HSP90			Heat Shock Protein	-25	SENSITIVE
DNA methyltransferas el	Resistant Phenotype Markers	HIGH RISK	DNA methylation	-45	HIGH RISK
DNA demethylase			DNA methylation	0	LOW RISK
06-methyl-DNA- tran			DNA methylation	-40	HIGH RISK
Histonedeacetyla se-			DNA coiling (nucleosome)	0	LOW RISK
HAT			Histone acetyl transferase	0	LOW RISK
CXCR4			Resistant Phenotype	15	HIGH RISK
CXCL12			Resistant Phenotype	20	HIGH RISK
Gamma GC			Resist to alkylating drugs	0	LOW RISK
HDAC			Histone deacetylase	0	LOW RISK
PARP (1-17)	DNA Repair Related Gene	HIGH RISK	DNA repair mechanism	30	HIGH RISK

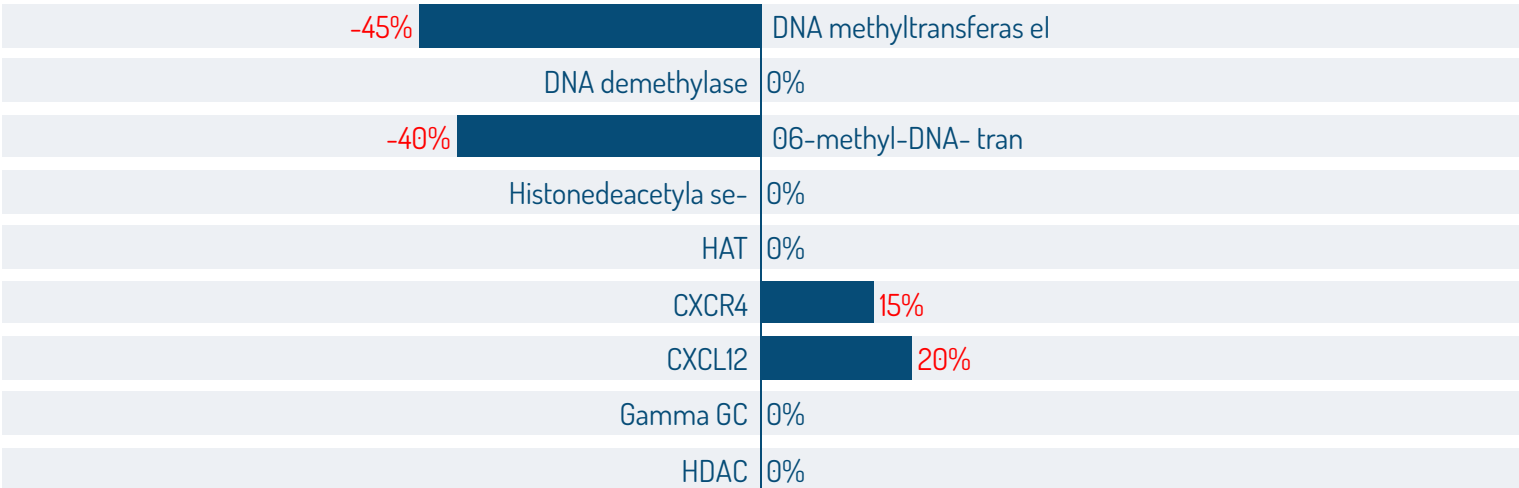
Signal transduction



Radiotherapy / Hyperthermia sensitivity



Resistant Phenotype Markers



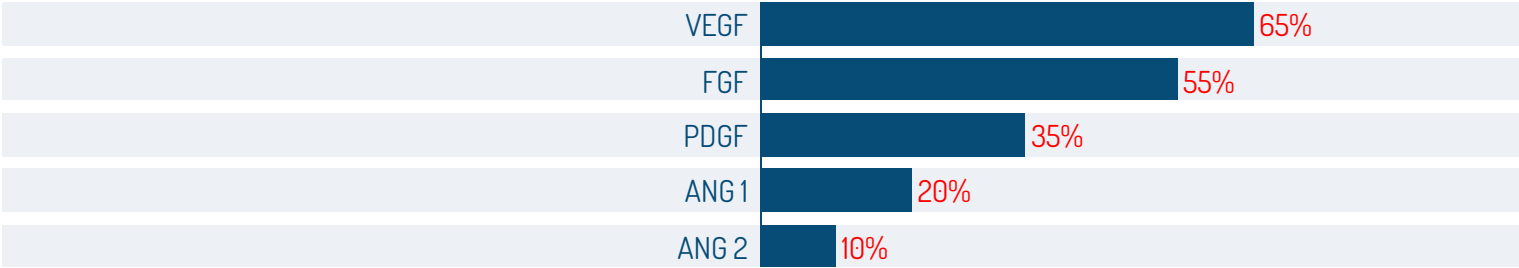
DNA Repair Related Gene



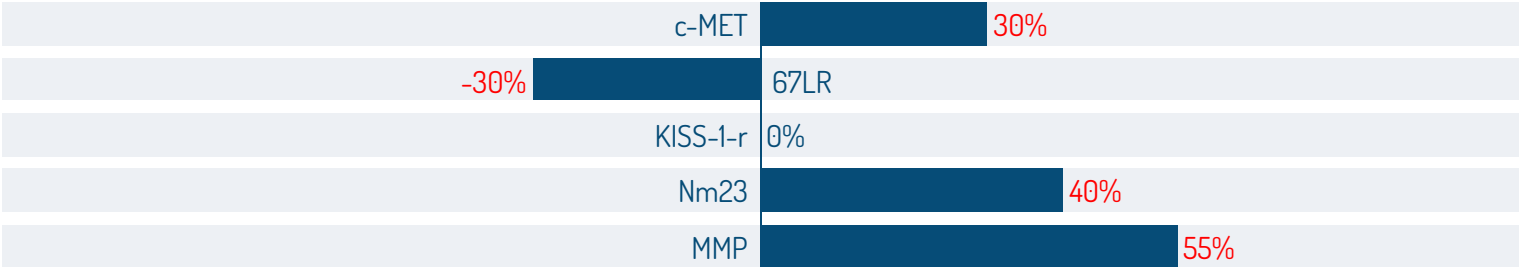
ANGIOGENESIS - METASTASES

NAME	FUNCTION	CLINICAL RISK	RELATED	RESULTS %	OUTCOME
VEGF	Angiogenesis	HIGH RISK	Angiogenesis	65	HIGH RISK
FGF			Angiogenesis	55	HIGH RISK
PDGF			Angiogenesis	35	HIGH RISK
ANG 1			Angiogenin I	20	HIGH RISK
ANG 2			Angiogenin II	10	HIGH RISK
c-MET	Migration invasion	HIGH RISK	Mesenchymal to epithelial transition	30	HIGH RISK
67LR			67 Laminin receptor	-30	HIGH RISK
KISS-1-r			Metastases regulator	0	LOW RISK
Nm23			Metastases regulator	40	HIGH RISK
MMP			Metastases	55	HIGH RISK

Angiogenesis



Migration invasion



CELL CYCLE REGULATION & IMMORTALIZATION / APOPTOSIS

NAME	FUNCTION	CLINICAL RISK	RELATED	RESULTS %	OUTCOME
E2F1	Increase Protein Synthesis	HIGH RISK	Transcr. Fact of TS & topo I	60	HIGH RISK
CDC6	Rapid Cell Cycle	LOW RISK	Initiation of DNA replication	0	LOW RISK
h-TERT	Immortalization	HIGH RISK	M2 crisis- aggressive phen	15	HIGH RISK
Bcl-2	Regulation of Apoptosis	HIGH RISK	Apoptosis	30	HIGH RISK
Bax			Apoptosis	0	LOW RISK
CD95 (fas-r)			Apoptosis related receptor	15	HIGH RISK
p27	Cell Cycle Rate	HIGH RISK	Cell arrest (G0)	0	LOW RISK
p53			Cell cycle regulator	-50	HIGH RISK
p16			Apoptosis	20	HIGH RISK
CDK4/6	Cell cycle regulator	HIGH RISK	Cyclin-dependent kinase	20	HIGH RISK

Increase Protein Synthesis



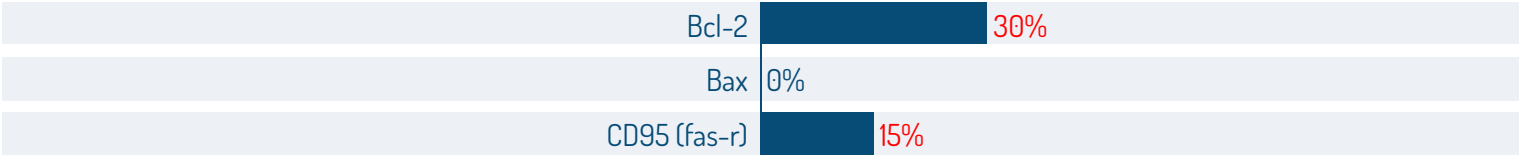
Rapid Cell Cycle



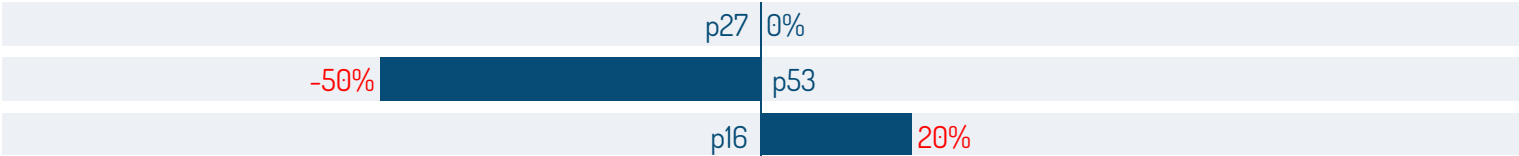
Immortalization



Regulation of Apoptosis



Cell Cycle Rate



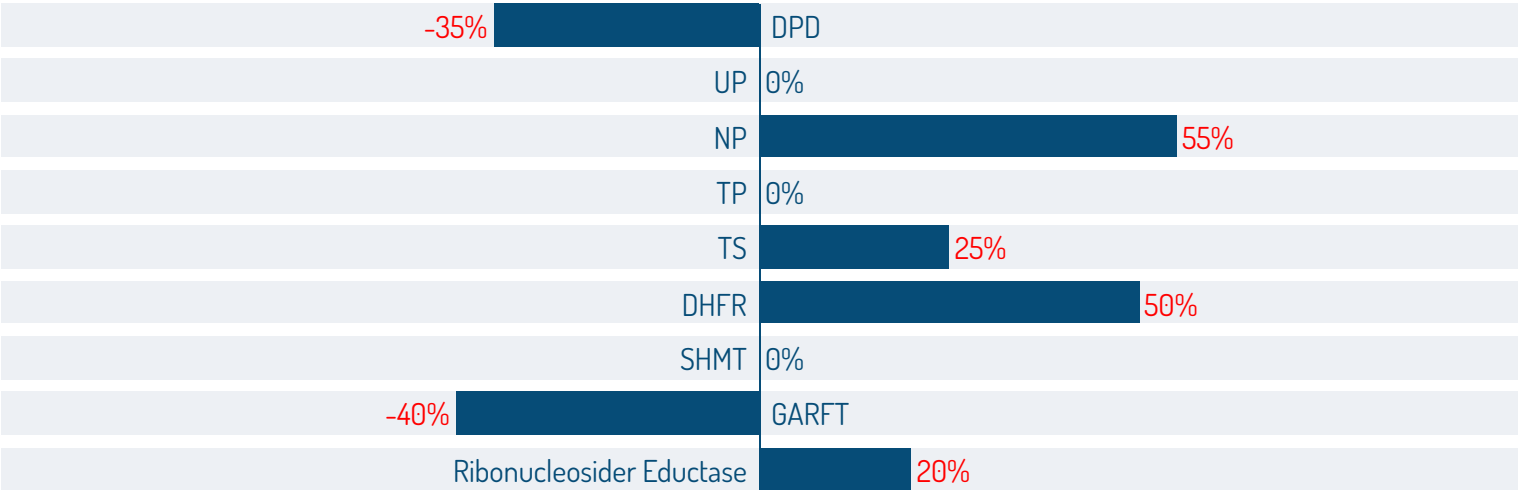
Cell cycle regulator



DRUG METABOLISMS & TARGETS

NAME	FUNCTION	CLINICAL RISK	RELATED	RESULTS %	OUTCOME
DPD	Nucleoside Import Transformation	HIGH RISK	Resist to 5FU	-35	HIGH RISK
UP			Resist to 5FU	0	LOW RISK
NP			Resist topyrim. Antagonist	55	HIGH RISK
TP			Resist to 5FU	0	LOW RISK
TS			Rapid cell cycle (THFA)	25	HIGH RISK
DHFR			Rapid cell cycle (THFA)	50	HIGH RISK
SHMT			Rapid cell cycle (THFA)	0	LOW RISK
GARFT			Rapid cell cycle (THFA)	-40	HIGH RISK
Ribonucleosider Eductase			DNA synthesis	20	HIGH RISK
CESI&2 (carboxyesterase)	Activation of Camptothecin	HIGH RISK	Resist to camptothecin	40	HIGH RISK
CypB1	Xenobiotic	HIGH RISK	Xenobiotic metabolism	15	HIGH RISK
ERCC1	DNA Repair Related Gene	HIGH RISK	DNA repair mechanism	0	LOW RISK
RRM1			Nucleotide polymerizations	30	HIGH RISK

Nucleoside Import Transformation



Activation of Camptothecin



Xenobiotic



DNA Repair Related Gene



MARKERS

NAME	FUNCTION	CLINICAL RISK	RELATED	RESULTS %		OUTCOME
CD33	Immune system regulation	LOW RISK	Myeloid Cell origin		0	LOW RISK
CD52	Immune system regulation	LOW RISK	Leukaemia Marker		0	LOW RISK
CD20	Development and differentiation of B cells into plasma cells	LOW RISK	Lymphoma Related Antigen		0	LOW RISK
EpCAM (EpCAM+ve)	Cell-cell adhesion	HIGH RISK	Epithelial Marker (1.8 cells/ml)		15	HIGH RISK
PD-L1	Immune system regulation	LOW RISK	Immunoregulatory Factor		0	LOW RISK
PD 1	Immune system regulation	LOW RISK	Immunoregulatory Factor		0	LOW RISK
PD-L2	Immune system regulation	LOW RISK	Immunoregulatory Factor		0	LOW RISK

Immune system regulation



Immune system regulation



Development and differentiation of B cells into plasma cells



Cell-cell adhesion



Immune system regulation



Immune system regulation

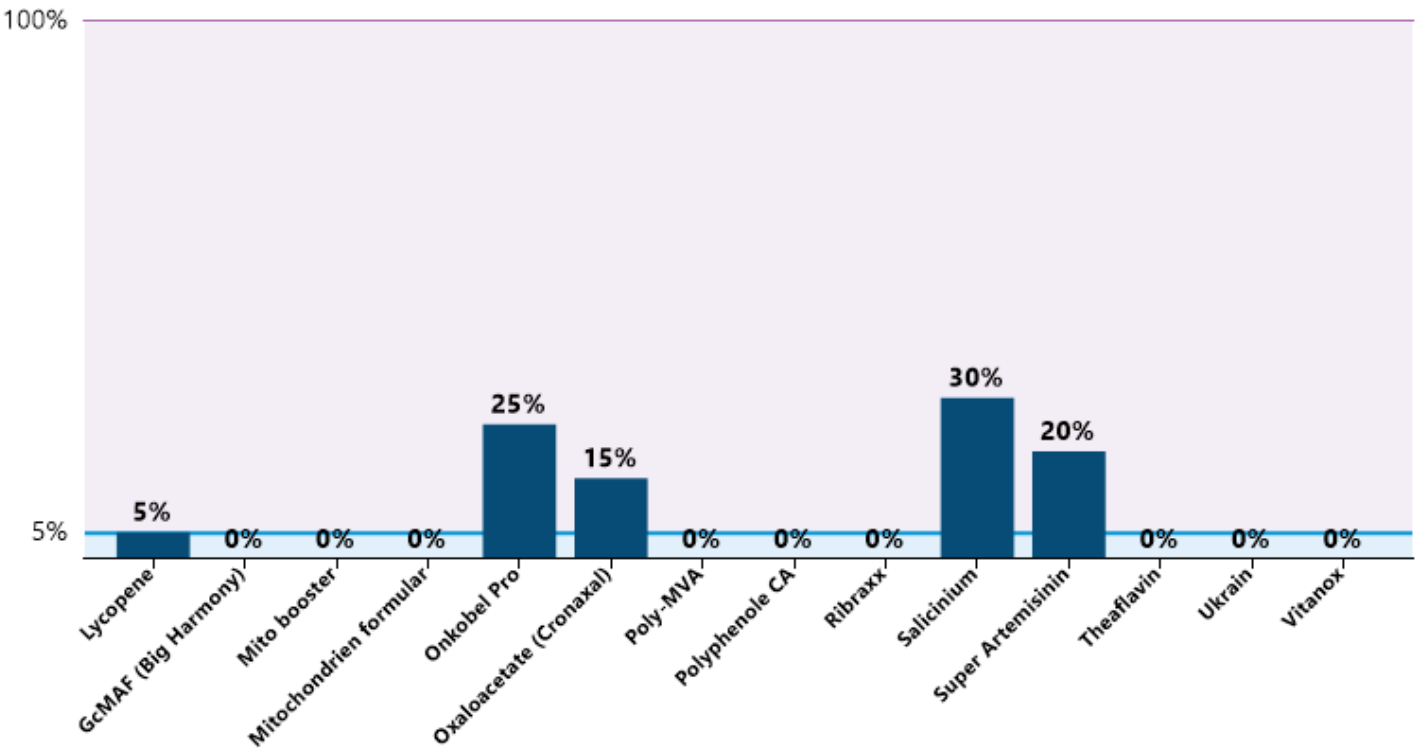
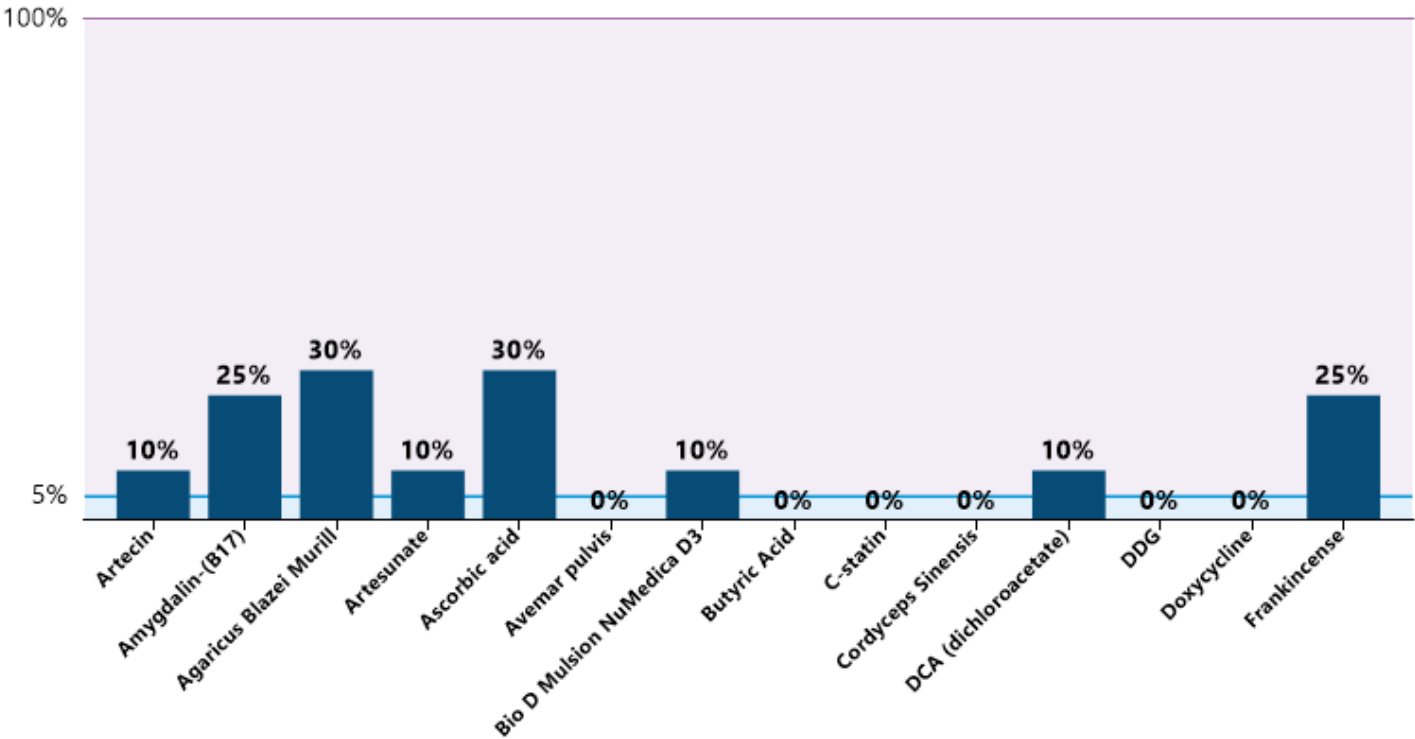


Immune system regulation



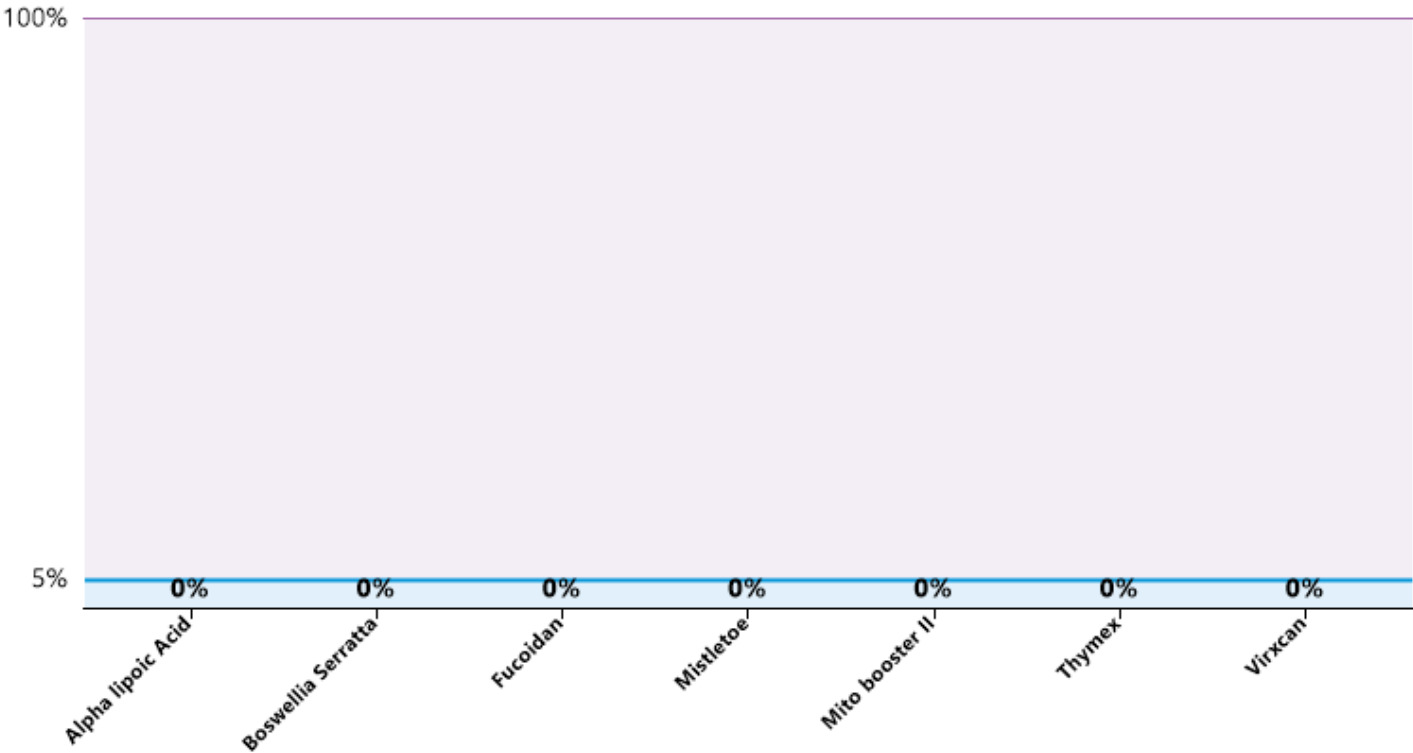
No sensitivity
Sensitivity

Class I (Cytotoxic Agents)

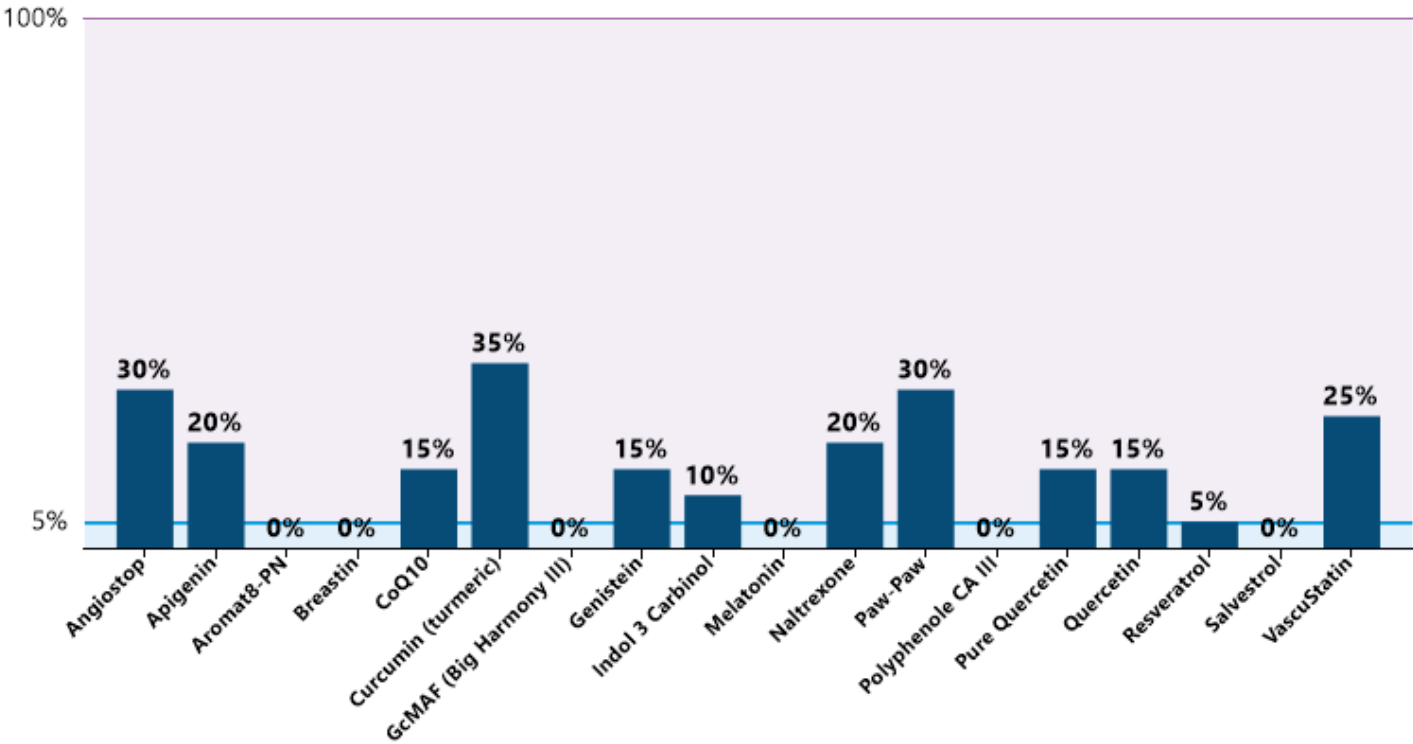


No sensitivity
Sensitivity

Class II (Immunostimulants / Immunomodulators)



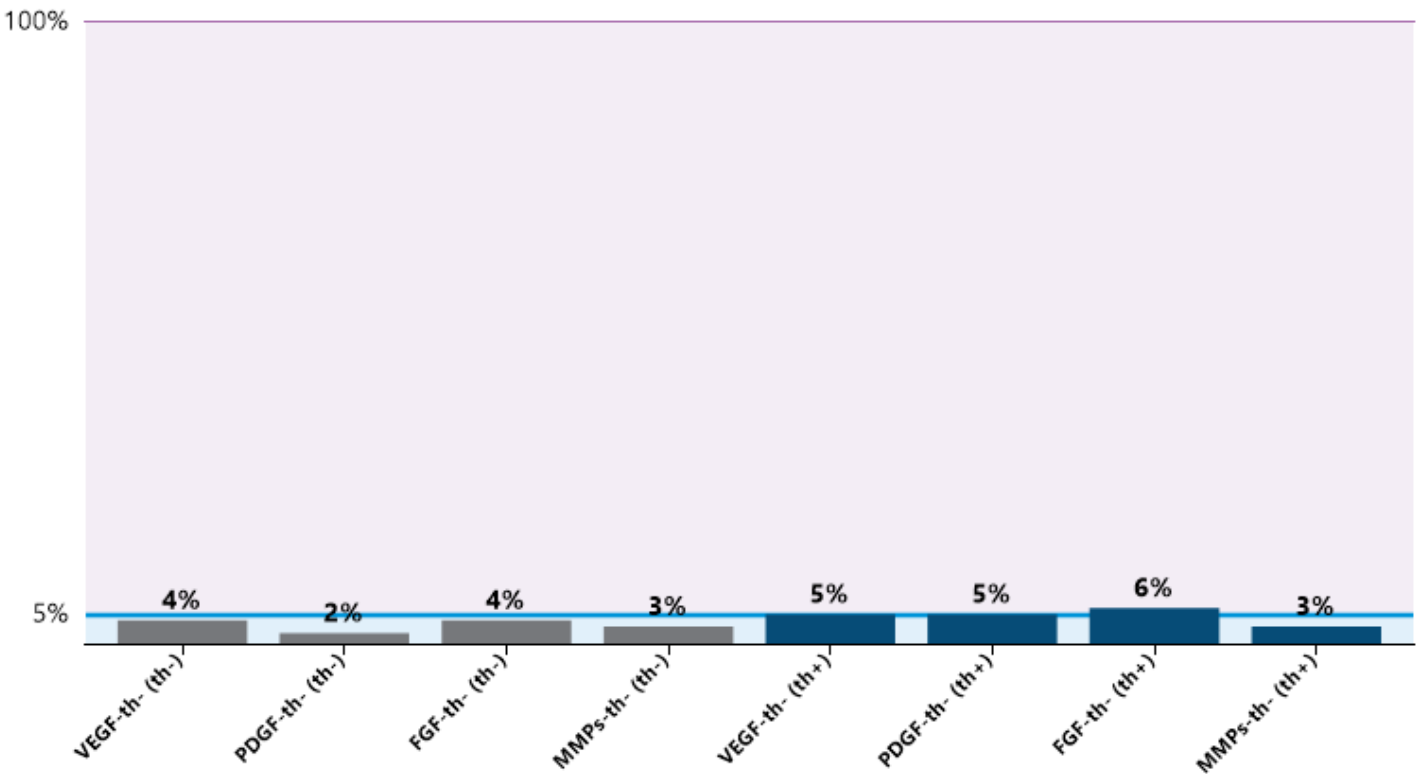
Class III (PK Inhibitors)



No sensitivity Sensitivity

Malignant Cells - Thalidomide

th- th+



Information

Laboratory Process

- Isolation of the malignant cells using flow cytometry and negative selection. The isolated cells were expanded and they splitter in two, from which, one part is going to viability assays and the other is going for transcriptomic micro-Arrays
- Isolation of mRNA
- Quality control of integrity of mRNA
- Reversed transcription of mRNA to cDNA
- Hybridisation of cDNA with micro-Arrays all genome transcriptomic micro-Arrays slide
- Analysis of the data and detection of repeatable patterns
- Normalization and assessment of clinical relevant probes

This Test report is issued based on testing the sample / specimen examined by the Laboratory. Modification of data, selective breeding and using portions of this test report is forbidden. The laboratory assumes no liability for improper use or improper interpretation of the results.

Clinical relevant genes related with:

Isolation of mRNA	p53, p21, p16, DHFR, TS, SHMT
Drug targets	Topo I & II, TS, DHFR, ribonucleotide reductase etc.
Signal Transduction Pathway	EGFr, PDGFr, etc.
Epigenetic aberration	Dnmt1, DNA demethylase, etc.
Angiogenesis	VEGF-r, FGFr, PDGFr
Growth signal	c-erb-B1, c-erb-B2, bar-abl, etc
Repair after physical application (radiation, hyperthermia)	HSP27, HSP70, HSP90, HIF1a, etc.

Sincerely,



Dr. Ioannis Papasotiriou MD, PhD, SCym

GROWTH FACTORS PROLIFERATION STIMULI

FUNCTION	NAME	NAME OFFICIAL	RELATED
Preprotein for Cellular stress	p180	RRBP1	Tyrosine kinase growth factor
Fusion Protein	Bcr-abl	BCR-ABL	Resist phenotype
Repair Related Gene	PTEN	PTEN	Tumor Suppressor Gene
Eicosanoid related protein	COX2 5-LOX	PTGS2 ALOX5	Tumour Growth Tumour Growth
Proteasome inhibitors	NfκB IκB (a,b,c)	NFKB1 NFKBIA/B	Transcription Factor NFκB Inhibitors
Proto-Oncogene	ALK EML-4-ALK NPM-ALK RET	ALK EML4-ALK NPM-ALK RET	Acute Leukemia kinase Fused EML-ALK Fused NPM-ALK Proto-Oncogene
Growth Factor Receptor	SS-r CD 117 (c-kit) IGF-r 1 IGF-r 2 EGF C-erb-B2 C-erb-B1	SSTR3/5 KIT IGF1R IGF2R EGF ERBB2 EGFR	Somatostatin receptor Proliferate growth factor receptor Insulin like growth factor receptor Insulin like growth factor receptor Tumour Growth Receptor tyrosine-protein kinase Epithelial Growth Factor Recept...
Signal Transduction Pathway	JAK1/2 c-Jun c-Fos Ras-Raf-MEK-ERK mTOR	JAK1/2 FOS JUN ERK1/2 MTOR	Single transduction pathway Proto-Oncogene Proto-Oncogene Single transduction pathway Single transduction pathway
Hormone Receptors	Progesterone-Receptor Estrogen-Receptor NR3C4-A NR3C4-B	PGR ESR1 NR3C4A NR3C4B	Growth Factor Receptor Growth Factor Receptor Androgen Receptor Androgen Receptor

SELF REPAIR - RESISTANCE

FUNCTION	NAME	NAME OFFICIAL	RELATED
Signal transduction	TGF-β	TGFB2	Tumour Growth
Radiotherapy / Hyperthermia se...	HSP27 HSP72 HSP90	HSPB1 HSPA1A HSP90AA1	Heat Shock Protein Heat Shock Protein Heat Shock Protein
Resistant Phenotype Markers	DNA methyltransferase I DNA-demethylase O6-methyl-DNA-tran Histone-deacetylase-dipeptide HAT CXCR4 HDAC CXCL12 Gamma GC	DNMT1 TET1 MGMT HDAC1 HAT1 CXCR4 HDAC2 CXCL12 GGCX	DNA methylation DNA methylation DNA methylation DNA Coiling Histone acetyl transferase Resist phenotype Histone deacetylase Resist phenotype Resist to alkylating drugs

ANGIOGENESIS

FUNCTION	NAME	NAME OFFICIAL	RELATED
Angiogenesis	VEGF	VEGFA	Angiogenesis
	FGF	FGF1(3)	Angiogenesis
	PDGF	PDGFA(2)	Angiogenesis
	ANG 1	ANGPT1	Angiopoietin
	ANG 2	ANGPT2	Angiopoietin

DRUG METABOLISMS & TARGETS

FUNCTION	NAME	NAME OFFICIAL	RELATED
Nucleoside Import Transformation	DPD	DPYD	Resist to 5FU
	UP	UPP1	Resist to 5FU
	NP	PNP	Purine Nucleoside Phosphorylase
	TP	TYMP	Resist to 5FU
	TS	TYMS	Rapid cell cycle (THFA)
	DHFR	DHFR	Rapid cell cycle (THFA)
	SHMT	SHMT1	Rapid cell cycle (THFA)
	GARFT	GART	Rapid cell cycle (THFA)
	Ribonucleoside reductase	RRM1	DNA synthesis
Activation of Camptothecin	CES1-2	CES1-2	Resist to camptothecin
Xenobiotic	CypB1	CYB1B1	Xenobiotic metabolism
DNA Repair Related Gene	ERCC1	ERCC1	DNA repair mechanism
	RRM1	RRM1	Nucleotide polymerizations

MARKERS

FUNCTION	NAME	NAME OFFICIAL	RELATED
markers	CD33	CD33	Myeloid Cell origin
	CD52	CD52	Leukaemia Marker
	CD20	CD20	Lymphoma Related Antigen
	EpCAM	EPCAM	Epithelial Marker
	PD-L1	CD274	Immunoregulatory Factor
	PD 1	PDCD1	Immunoregulatory Factor
	PD-L2	PDCD1LG2	Immunoregulatory Factor

- a. Lin D, Shen L, Luo M, Zhang K, Li J, Yang Q, Zhu F, Zhou D, Zheng S, Chen Y, Zhou J. Circulating tumor cells: biology and clinical significance. *Signal Transduct Target Ther*. 2021 Nov 22;6(1):404. doi: 10.1038/s41392-021-00817-8. PMID: 34803167; PMCID: PMC8606574.
- b. Bhagwat N, Carpenter EL. Flow Cytometric Methods for Circulating Tumor Cell Isolation and Molecular Analysis. *Adv Exp Med Biol*. 2017;994:105-118. doi: 10.1007/978-3-319-55947-6_5. PMID: 28560670.
- c. Papasotiriou I, et al. Detection of Circulating Tumor Cells in Patients with Breast, Prostate, Pancreatic, Colon and Melanoma Cancer: A Blinded Comparative Study Using Healthy Donors. *Journal of Cancer Therapy*. 2015;6:543-553. <http://dx.doi.org/10.4236/jct.2015.67059>.
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- e. Pisanidou V, Apostolou P, Beis G, Hatzidaki E, Papasotiriou I. Cancer Comprehensive Analysis in Gastric Carcinoma: Benefits and New Perspectives. *Case Rep Oncol*. 2021 Nov 25;14(3):1682-1690. doi: 10.1159/000520359. PMID: 35082626; PMCID: PMC8739675.
- f. Guadagni S, Fiorentini G, De Simone M, Masedu F, Zoras O, Mackay AR, Sarti D, Papasotiriou I, Apostolou P, Catarci M, Clementi M, Ricevuto E, Bruera G. Precision oncotherapy based on liquid biopsies in multidisciplinary treatment of unresectable recurrent rectal cancer: a retrospective cohort study. *J Cancer Res Clin Oncol*. 2020 Jan;146(1):205-219. doi: 10.1007/s00432-019-03046-3. Epub 2019 Oct 16. PMID: 31620896; PMCID: PMC6942036.
- g. Yang C, Xia BR, Jin WL, Lou G. Circulating tumor cells in precision oncology: clinical applications in liquid biopsy and 3D organoid model. *Cancer Cell Int*. 2019 Dec 18;19:341. doi: 10.1186/s12935-019-1067-8. PMID: 31866766; PMCID: PMC6918690.
- h. Toloudi M, Ioannou E, Chatziioannou M, Apostolou P, Kiritsis C, Manta S, Komiotis D, Papasotiriou I. Comparison of the growth curves of cancer cells and cancer stem cells. *Curr Stem Cell Res Ther*. 2014 Mar;9(2):112-6. doi: 10.2174/1574888x0902140121163539. PMID: 24359142.
- i. Guadagni S, Masedu F, Fiorentini G, Sarti D, Fiorentini C, Guadagni V, Apostolou P, Papasotiriou I, Parsonidis P, Valenti M, Ricevuto E, Bruera G, Farina AR, Mackay AR, Clementi M. Circulating tumour cell gene expression and chemosensitivity analyses: predictive accuracy for response to multidisciplinary treatment of patients with unresectable refractory recurrent rectal cancer or unresectable refractory colorectal cancer liver metastases. *BMC Cancer*. 2022 Jun 16;22(1):660. doi: 10.1186/s12885-022-09770-3. PMID: 35710393; PMCID: PMC9202660.
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