

Patient NAME Mr John Doe	DATE OF BIRTH 1992-Jun-12	DISEASE Colorectal	STAGE II	Physician NAME Administrator
SPECIMEN	VIAL IDs 4			

REPORT SUMMARY

CTCs COUNT: Isolated 2 cells/ml , SD +/- 0.3 cells

GENE EXPRESSION

Expression

Over expression: IkB(a,b,c), NFkB, HDAC, ANG 1
Down regulation: COX2, HSP90, HSP72

NATURAL SUBSTANCES SENSITIVITY

Class I	Class II	Class III
Cytotoxic Agents	Immunostimulants / Immunomodulators	PK Inhibitors
Artecin, Artesunate, Bio D Mulsion NuMedica D3, Butyric Acid, DCA (dichloroacetate), Doxycycline, Frankincense, Lycopene, Colloidal Silver	Boswellia Serratta, Fucoidan, Astaxanthin, Reishi Pure	Apigenin, Indol 3 Carbinol, Melatonin

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

Conclusion Results

- 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

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	-50	HIGH RISK
COX2	Eicosanoid related protein	HIGH RISK	Tumour Growth	-45	HIGH RISK
5-LOX			Tumour Growth	0	LOW RISK
NFkB	Proteasome inhibitors	HIGH RISK	Transcription fact	10	HIGH RISK
IkB(a,b,c)			Inhibitor of NFkB	20	HIGH RISK
ALK	Proto-Oncogene	HIGH 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	15	HIGH RISK

Preprotein for Cellular stress

p180 | 0%

Fusion Protein

Bcr-abl | 0%

Repair Related Gene

-50%

PTEN

Eicosanoid related protein

-45%

COX2

5-LOX | 0%

Proteasome inhibitors

NFkB | 10%

IkB(a,b,c) | 20%

Proto-Oncogene

ALK | 0%

EML-4-ALK | 0%

NPM-ALK | 0%

RET | 15%

Growth Factors Proliferation Stimuli

NAME	FUNCTION	CLINICAL RISK	RELATED	RESULTS %	OUTCOME
SS-r	Growth Factor Receptor	LOW 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	0	LOW RISK
IGF-r-2			Insulin like growth factor receptor	0	LOW RISK
EGF			Tumour Growth	0	LOW RISK
c-erb-B1			Her1	0	LOW 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	65	HIGH RISK
c-Fos			Proto-Oncogene	40	HIGH RISK
Ras/Raf/MEK/Er k			Transduction pathway	20	HIGH RISK
mTOR			Transduction pathway	50	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

SS-r	0%
CD 117(c-kit)	0%
IGF-r 1	0%
IGF-r-2	0%
EGF	0%
c-erb-B1	0%
c-erb-B2	0%

Signal Transduction Pathway

JAK 1/2	0%
c-Jun	65%
c-Fos	40%
Ras/Raf/MEK/Er k	20%
mTOR	50%

Hormone Receptors

Progesterone Receptor	0%
Estrogene Receptor	0%
NR3C4-A	0%
NR3C4-B	0%

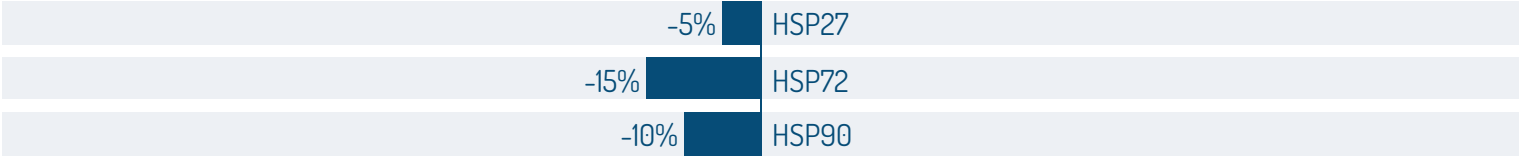
SELF REPAIR – RESISTANCE

NAME	FUNCTION	CLINICAL RISK	RELATED	RESULTS %	OUTCOME
TGF- β	Signal transduction	LOW RISK	Tumour Growth	0	LOW RISK
HSP27	Radiotherapy / Hyperthermia sensitivity	SENSITIVE	Heat Shock Protein	-5	SENSITIVE
HSP72			Heat Shock Protein	-15	SENSITIVE
HSP90			Heat Shock Protein	-10	SENSITIVE
DNA methyltransferases	Resistant Phenotype Markers	HIGH RISK	DNA methylation	0	LOW RISK
DNA demethylase			DNA methylation	0	LOW RISK
5mC-methyl-DNA- transferase			DNA methylation	0	LOW RISK
Histone deacetylase			DNA coiling (nucleosome)	65	HIGH RISK
HAT			Histone acetyl transferase	0	LOW RISK
CXCR4			Resistant Phenotype	-30	HIGH RISK
CXCL12			Resistant Phenotype	0	LOW RISK
Gamma GC			Resist to alkylating drugs	0	LOW RISK
HDAC			Histone deacetylase	60	HIGH RISK
PARP (1-17)	DNA Repair Related Gene	HIGH RISK	DNA repair mechanism	40	HIGH RISK

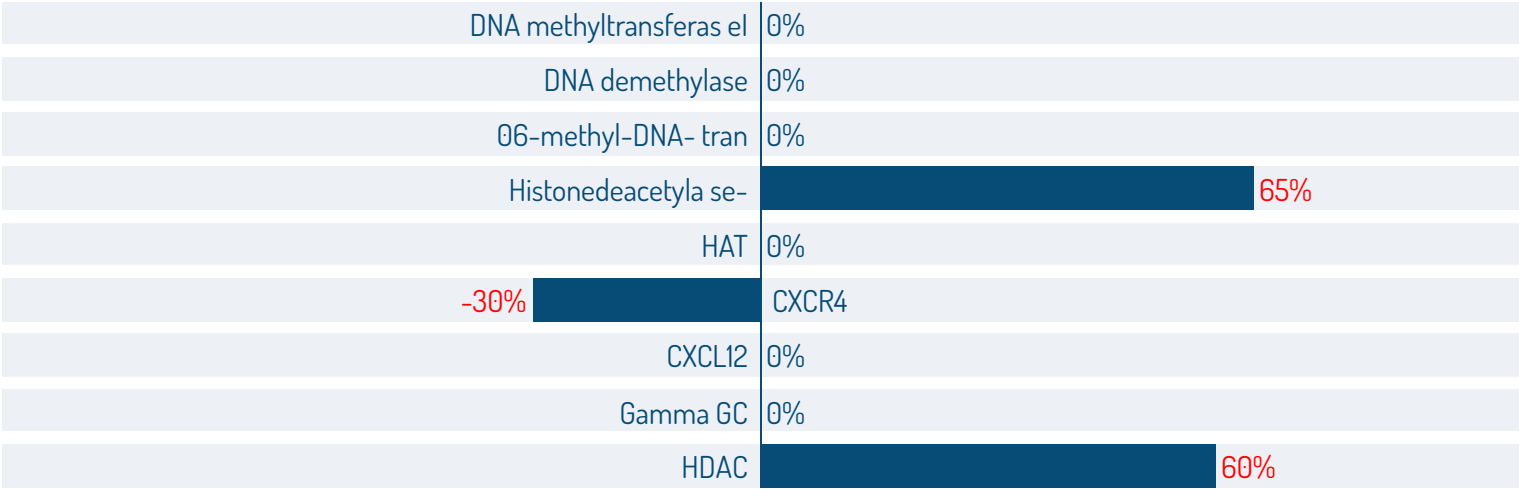
Signal transduction



Radiotherapy / Hyperthermia sensitivity



Resistant Phenotype Markers



DNA Repair Related Gene



ANGIOGENESIS - METASTASES

NAME	FUNCTION	CLINICAL RISK	RELATED	RESULTS %	OUTCOME
VEGF	Angiogenesis	HIGH RISK	Angiogenesis	0	LOW RISK
FGF			Angiogenesis	0	LOW RISK
PDGF			Angiogenesis	0	LOW RISK
ANG 1			Angiogenin I	25	HIGH RISK
ANG 2			Angiogenin II	0	LOW RISK
c-MET	Migration invasion	HIGH RISK	Mesenchymal to epithelial transition	0	LOW RISK
67LR			67 Laminin receptor	-35	HIGH RISK
KISS-1-r			Metastases regulator	0	LOW RISK
Nm23			Metastases regulator	0	LOW RISK
MMP			Metastases	0	LOW RISK

Angiogenesis

VEGF	0%
FGF	0%
PDGF	0%
ANG 1	25%
ANG 2	0%

Migration invasion

c-MET	0%
67LR	-35%
KISS-1-r	0%
Nm23	0%
MMP	0%

CELL CYCLE REGULATION & IMMORTALIZATION / APOPTOSIS

NAME	FUNCTION	CLINICAL RISK	RELATED	RESULTS %	OUTCOME
E2F1	Increase Protein Synthesis	LOW RISK	Transcr. Fact of TS & topo I	0	LOW RISK
CDC6	Rapid Cell Cycle	LOW RISK	Initiation of DNA replication	0	LOW RISK
h-TERT	Immortalization	LOW RISK	M2 crisis- aggressive phen	0	LOW RISK
Bcl-2	Regulation of Apoptosis	HIGH RISK	Apoptosis	0	LOW RISK
Bax			Apoptosis	-10	HIGH RISK
CD95 (fas-r)			Apoptosis related receptor	-40	HIGH RISK
p27	Cell Cycle Rate	HIGH RISK	Cell arrest (G0)	-15	HIGH RISK
p53			Cell cycle regulator	45	HIGH RISK
p16			Apoptosis	0	LOW RISK
CDK4/6	Cell cycle regulator	LOW RISK	Cyclin-dependent kinase	0	LOW RISK

Increase Protein Synthesis



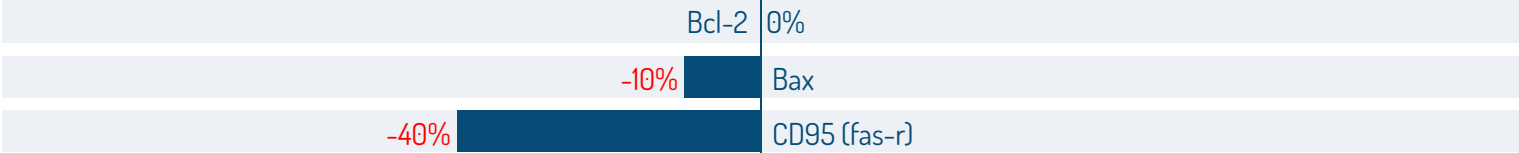
Rapid Cell Cycle



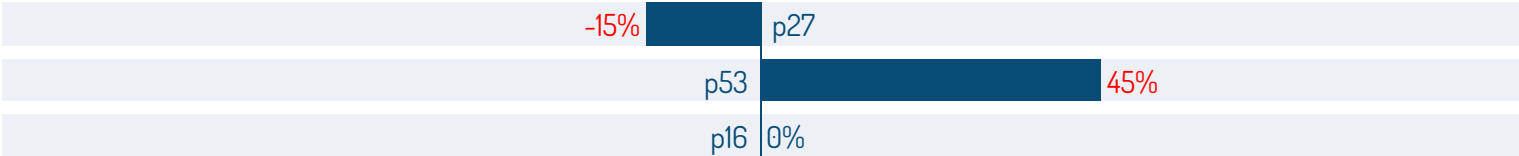
Immortalization



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	60	HIGH RISK
UP			Resist to 5FU	70	HIGH RISK
NP			Resist topyrim. Antagonist	55	HIGH RISK
TP			Resist to 5FU	55	HIGH RISK
TS			Rapid cell cycle (THFA)	0	LOW RISK
DHFR			Rapid cell cycle (THFA)	0	LOW RISK
SHMT			Rapid cell cycle (THFA)	0	LOW RISK
GARFT			Rapid cell cycle (THFA)	5	LOW RISK
Ribonucleosider Eductase			DNA synthesis	0	LOW RISK
CES1&2 (carboxyesterase)	Activation of Camptothecin	HIGH RISK	Resist to camptothecin	40	HIGH RISK
CypB1	Xenobiotic	LOW RISK	Xenobiotic metabolism	0	LOW RISK
ERCC1	DNA Repair Related Gene	HIGH RISK	DNA repair mechanism	50	HIGH RISK
RRM1			Nucleotide polymerizations	0	LOW RISK

Nucleoside Import Transformation

DPD	60%
UP	70%
NP	55%
TP	55%
TS	0%
DHFR	0%
SHMT	0%
GARFT	5%
Ribonucleosider Eductase	0%

Activation of Camptothecin

CES1&2 (carboxyesterase)	40%
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Xenobiotic

CypB1	0%
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DNA Repair Related Gene

ERCC1	50%
RRM1	0%

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	Cell-cell adhesion	LOW RISK	Epithelial Marker		0	LOW RISK
PD-L1	Immune system regulation	HIGH RISK	Immunoregulatory Factor		20	HIGH 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

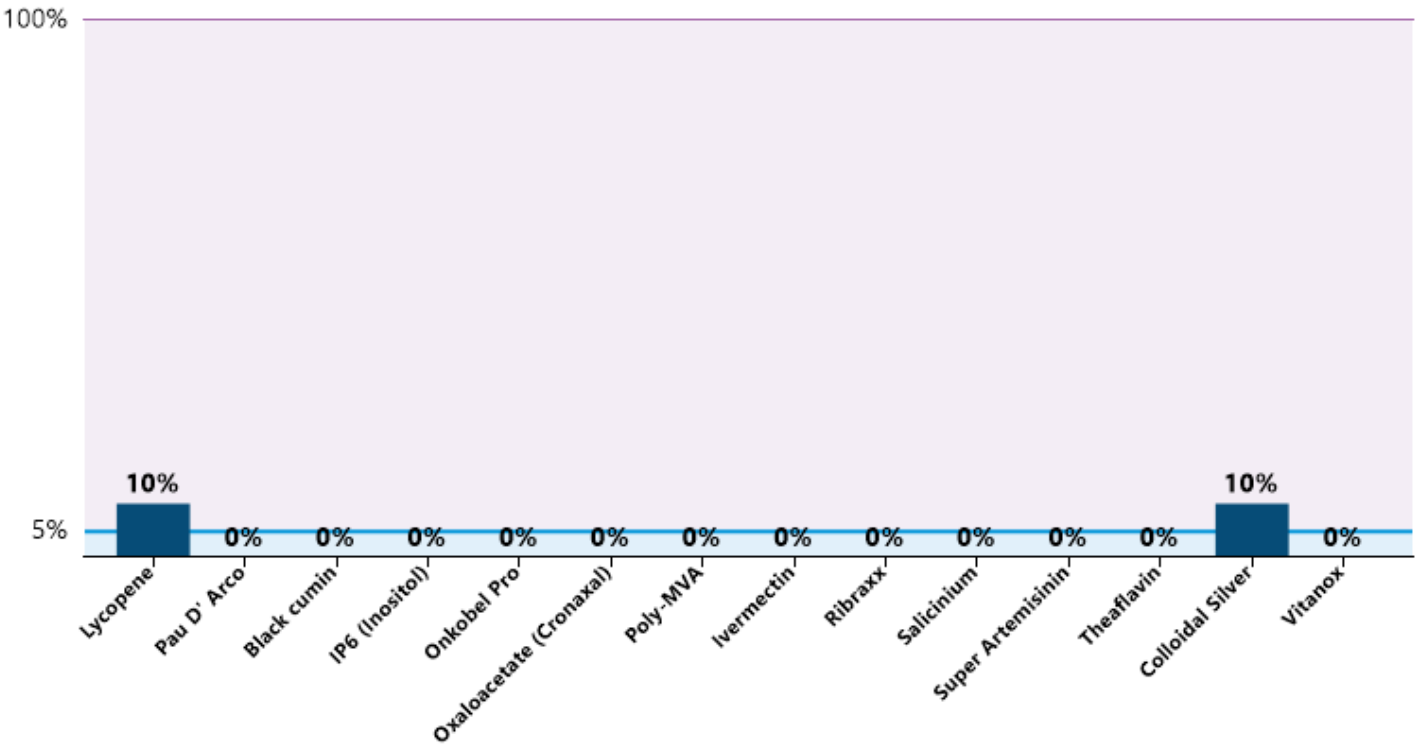
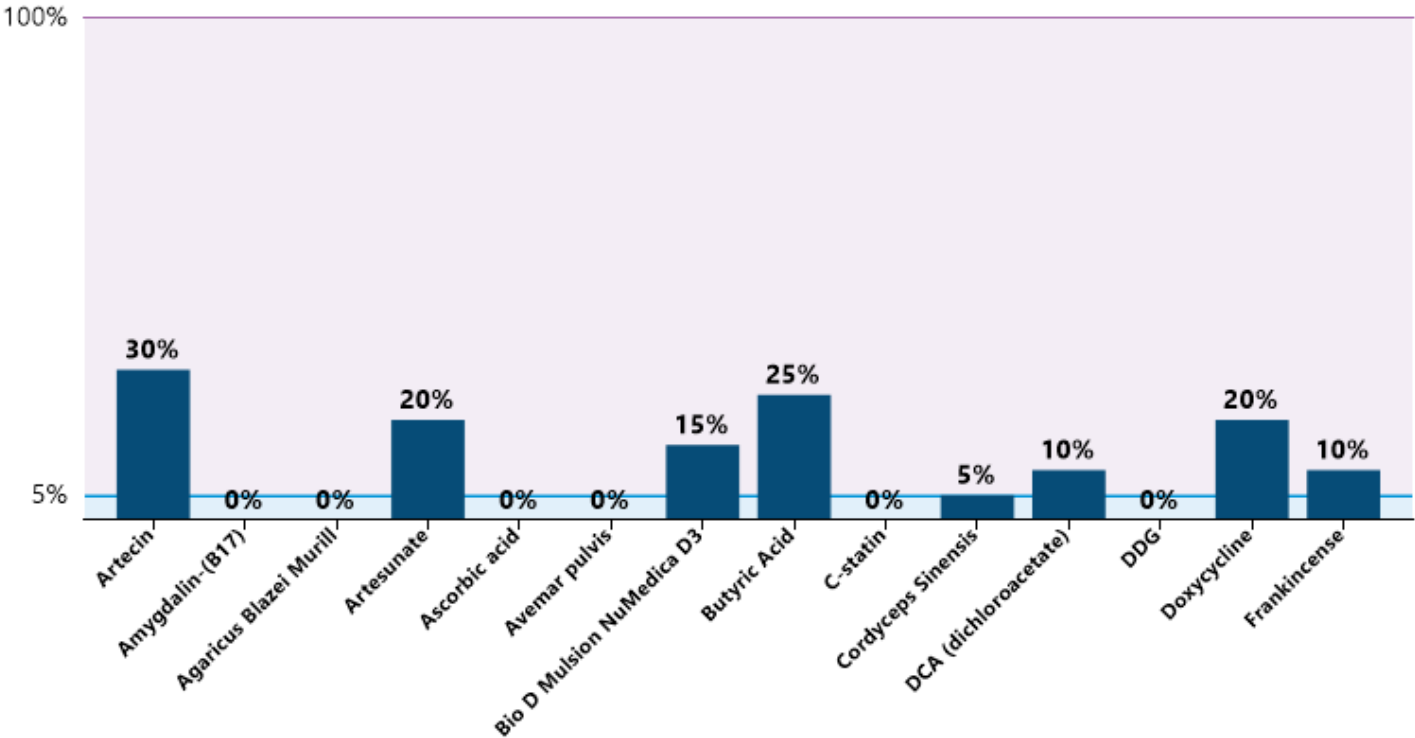


Immune system regulation



No sensitivity
 Sensitivity

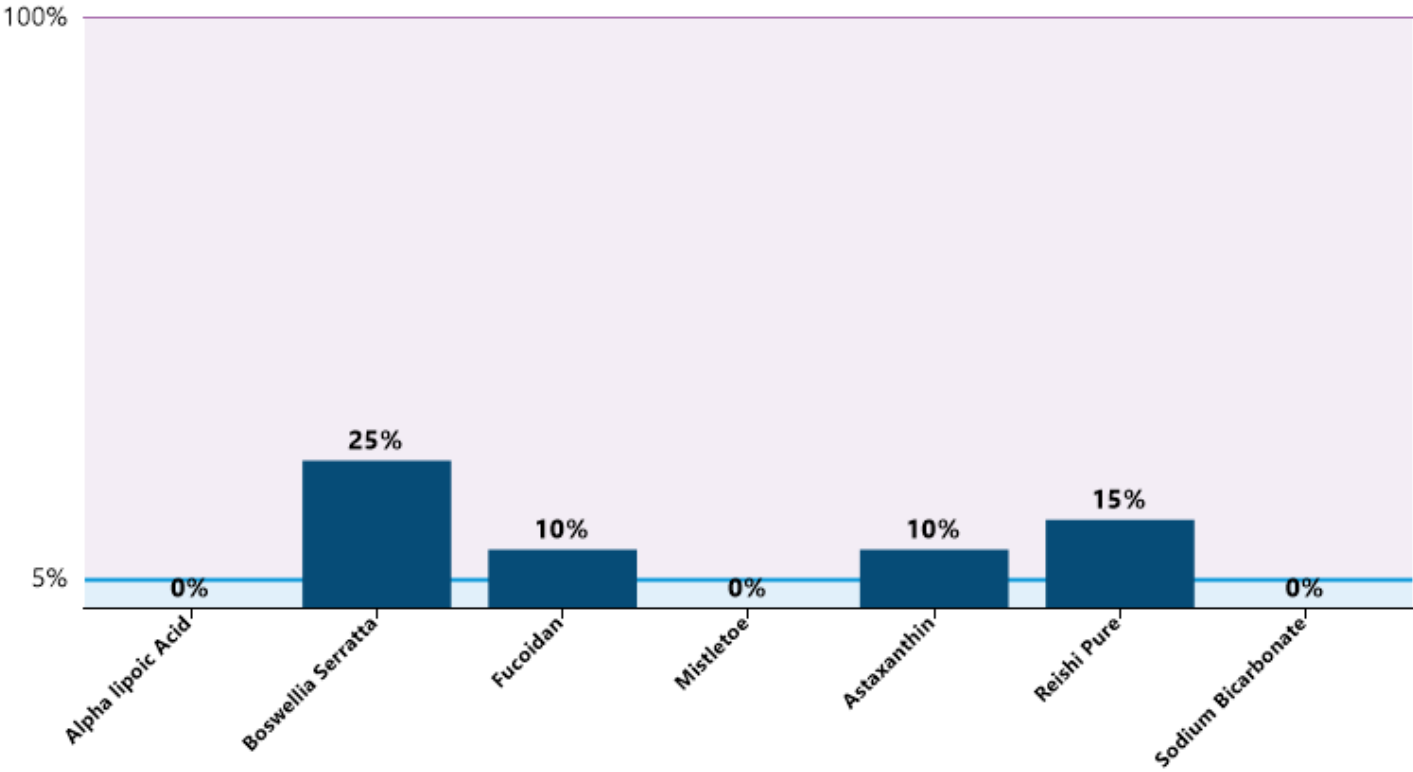
Class I (Cytotoxic Agents)



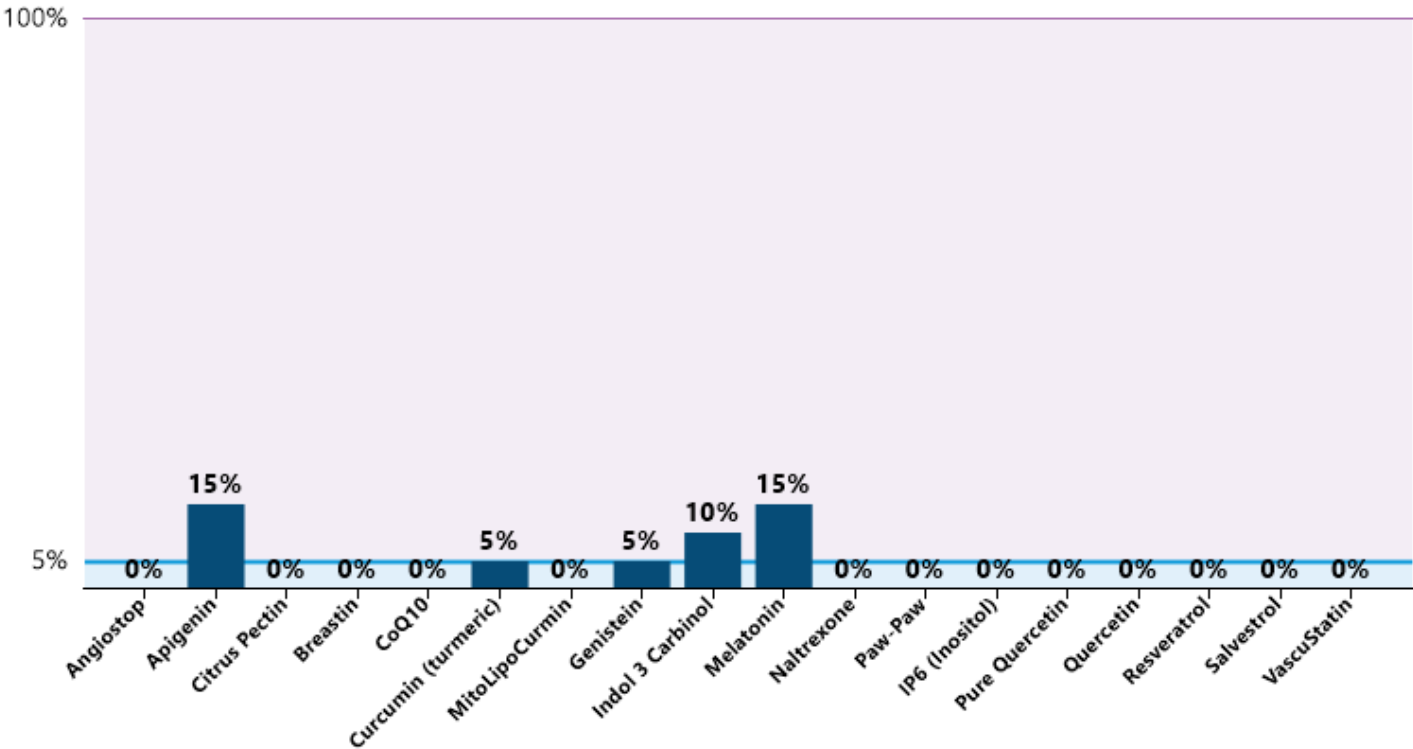
No sensitivity

Sensitivity

Class II (Immunostimulants / Immunomodulators)



Class III (PK Inhibitors)



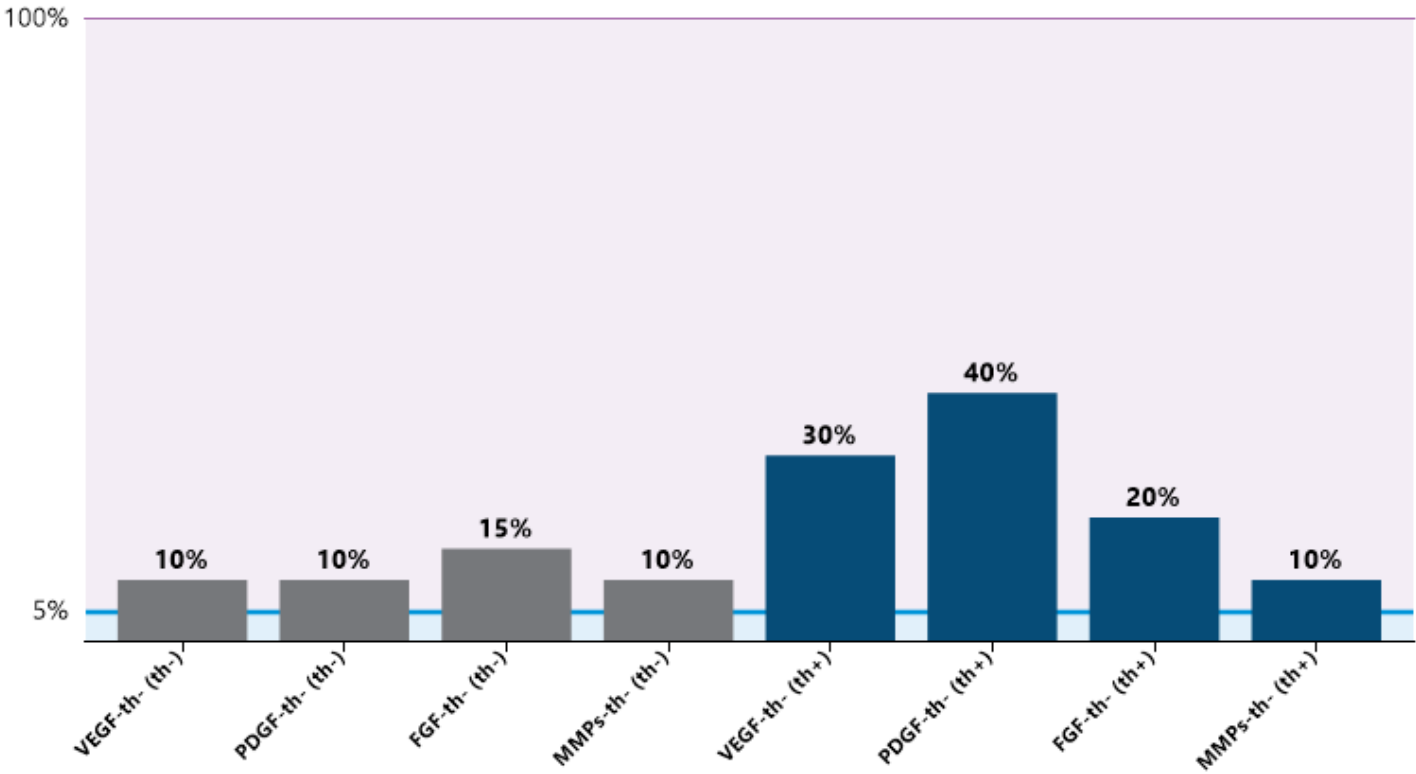
No sensitivity

Sensitivity

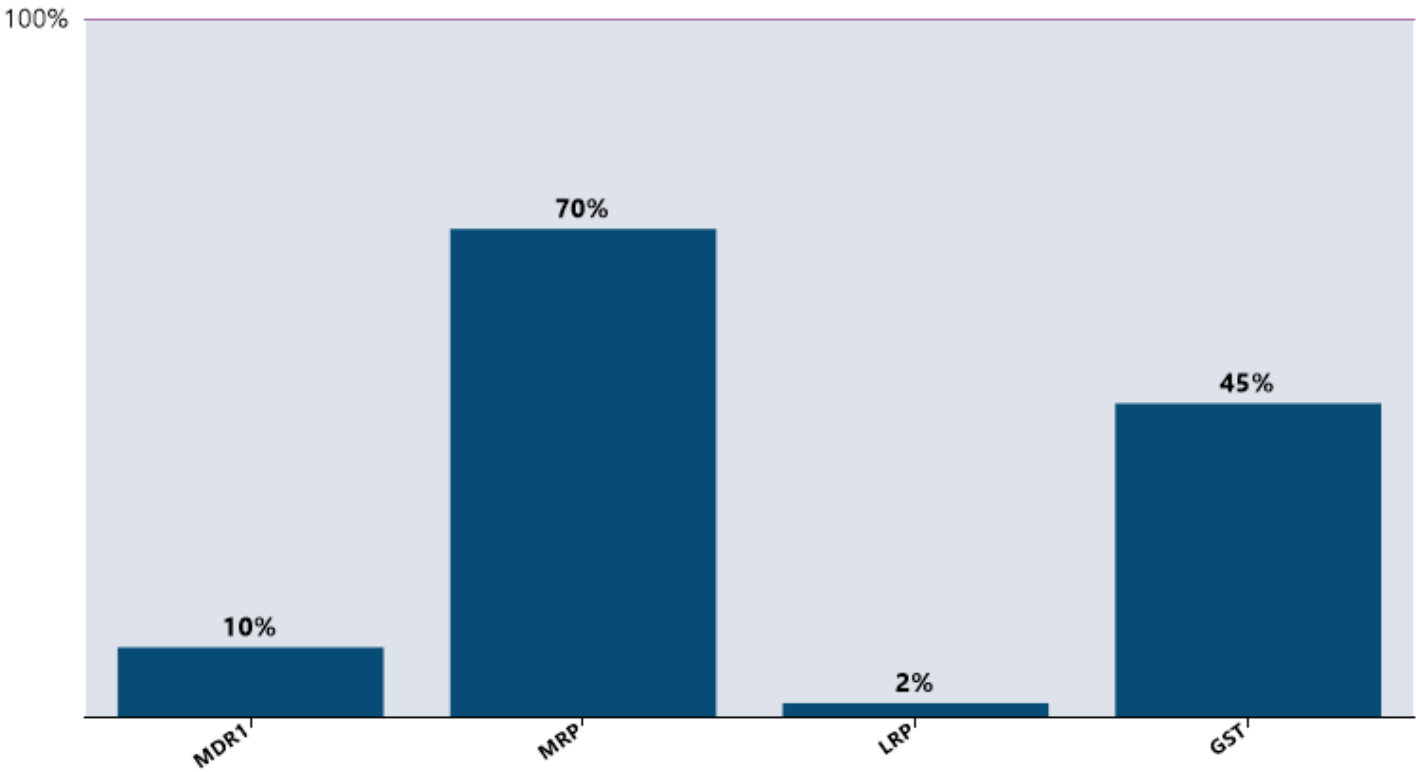
Malignant Cells - Thalidomide

th-

th+



Resistance Factors



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.

Expression Rates

Clinical relevant genes related with:

Related with cell cycle regulation	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 Epithermal 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. Hashem S, Ali TA, Akhtar S, Nisar S, Sageena G, Ali S, Al-Mannai S, Therachiyil L, Mir R, Elfaki I, Mir MM, Jamal F, Masoodi T, Uddin S, Singh M, Haris M, Macha M, Bhat AA. Targeting cancer signaling pathways by natural products: Exploring promising anti-cancer agents. *Biomed Pharmacother*. 2022 Jun;150:113054. doi: 10.1016/j.biopha.2022.113054. Epub 2022 Apr 30. PMID: 35658225.
- b. Nuzzo G, Senese G, Gallo C, Albiani F, Romano L, d'Ippolito G, Manzo E, Fontana A. Antitumor Potential of Immunomodulatory Natural Products. *Mar Drugs*. 2022 Jun 8;20(6):386. doi: 10.3390/md20060386. PMID: 35736189; PMCID: PMC9229642.
- c. Toloudi M, Apostolou P, Chatziioannou M, Papasotiriou I. Correlation between Cancer Stem Cells and Circulating Tumor Cells and Their Value. *Case Rep Oncol*. 2011 Jan 29;4(1):44–54. doi: 10.1159/000324403. PMID: 21526006; PMCID: PMC3082489.
- d. Dong S, Guo X, Han F, He Z, Wang Y. Emerging role of natural products in cancer immunotherapy. *Acta Pharm Sin B*. 2022 Mar;12(3):1163–1185. doi: 10.1016/j.apsb.2021.08.020. Epub 2021 Aug 21. PMID: 35530162; PMCID: PMC9069318.
- e. Ling T, Lang WH, Maier J, Quintana Centurion M, Rivas F. Cytostatic and Cytotoxic Natural Products against Cancer Cell Models. *Molecules*. 2019 May 26;24(10):2012. doi: 10.3390/molecules24102012. PMID: 31130671; PMCID: PMC6571673.
- f. 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.
- g. Gairola K, Gururani S, Bahuguna A, Garia V, Pujari R, Dubey SK. Natural products targeting cancer stem cells: Implications for cancer chemoprevention and therapeutics. *J Food Biochem*. 2021 Jul;45(7):e13772. doi: 10.1111/jfbc.13772. Epub 2021 May 24. PMID: 34028051.