- Integrative Oncology -

Personalised Cancer Care via CTCs & Chemosensitivity Testing

Peter Gotis B. Sc, Lab Director – NutriPATH Australia

HEAT, Bangkok, September 2019

Disclosures:

- 1995 – 2010 Director, PATHLAB Australia, Australia
- 2003 – 2011 Director, Age Diagnostic Laboratories, USA
- 2011 – present Lab Director, NutriPATH Integrative Pathology, Australia

Acknowledgement:

- Dr Ioannis Papasotiriou
  Medical Director –
  Research Genetic Cancer Centre

Structure of this presentation

An introduction to Personalised Oncology using CTCs

- Methodologies
  - Isolation Techniques
  - Gene Expression & Chemosensitivity Testing
    1. Conventional Cytotoxic Agents
    2. Immune System Regulators
    3. Natural/Biological Substances
- Combining Pharmacogenomics
  - Pharmacology (PD vs PK)
  - used to “Fine Tune” the Targeted Personalised Therapy
- Formulation of the Personalised Treatment Protocol

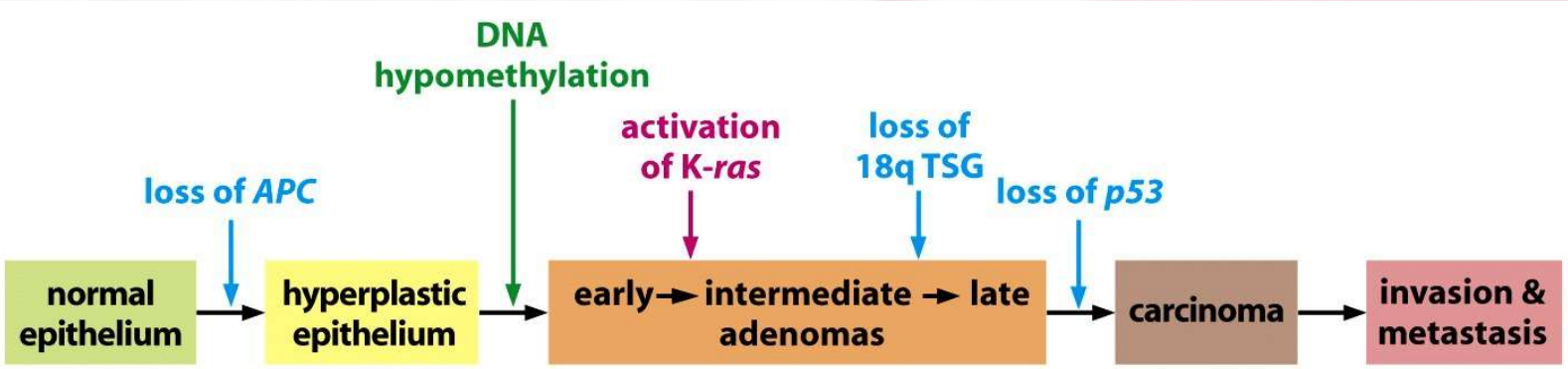
CARCINOGENESIS STEPS

INITIATION
- Viral interference
- Chemical interference
- Radiation influence

PROMOTION
- Cell cycle instability
- DNA repair aberration
- Apoptosis instability
- Both DNA and mRNA

PROGRESS
- Invasion
- Neo-angiogenesis
- EMT and MET

VOGELSTEIN MODEL OF DEVELOPING COLON CANCER

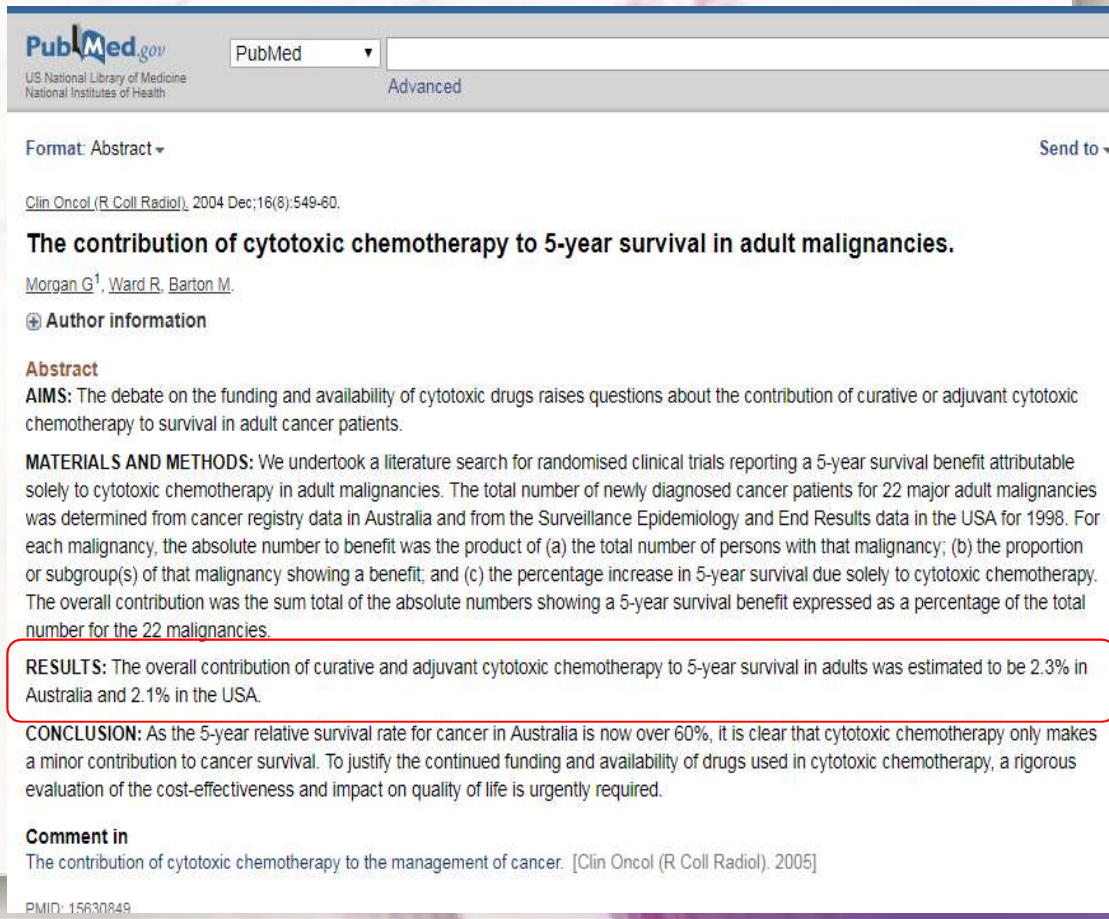


Recent Rate of success

- For Adjuvant chemotherapy the success rate for the 5 major types of malignancy varies from 2.1% to 2.3% in 5 years.

  Royal North Shore Hospital Clin Oncol (R Coll Radiol) 2005 Jun;17(4):294

- For curative stage of disease the success rate varies between 5 to 7.5% for the same 5 types of malignancies.



PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed ▾ Advanced

Format: Abstract ▾ Send to ▾

Clin Oncol (R Coll Radiol). 2004 Dec;16(8):549-60.

The contribution of cytotoxic chemotherapy to 5-year survival in adult malignancies.

Morgan G[1], Ward R, Barton M.

⊕ **Author information**

Abstract

AIMS: The debate on the funding and availability of cytotoxic drugs raises questions about the contribution of curative or adjuvant cytotoxic chemotherapy to survival in adult cancer patients.

MATERIALS AND METHODS: We undertook a literature search for randomised clinical trials reporting a 5-year survival benefit attributable solely to cytotoxic chemotherapy in adult malignancies. The total number of newly diagnosed cancer patients for 22 major adult malignancies was determined from cancer registry data in Australia and from the Surveillance Epidemiology and End Results data in the USA for 1998. For each malignancy, the absolute number to benefit was the product of (a) the total number of persons with that malignancy; (b) the proportion or subgroup(s) of that malignancy showing a benefit; and (c) the percentage increase in 5-year survival due solely to cytotoxic chemotherapy. The overall contribution was the sum total of the absolute numbers showing a 5-year survival benefit expressed as a percentage of the total number for the 22 malignancies.

RESULTS: The overall contribution of curative and adjuvant cytotoxic chemotherapy to 5-year survival in adults was estimated to be 2.3% in Australia and 2.1% in the USA.

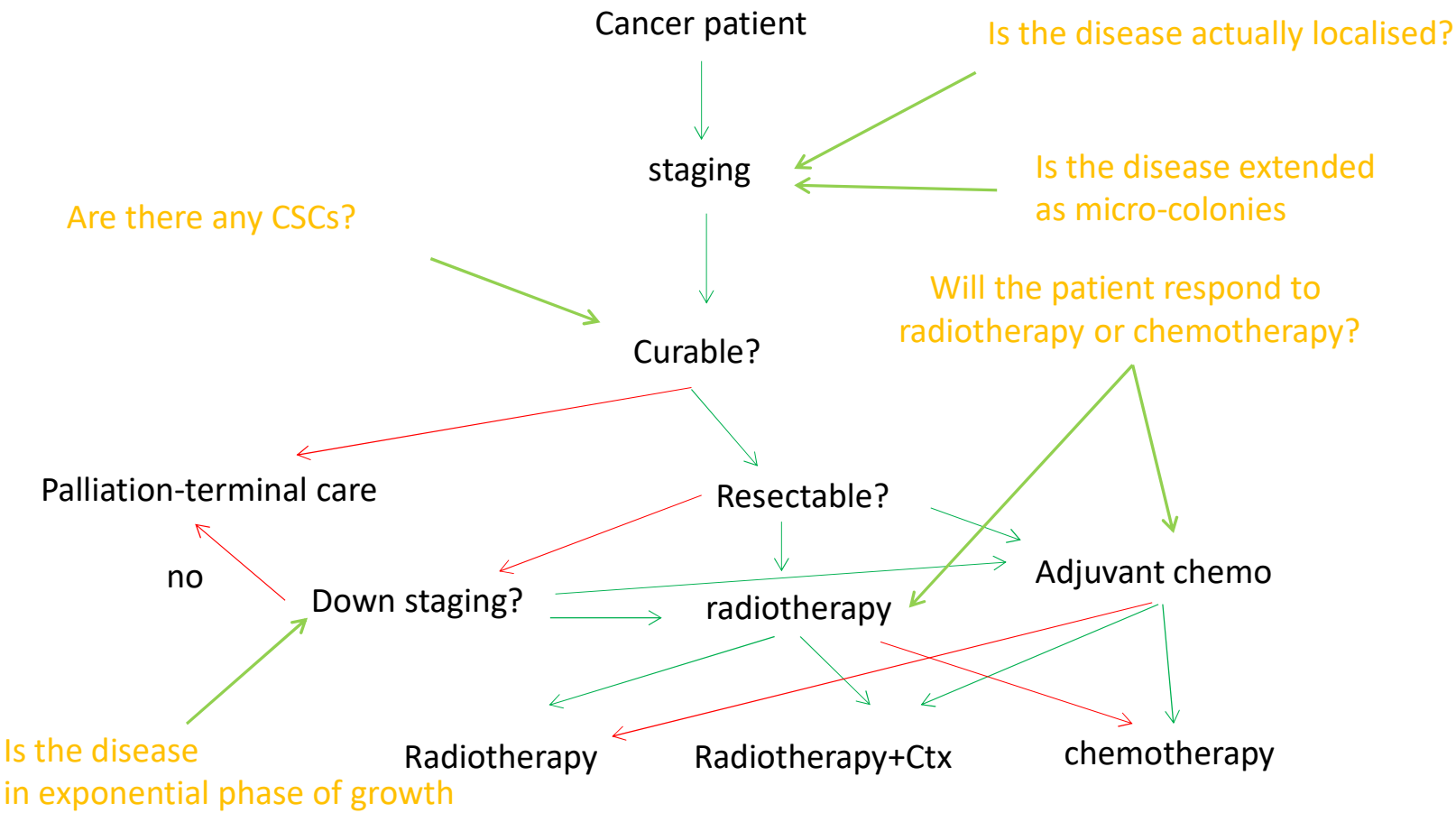
CONCLUSION: As the 5-year relative survival rate for cancer in Australia is now over 60%, it is clear that cytotoxic chemotherapy only makes a minor contribution to cancer survival. To justify the continued funding and availability of drugs used in cytotoxic chemotherapy, a rigorous evaluation of the cost-effectiveness and impact on quality of life is urgently required.

Comment in

The contribution of cytotoxic chemotherapy to the management of cancer. [Clin Oncol (R Coll Radiol). 2005]

PMID: 15630849

Dead-End in empirical treatment

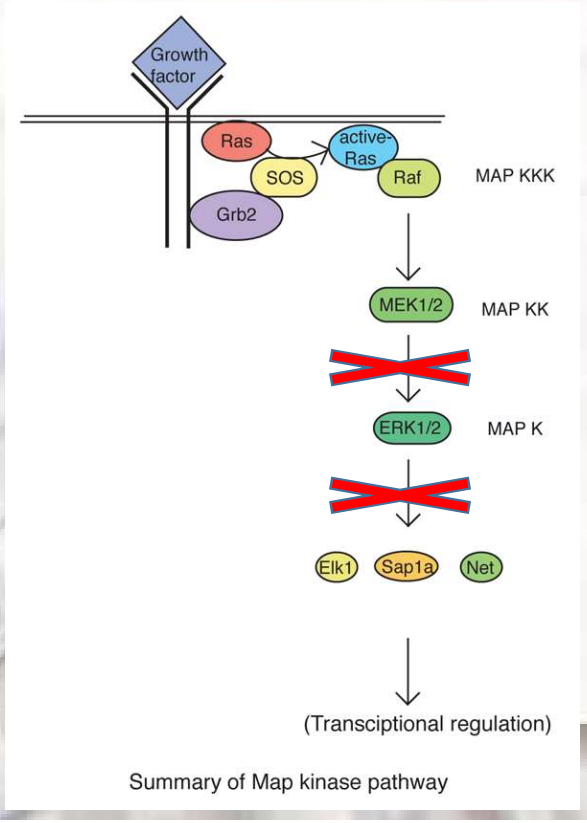


Possible Reasons and causes

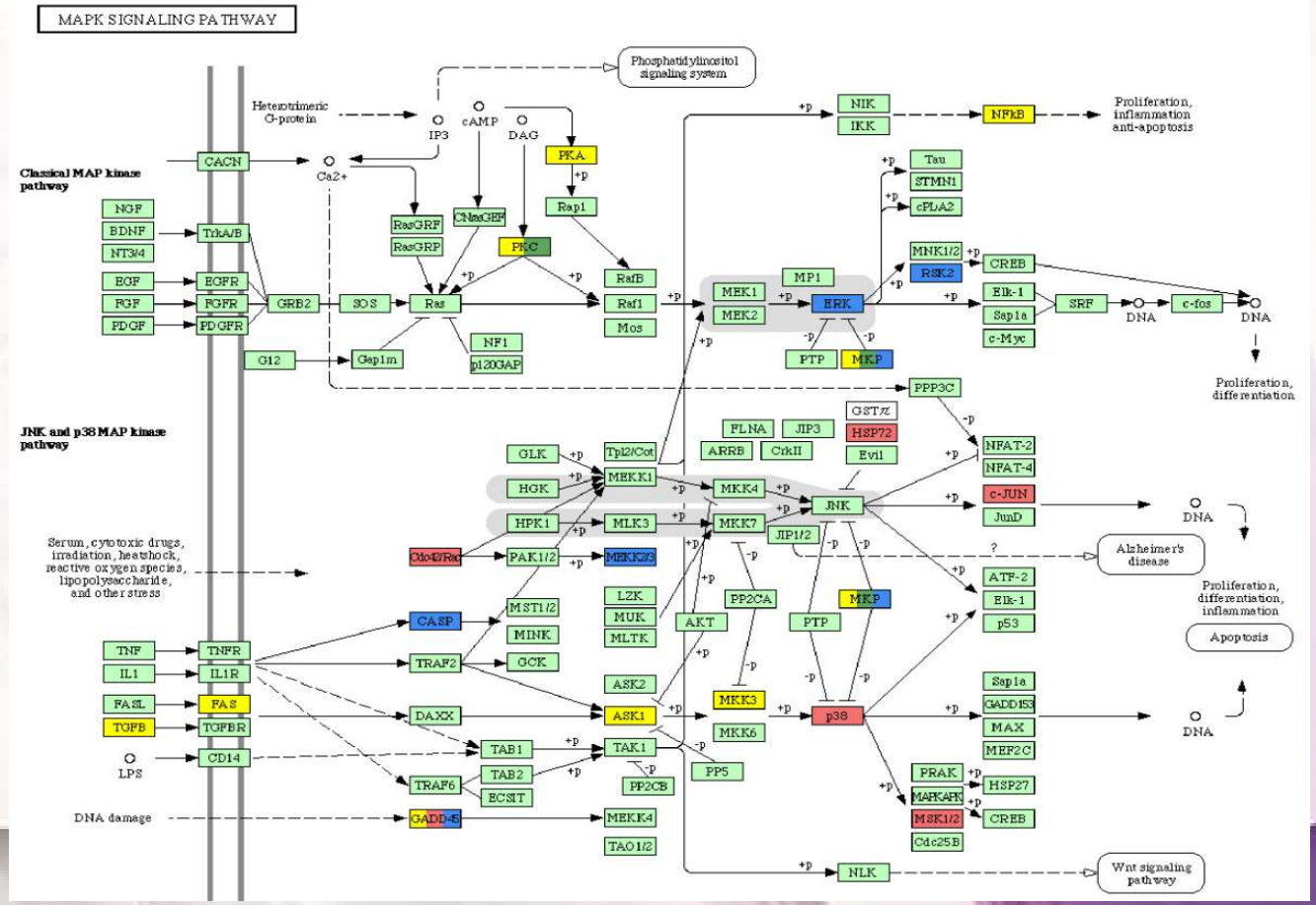
1. Lack sensitivity to detect the MRD (Minimal Residual Disease)
2. Inability to discriminate the actual important cells from the irrelevant.
3. Inability to detect the genetic instability of malignant cells.
4. Inability to distinguish which cells may change and become the driving entity and which may not.

How reliable are the current biomarkers?

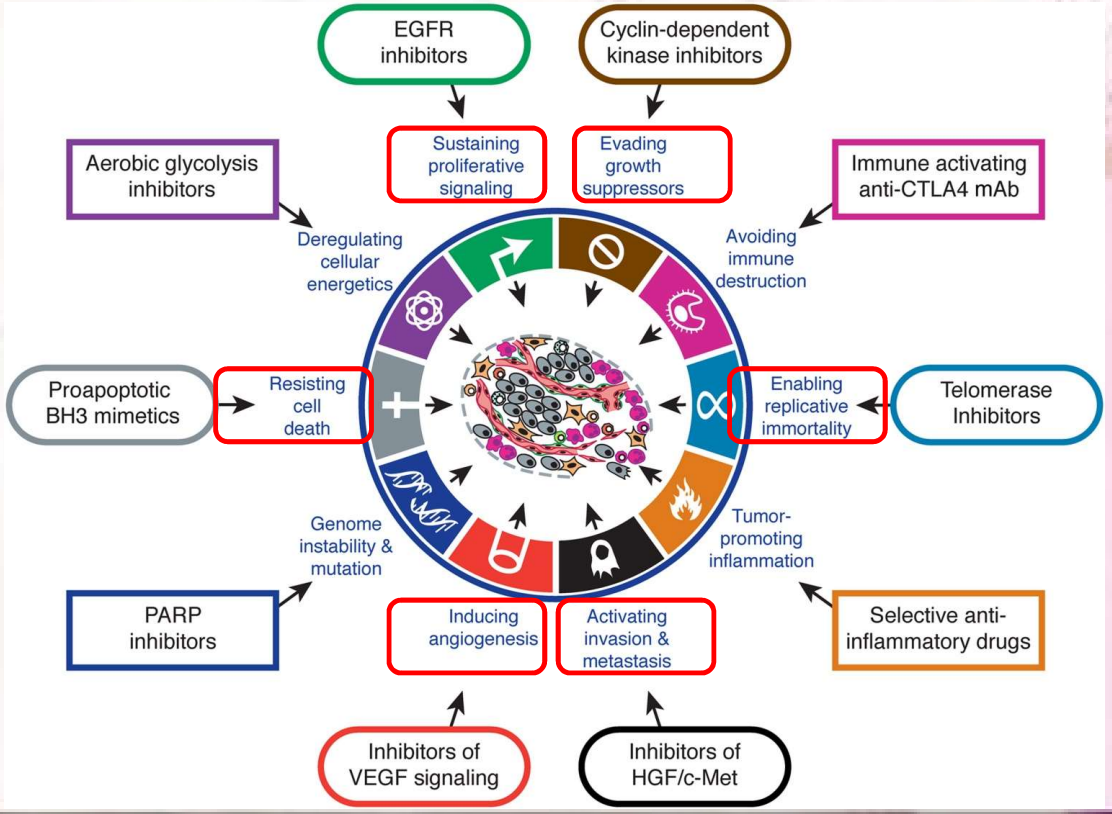
- The Assumption is... (The cascade is linear)
- The Reality though… (Complex cross talking and signalling)



Summary of Map kinase pathway



Cancer Hallmarks



Weinberg et al (2014)

Tumor Physiology (CTCs)

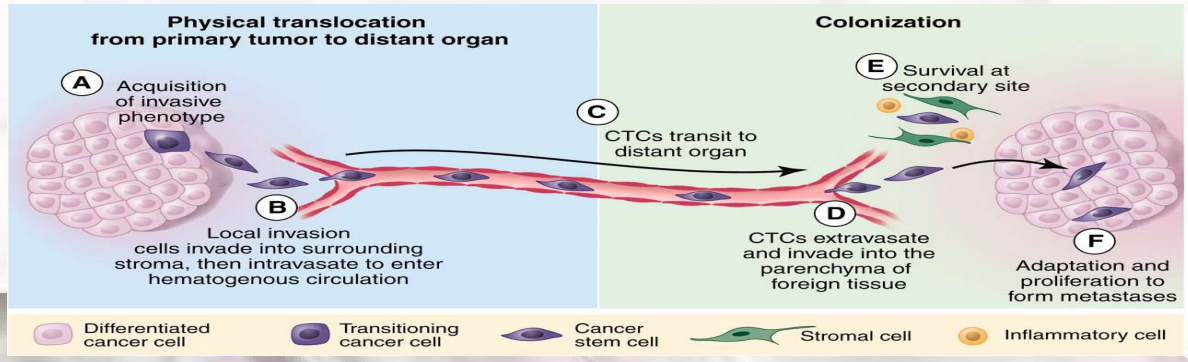
1. A tumor is composed of 2 groups of cells

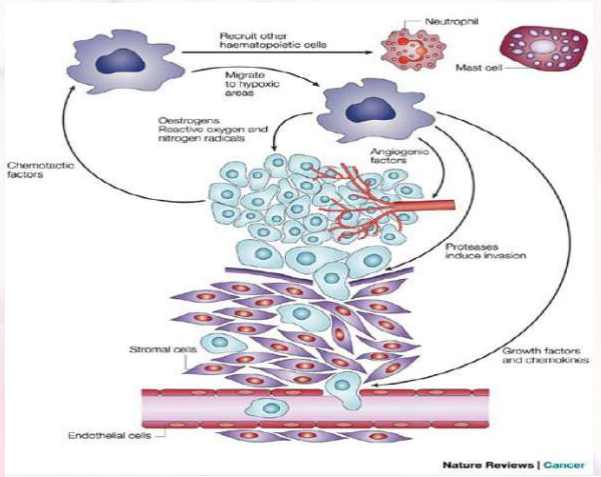
   a. The stroma cells composed from fibroblast, lymphocytes, endothelial cells etc

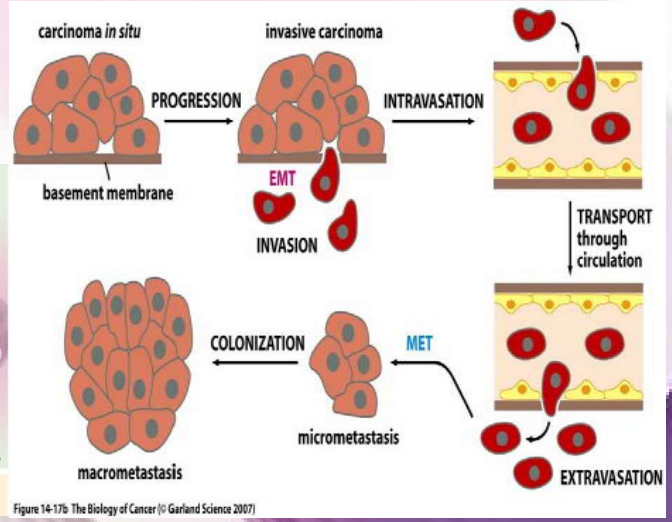
   b. The cancer cells a heterogeneous composition of subpopulations with different features and aggressive behavior.

2. One of these subpopulations are the Cancer Stem Cell like cells (CSCs). They are progenitors to tumours and generator of metastases.

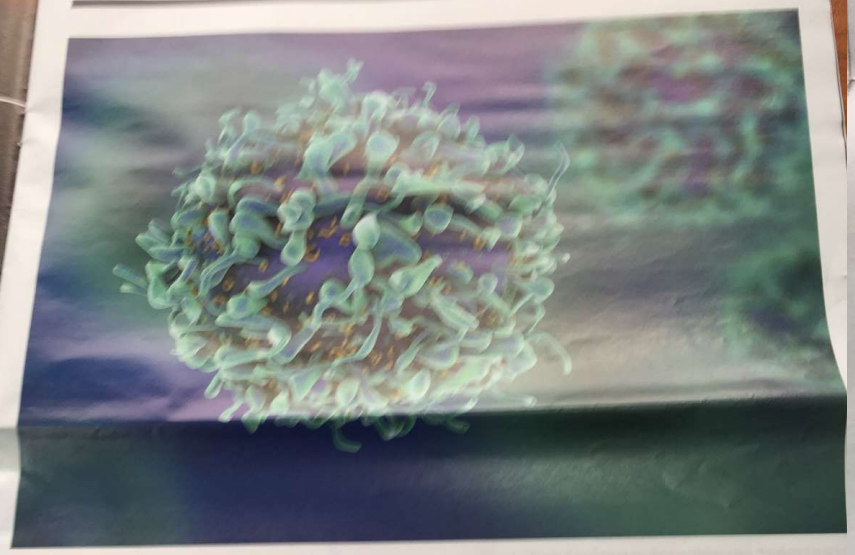
3. This subpopulation has the ability to invade the surrounding organs, enter the circulation (blood vessel or lymphatics) and engraft to distant organs in order to generate metastases and relapses.







Due to this heterogeneity and plasticity of the disease, we need to review the personalized approach as a therapeutic concept



New developments in the management of lymphoma

Dr Shafqat Inam
Dr John Gibson

INTRODUCTION

BIOLOGY OF LYMPHOPOIESIS

INSIDE

characterised by ... neoplastic Reed-Sternberg cell (see figure 3) within a dense inflammatory infiltrate.[7]

Non-Hodgkin lymphoma has numerous subtypes based on the origin and stage of development of the neoplastic lymphocyte. These are broadly classified as B-cell or T-cell lymphomas.

B-cell lymphomas can be divided into two broad groups based on their biological behaviour and treatment approaches.[8] Aggressive B-cell non-Hodgkin lymphoma (the prototypical example is diffuse large B-cell lymphoma) presents with more proliferative disease, although it is considered curable with a defined course of treatment, while indolent lymphomas (the most common being follicular lymphoma) are slow growing and may require treatment multiple times over many years. B-cell non-Hodgkin lymphomas can involve lymph nodes as well as almost any organ in the body, including the bone marrow.

Cancers primarily of the lymphoid cells in the bone marrow are known as leukaemias, though there can be overlap with lymphomas with pre-

ment encompassing patient and ... ease factors is crucial for achieving the best clinical outcome.

The patient's age, comorbidities and preferences will guide the intensity of treatment and specific drug choices.[11] For example, drug dosing will often need to be reduced to account for liver or renal impairment, and certain chemotherapy regimens are too toxic to deliver safely above a certain age threshold. Important disease factors are the histopathological subtype of lymphoma and risk stratification, which uses grade, stage, serum biomarkers and imaging.

An individualised approach to treatment encompassing patient and disease factors is crucial for achieving the best clinical outcome.

Patients with aggressive lymphomas will generally require treatment soon after diagnosis, while patients with more indolent lymphomas may undergo a period of observation ('watch and wait') until they develop a clinical indication that requires treatment. During initial therapy, there is close clinical monitoring of response, and often also interim PET scanning to confirm response (see figure 4).[12] A ...

Empirical vs Personalized treatment Pros & Cons

	Empirical Mode of Therapy	Personalised Treatment Plan
ADVANTAGES	• Low Cost (Short Term) • Fast Application • Applicable to the masses • No need for physician training in PK and PD	• High Rate of Success • Long Term Cost Effective • Shortening of hospital admission and residence of a patient
DISADVANTAGES	• High Rate of Failure • No individuality in case treatment • No further option after last line of therapy	• Higher Cost than Empirical • Need a series of analyses to be performed

What we need to consider for a true applied personalized approach

- Precise information which reflects/shows/confirms the downstream outcome
- Multimodal data is required; not only at a genomic level, but also in :
  1. Epigenetic (gene expression)
  2. Proteomics
  3. Glucoproteomics
  - At only the genomic level, we don't know whether gene expression is present
- A more complete Pharmacological analysis (PD and PK)
  - What the drug does to the disease (Pharmacodynamics - PD)
  - How the body utilises the drug (Pharmacokinetics - PK)

How can we find a needle in a hay-stack?

- Average No. CTCs in blood is 10-30cell/50,000 events (RBC and platelets have been subtracted)
- Selection/isolation process is critical

What we need to preserve during isolation and detection of CTCs

1. High purity of CTCs — Free of interferring debris and non-relevant ce
2. Viable/Live CTCs — Vital for Chemosensitivity and Genetic Express
3. Isolate all CTC subsets (The important cells)

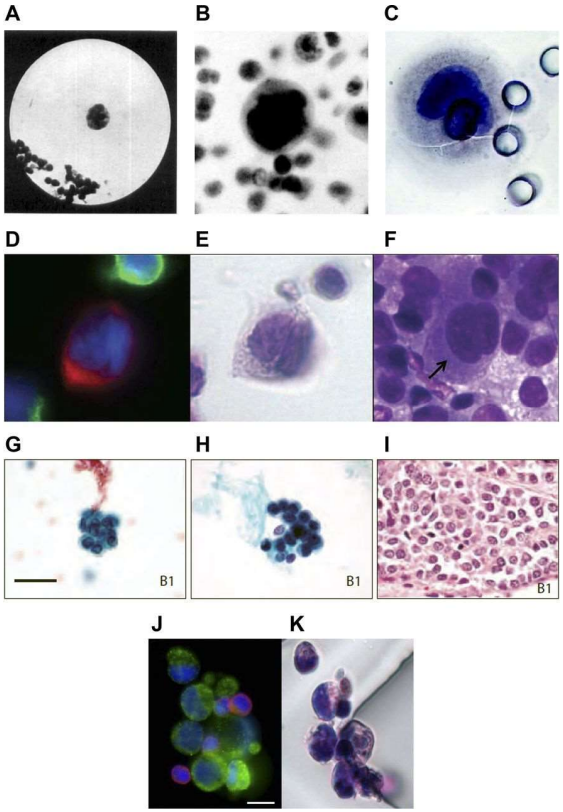
Detect the disease-relevant CTCs

Detect CTCs subset with stemness (resistance) properties

Pin point the subclasses of CTCs with plasticity properties (EMT-MET)

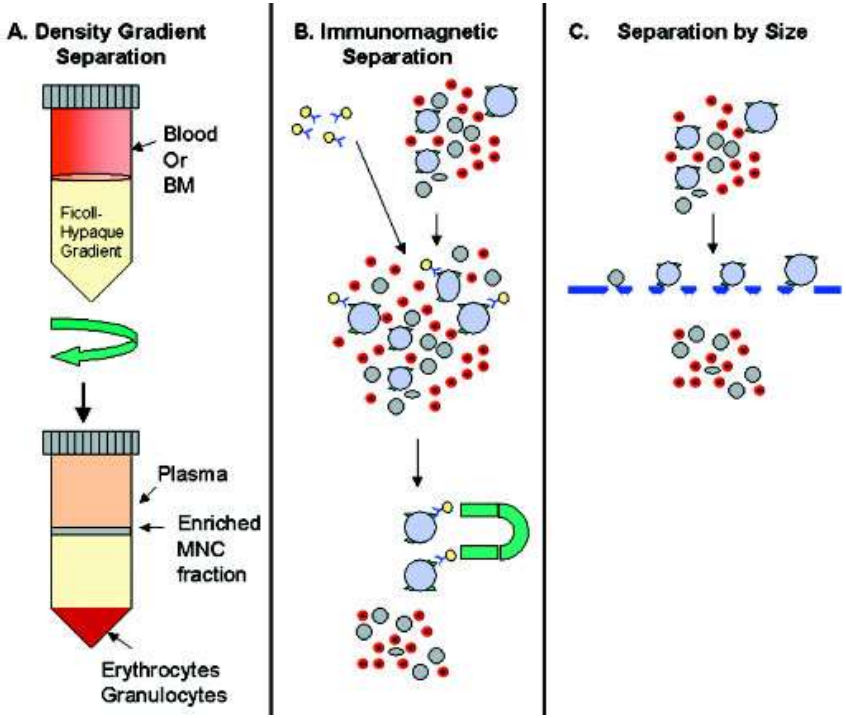
CHOOSING THE RIGHT METHOD

MICROSCOPY/STAINING BASED METHOD



- FIXATION OF THE SAMPLE
- POSITIVE SELECTION METHOD
- CELLS ARE DAMAGED DURING PROCESS

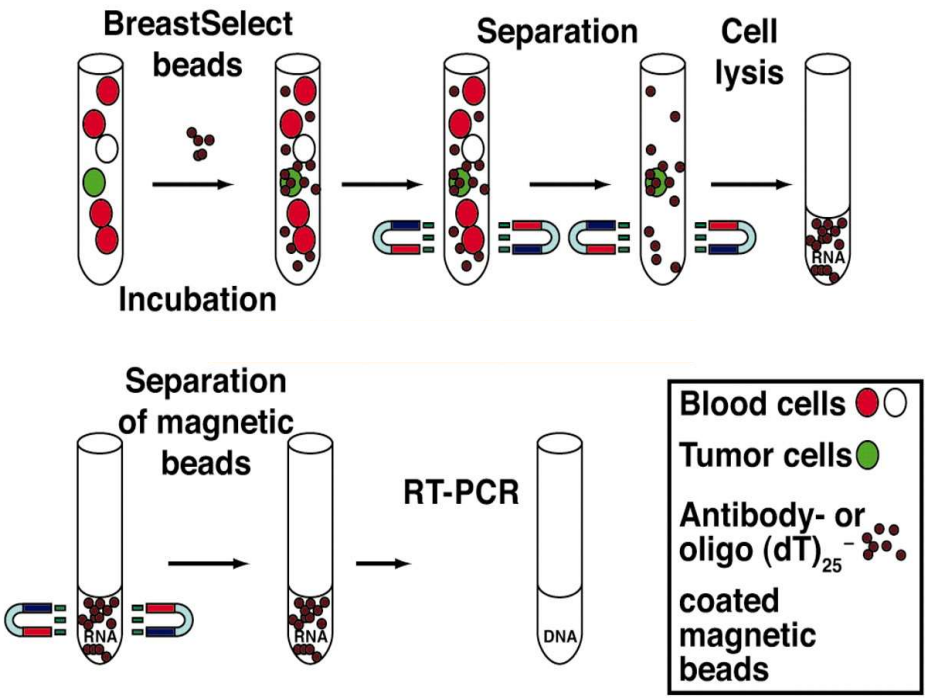
GRADIENT BASED METHOD



- SEPARATION BASED ON SIZE
- ENRICHMENT METHOD
- NOT ALL CELLS ARE CAPTURED DURING PROCESS

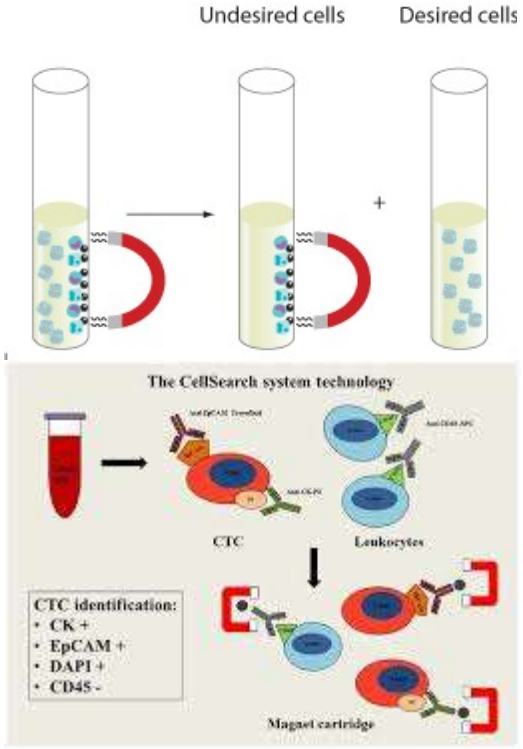
CHOOSING THE RIGHT METHOD

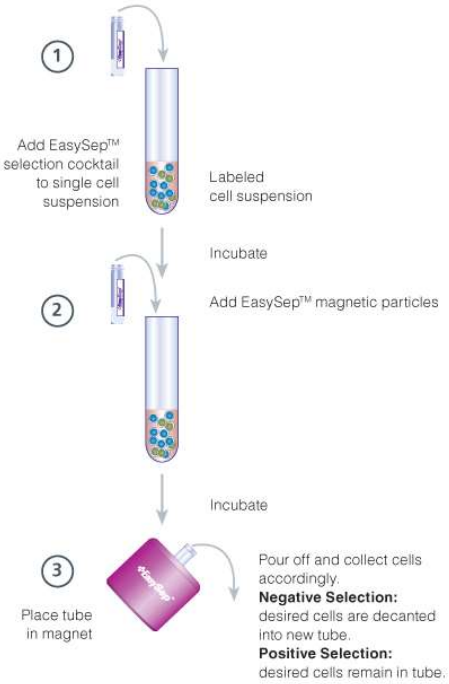
PCR BASED METHOD



- ANTIBODY CAPTURE
- POSITIVE SELECTION METHOD
- LIMITATIONS ON CELL MARKERS
- NO FURTHER APPLICATION POSSIBLE

BEAD BASED METHOD





- panCK or Epcam ID THROUGH PCR
- POSITIVE SELECTION METHOD
- CELLS DESTROYED IN THE PROCESS
- NO FURTHER APPLICATION POSSIBLE

FLOW CYTOMETRY and CTCs

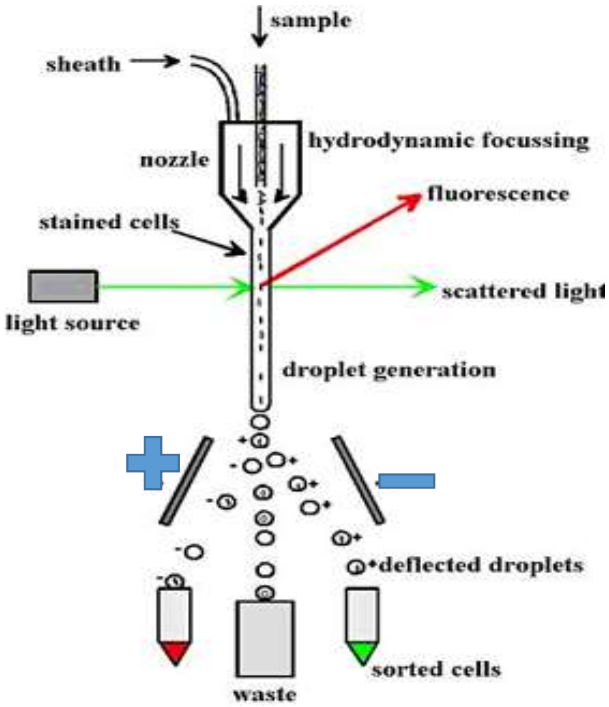
- FC can provide information about quantity and quality of CTCs
- There are two isolation processes to detect CTCs using FC:

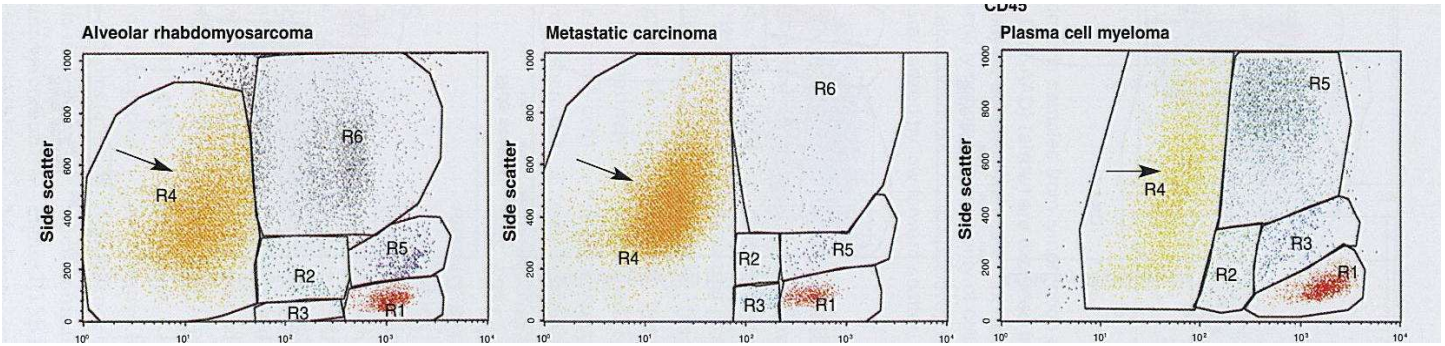
  Positive selection (Capture the cells you want; discard the others)

  Negative selection (Capture the cells you don't want and discard; the remainder are the cells you need).

- Combining these selection processes allows for

  Higher CTC capture rate,
  Higher CTC purity level
  Viable CTCs for further use





CTC Comparative Methods

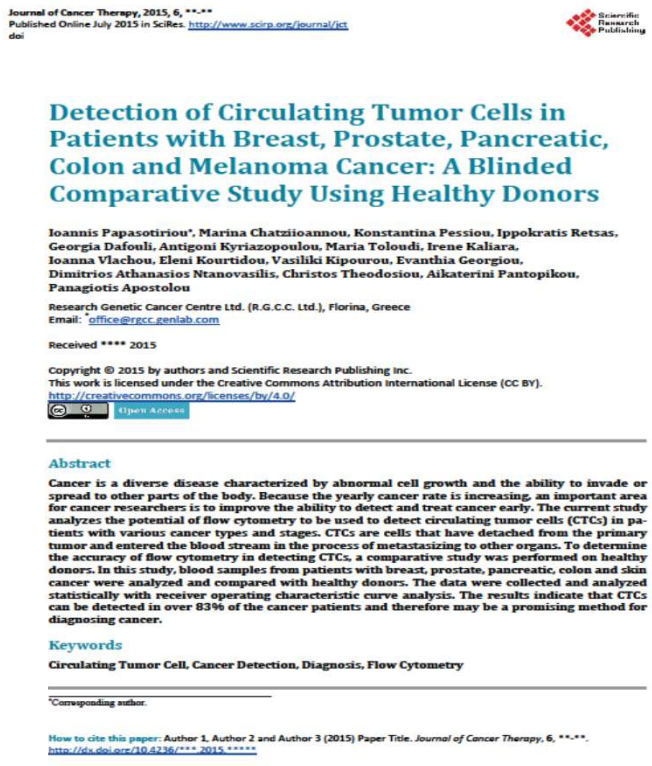
	Bead Based Method	PCR Based Method	Flow Cytry, Genomic & Viability Assays	Microscopy Based Method	Gradient Method
	Cell Search		RGCC	Maintrac	
Isolation Method	Magnetic Beads (antibodies with iron particles)	Method requires destroying cells to identify marker (panCK or Epcam)	Flow Cytometry sorting with interrogation in droplets, in ratio of droplet/cell (1:1)	Immobilizing cells on a slide and staining them	Cells are isolated based on size
CTC Purity	Enrichment method, NOT isolation Method	There are no cells any more	>99%, Isolation Method	CTCs are simply stained, not isolated	Enrichment Method
Viability of CTCs	70 – 85%	0%, No Cells	>99%	0%, No Cells	Uncertain/Questionable
Quality of CTCs for further analysis	Inappropriate for further molecular analysis due to lymphocyte contamination	Very limited	Appropriate for further molecular analysis as there is no "noise".	CTCs are no longer viable	Not recommended
Selection of CTCs	Based mainly on Positive selection of CTCs, limited to a few markers	Based on Positive selection	Based on Negative AND Positive selection in order to firstly identify and secondly phenotype the CTCs	Possible	Based on size
Further Abilities			Identification of heterogeneity of CTCs	Identification of heterogeneity is marker dependent	Identification of heterogeneity of CTCs
Additional Features	Method only enumerates CTCs	Method only enumerates CTCs. Limited identification of other CTC features	Method allows gene expression assays to be performed to determine features vital for therapy scheduling	Method for detection and enumeration only	

Accuracy and clinical relevance of the CTC analysis

Journal of Cancer Therapy, 2015, 6, 543-553
Published Online July 2015 in SciRes. http://www.scirp.org/journal/jct
http://dx.doi.org/10.4236/jct.2015.67059

5. Conclusion

In conclusion, this study demonstrates that it is possible to detect CTCs with higher sensitivity (86.2%) and specificity (83.9%) compared with routine clinical methodologies. The parameters may vary depending on the antibody panel used; however, using flow cytometry to identify CTCs has proven to be efficient. These results suggest that further studies are required to improve the accuracy by which CTCs and CTC subtypes can be identified by flow cytometry and thereby improve our ability to detect and follow the progression of cancer.



Journal of Cancer Therapy, 2015, 6, **-**
Published Online July 2015 in SciRes. http://www.scirp.org/journal/jct
doi

Scientific Research Publishing

Detection of Circulating Tumor Cells in Patients with Breast, Prostate, Pancreatic, Colon and Melanoma Cancer: A Blinded Comparative Study Using Healthy Donors

Ioannis Papasotiriou*, Marina Chatziioannou, Konstantina Pessiou, Ippokratis Retsas, Georgia Dafouli, Antigoni Kyriazopoulou, Maria Toloudi, Irene Kaliara, Ioanna Vlachou, Eleni Kourtidou, Vasiliki Kipourou, Evanthia Georgiou, Dimitrios Athanasios Ntanovasilis, Christos Theodosiou, Aikaterini Pantopikou, Panagiotis Apostolou

Research Genetic Cancer Centre Ltd. (R.G.C.C. Ltd.), Florina, Greece
Email: *office@rgcc.genlab.com

Received **** 2015

Abstract

Cancer is a diverse disease characterized by abnormal cell growth and the ability to invade or spread to other parts of the body. Because the yearly cancer rate is increasing, an important area for cancer researchers is to improve the ability to detect and treat cancer early. The current study analyzes the potential of flow cytometry to be used to detect circulating tumor cells (CTCs) in patients with various cancer types and stages. CTCs are cells that have detached from the primary tumor and entered the blood stream in the process of metastasizing to other organs. To determine the accuracy of flow cytometry in detecting CTCs, a comparative study was performed on healthy donors. In this study, blood samples from patients with breast, prostate, pancreatic, colon and skin cancer were analyzed and compared with healthy donors. The data were collected and analyzed statistically with receiver operating characteristic curve analysis. The results indicate that CTCs can be detected in over 83% of the cancer patients and therefore may be a promising method for diagnosing cancer.

Keywords

Circulating Tumor Cell, Cancer Detection, Diagnosis, Flow Cytometry

*Corresponding author.

How to cite this paper: Author 1, Author 2 and Author 3 (2015) Paper Title. *Journal of Cancer Therapy*, 6, **-**.
http://dx.doi.org/10.4236/*** 2015 *****

Basic CTCs Assessments

CTC Testing: As a Prognostic Tool

- **CTC Count:** Used for detection of ***early relapse*** and as follow-up tool.
  Provides information about the presence & concentration of CTCs.

- **CTC Typing:** Used as a prognostic and follow up tool
  Used for guidance to define the primary tumor when it is unknown.
  Provides information about the presence & concentration of CTCs,
  Provides information about the immunophenotype
  - Haematological or Non-haematological (solid tissue)
  - Organ of Primary Origin (if Non-haematological)
  - Resistance capabilities
  - Metastatic risk

Example of CTC Count

CTC count:
Used for detection of ***early relapse and*** as follow-up tool.

Provides information about the presence & concentration of CTCs.

Florina , 29/10/2018

Dear Colleague,

We send you the results from the analysis on a patient suffering from breast carcinoma stage II. The sample that was sent to us for analysis was a sample of 10ml 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 and negative selection using multiple cell markers.

The results during the isolation procedure are presented below:

Table of markers:

CD45 positive cells (Hematologic origin cells)		CD45 negative cells (non Hematologic origin)	
CD15	NEGATIVE	CD133	POSITIVE
		CD44	POSITIVE

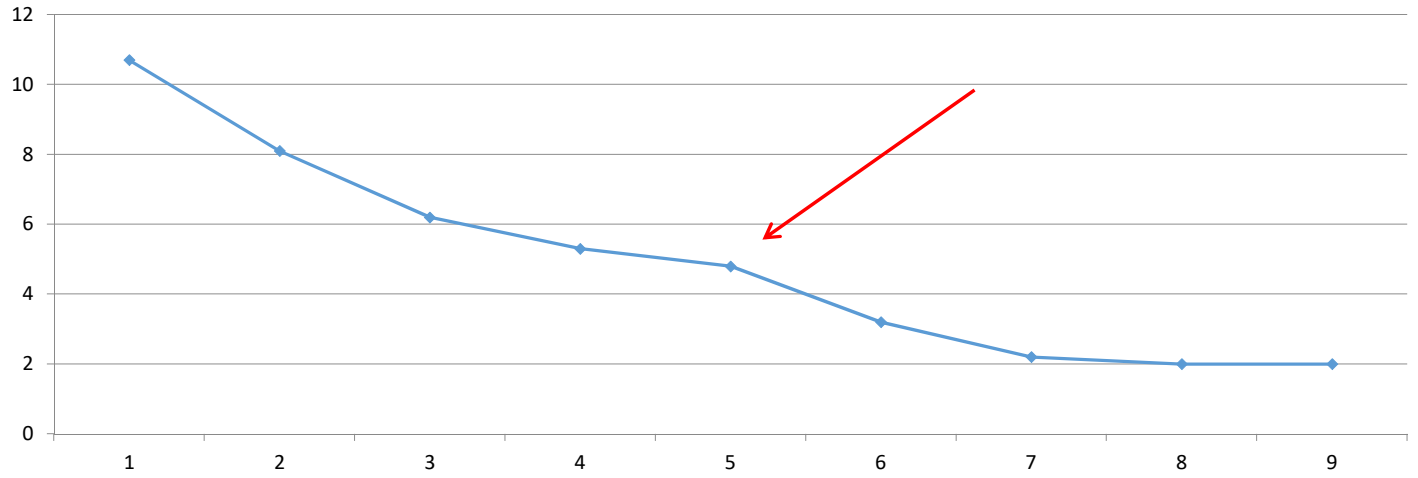
Index of marker: CD45: Hematologic origin cell marker, **CD133, CD44:** tumor stem cell marker, **CD15:** hematological malignancy marker.

The final results after the isolation procedure are presented below: We notice that after isolation procedure there are remaining malignant cells. The concentration of these cells was isolated 2.8cells/7.5ml, SD +/- 0.3cells.

Index of circulating cells number: (If Over limit: Advanced or Progression of Disease, If Less than limit: Early disease or disease is responding to a treatment plan).
Breast cancer: < 5cells /7.5ml , Prostate cancer < 20cells/ml , Sarcoma: <15cells/6.5ml, Colon cancer: <5cells/ml, Lung cancer (Lc=0, r=0.99): <10cell/ml. All cancer types other than those listed above should be <5 cells/ml.

CTC Testing: As a Follow Up Tool

1 oncotrail (3m)	8,1.cell/ml	5 oncotrail (15m)	3,2cell/ml
2 oncocount (6m)	6,2 cell/ml	6 oncocount (18m)	2,2cell/ml
3 oncotrail (9m)	5,3cell/ml	7 oncotrail (21m)	2cell/ml
4 oncocount (12m)	4,8cell/ml	8 oncocount (24m)	2cell/ml



Example of CTC Typing

Florina , 25/02/2019

Dear Colleague,

We send you the results from the analysis on a patient suffering from breast carcinoma stage II. The sample that was sent to us for analysis was a sample of 15ml 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 and negative selection using multiple cell markers.

The results during the isolation procedure are presented below:

Table of markers:

CD45 positive cells (Hematologic origin cells)		CD45 negative cells (non Hematologic origin)	
CD15	NEGATIVE	CD34	NEGATIVE
CD30	NEGATIVE	CD99	NEGATIVE
BCR-ABL	NEGATIVE	EpCam	Dim_POSITIVE
CD34	NEGATIVE	VHL mut	NEGATIVE
CD19	NEGATIVE	CD133	POSITIVE
		CD44	NEGATIVE
		Nanog	POSITIVE
		OKT-4	Dim_POSITIVE
		Sox-2	NEGATIVE
		PSMA	NEGATIVE
		c-MET	NEGATIVE
		CD31	NEGATIVE
		CD19	NEGATIVE
		MUC-1	POSITIVE
		CD63	NEGATIVE
		panCK	POSITIVE

Index of marker: CD45:Hematologic origin cell marker, **BCR-ABL, CD30**: hematologic malignancy marker, **CD133, Sox-2, OKT-4, Nanog, CD44**: tumor stem cell marker, **CD15**: hematological malignancy marker, **CD19 (CD45 negative cells – Non Hematologic origin cells)**: hematological malignancy, **CD19 (CD45 positive cells – Hematologic origin cells)**: lung neuroendocrine malignancy, **CD31**: endothelial cell membrane antigen, **CD34**: hematological stem cell and blast cell marker, epithelioid sarcoma marker, **CD63**: melanoma cell marker, **CD99**: sarcoma marker, **EpCam**: epithelial origin marker, **MUC-1**: Breast cancer antigen, **PSMA**: prostate specific cancer stem cell membrane antigen, **VHL mut**: renal carcinoma marker, **c-MET**: membrane antigen that regulates the mesenchymal to epithelial transition, **panCK**: epithelial origin cell marker.

The final results after the isolation procedure are presented below: We notice that after isolation procedure there are remaining malignant cells. The concentration of these cells was isolated 2cells/7.5ml, SD +/- 0.3cells.

Index of circulating cells number: (If Over limit: Advanced or Progression of Disease, If Less than limit: Early disease or disease is responding to a treatment plan).
Breast cancer: < 5cells /7.5ml , Prostate cancer < 20cells/ml , Sarcoma: <15cells/6.5ml, Colon cancer: <5cells/ml, Lung cancer (Lc=0, r=0.99): <10cell/ml. All cancer types other than those listed above should be <5 cells/ml.

Markers

Nanog
OKT4, Sox2
CD133 → Stemness (Resistance) markers

C-MET → Micro-metastases marker

PSMA,
PSA, → Prostate origin of CTCs

panCk
CD31
EpCam → Epithelial origin marker

CD63 → Melanoma marker

Vimentin
Synaptophysin → Mesenchymal and Endocrine markers

CD44 → Stemness related marker

MUC-1 → Breast carcinoma marker

VHL → Renal carcinoma marker

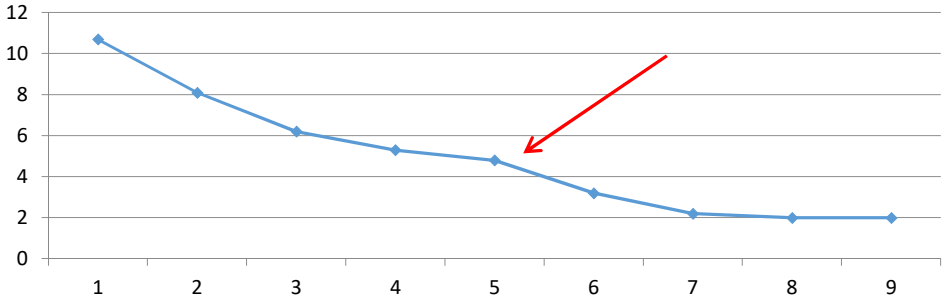
Phenotype (at the beginning)

CD45 NEGATIVE cells (Hematologic origin cells)		CD45 NEGATIVE cells (non Hematologic origin)	
CD15	NEGATIVE	CD34	NEGATIVE
CD30	NEGATIVE	CD99	NEGATIVE
BCR-ABL	NEGATIVE	EpCam	POSITIVE
CD34	NEGATIVE	VHL mut.	NEGATIVE
CD19	NEGATIVE	**CD133**	**POSITIVE**
		Nanog	**POSITIVE**
		Okt-4	**POSITIVE**
		Sox-2	**POSITIVE**
		PSMA	NEGATIVE
		c-MET	**POSITIVE**
		CD31	NEGATIVE
		CD19	NEGATIVE
		MUC-1	NEGATIVE
		CD44	NEGATIVE
		PAN-CK	POSITIVE

Phenotype (after 24 months)

CD45 NEGATIVE cells (Hematologic origin cells)		CD45 NEGATIVE cells (non Hematologic origin)	
CD15	NEGATIVE	CD34	NEGATIVE
CD30	NEGATIVE	CD99	NEGATIVE
BCR-ABL	NEGATIVE	EpCam	POSITIVE
CD34	NEGATIVE	VHL mut.	NEGATIVE
CD19	NEGATIVE	**CD133**	**POSITIVE**
		Nanog	**POSITIVE**
		Okt-4	**POSITIVE**
		Sox-2	**NEGATIVE**
		PSMA	NEGATIVE
		c-MET	**NEGATIVE**
		CD31	NEGATIVE
		CD19	NEGATIVE
		MUC-1	NEGATIVE
		CD44	NEGATIVE
		PAN-CK	POSITIVE

1 oncotrail (3m)	**8,1.cell/ml**	5 oncotrail (15m)	**3,2cell/ml**
2 oncocount (6m)	**6,2 cell/ml**	6 oncocount (18m)	**2,2cell/ml**
3 oncotrail (9m)	**5,3cell/ml**	7 oncotrail (21m)	**2cell/ml**
4 oncocount (12m)	**4,8cell/ml**	8 oncocount (24m)	**2cell/ml**



Chemosensitivity Testing

CTC Testing: Chemosensitivity Testing

- **Used for patients with present macroscopic disease**

- **True Personalised Oncology Assessment**
  - to determine the epigenetic activity of each patient's cancer
    (fast/slow growing, resistant, metastatic, angiogenesis, immortal)
  - to determine the most efficacious agents for each patient's cancer
    "One size doesn't fit all"
  - Getting it right the First Time; the patient may not have a 2nd opportunity

- **Agents able to be assessed**
  Cytotoxic/Cytostatic agents
  Immune regulators
  Growth Factor inhibitors
  Natural Substances

Patient Performance Status

- We must firstly assess is the patient in suitable health to undertake this approach
- ECOG is an Internationally used staging guide.
- Score 0 – 2 Good

  Patient able to tolerate cytotoxic treatments
- Score 3+ Poor

  Patient would deteriorate with cytotoxic treatments
  Only consider Natural substances in this situation

ECOG Performance Status

These scales and criteria are used by doctors and researchers to assess how a patient's disease is progressing, assess how the disease affects the daily living abilities of the patient, and determine appropriate treatment and prognosis. They are included here for health care professionals to access.

ECOG PERFORMANCE STATUS*

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

* As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

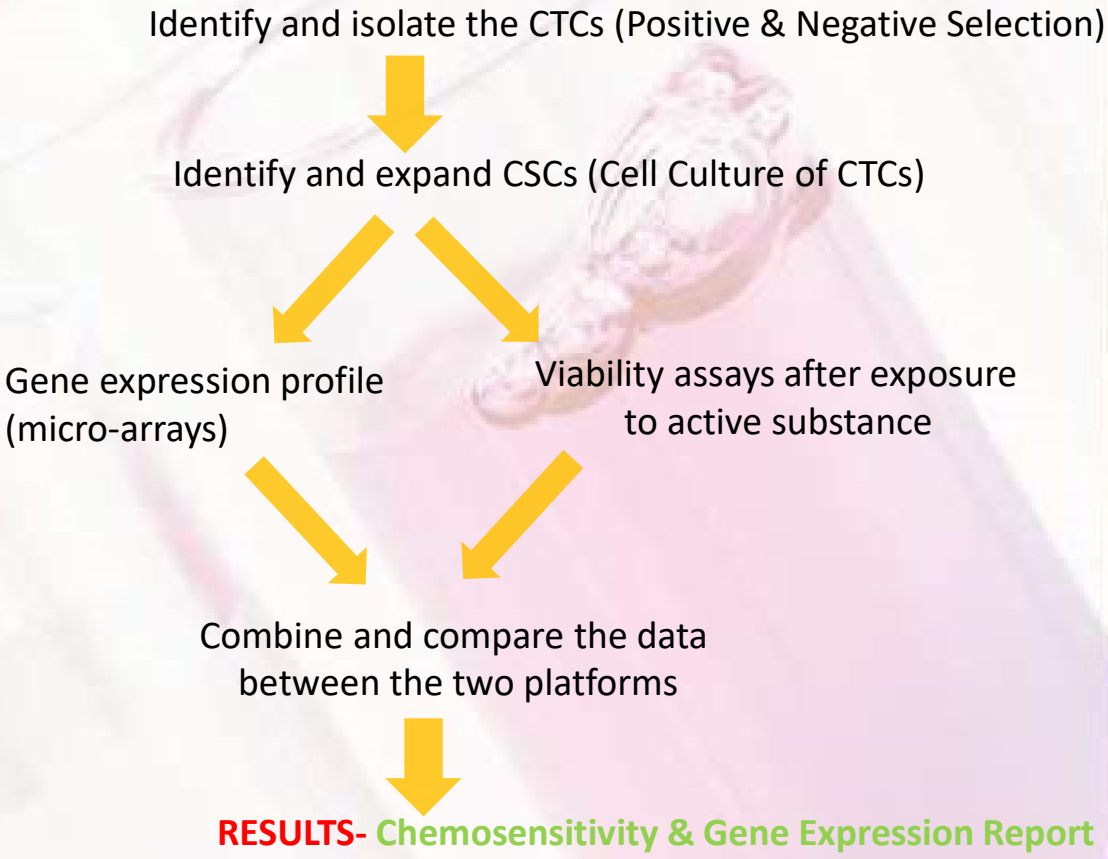
Steps of the process

- Use a dual Platform
  - Gene Expression Profile
  - Cytotoxicity/Viability study

- The Gene Expression profile when compared to the Protein Expression rate is not always a linear relationship
(influenced by post transcription processes etc)

- For a drug to have an effect it needs to reach the intracellular area (membrane permeability).

- In order to confirm the information that the Gene Expression micro-array generates, the cancer cells are exposed to the active substance of each drug and the cytotoxicity/viability is explored.
Assays are performed in triplicate.



Chemosensitivity Testing

- Gene Expression Profile -

Organizing the Gene Expression profile

- Related with cell cycle regulation: p53,p21, p16, DHFR, TS, SHMT
- Related with drug targets: Topo I and II, TS, DHFR, ribonucleotide reductase etc
- Related with signal transduction pathway: IGFr, EGF-r, PDGF-r etc
- Related with Epigenetic aberration: Dnmt1, DNA demethylase etc
- Related with Angiogenesis: VEGF-r, FGF-r, PDGF-r
- Related with growth signal: c-erb-B1, c-erb-B2, bcr-abl etc
- Related with repair after physical application (Radiation, hyperthermia): HSP 27, HSP70, HSP 90, HIF1a etc

Why are we focusing on these markers?

- The markers are meant to answer clinical questions.
  1. Is the cancer fast or slow growing?
  2. Is the cancer resistant in phenotype?
  3. Has the cancer a high rate of metastases?
  4. Is the cancer sensitive to radiotherapy/hyperthermia/ablation?

Why are we focusing on these markers?

- The markers are meant to answer clinical questions.
  1. Is the cancer fast or slow growing?
  - **P27**: Increased level means slow growing tumor
  - **P21**: Increased level means slow growing tumor
  - **CDK4/CDK6**: Increased level means fast growing tumor

Why are we focusing on these markers?

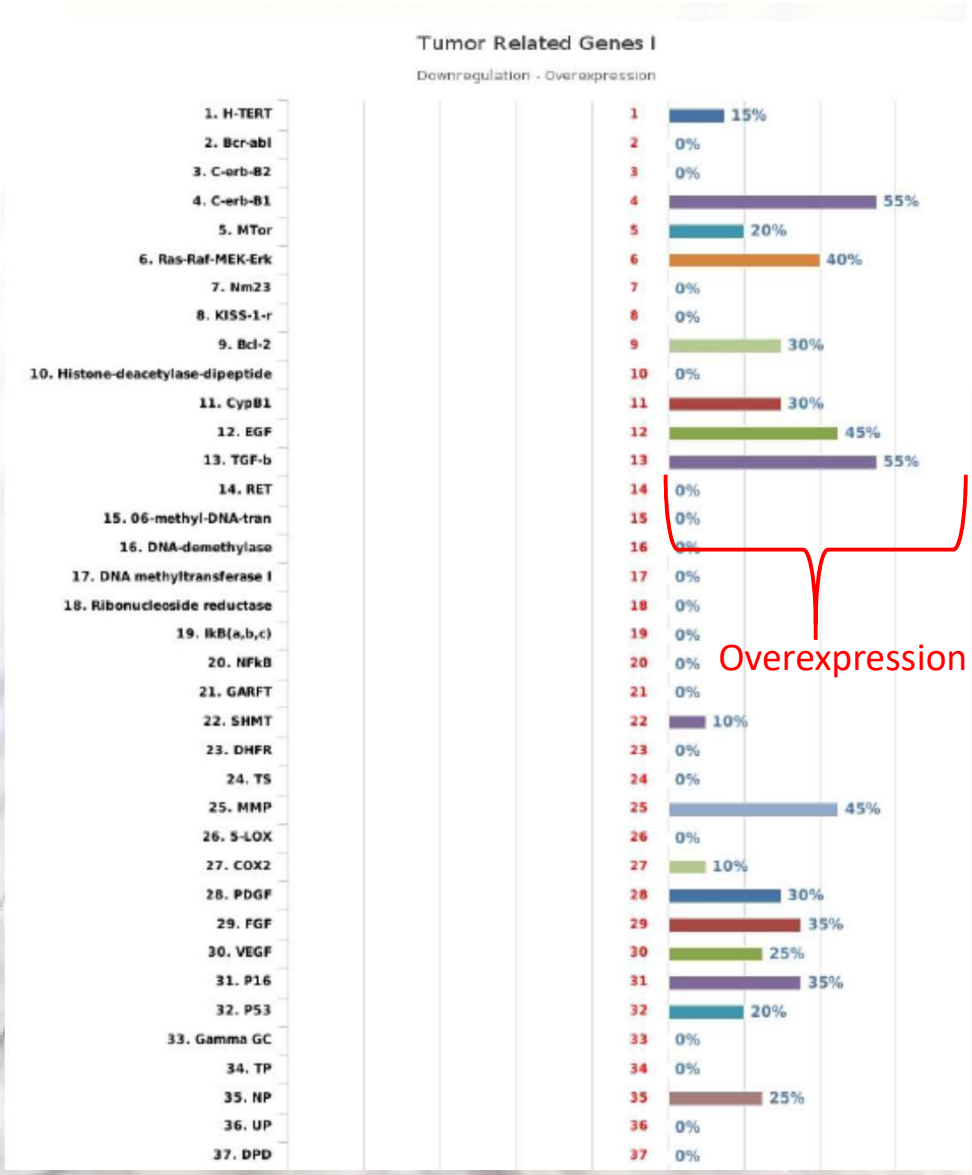
- The markers are meant to answer clinical questions.
  1. Is the cancer fast or slow growing?
  2. Is the cancer resistant in phenotype?
  - **MDR1, MRP**: Increased level means resistance to many chemical drugs and natural substances.
  - **HAT, DNMT1**: Increased level means resistant phenotype

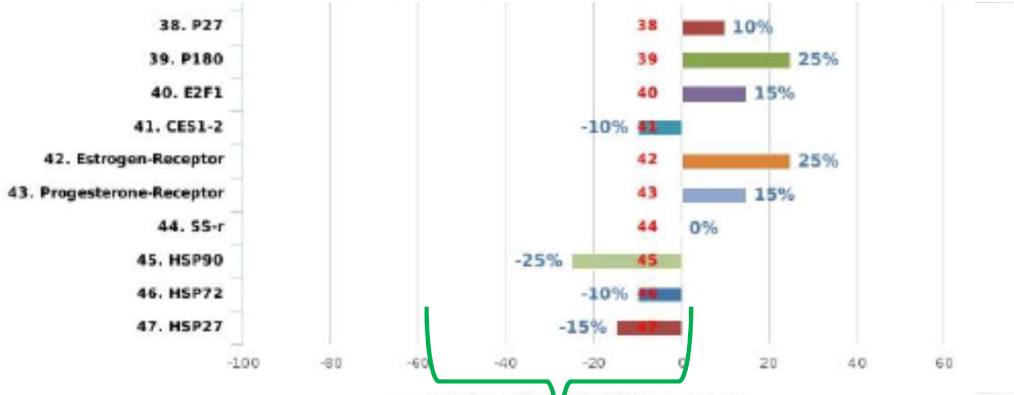
Why are we focusing on these markers?

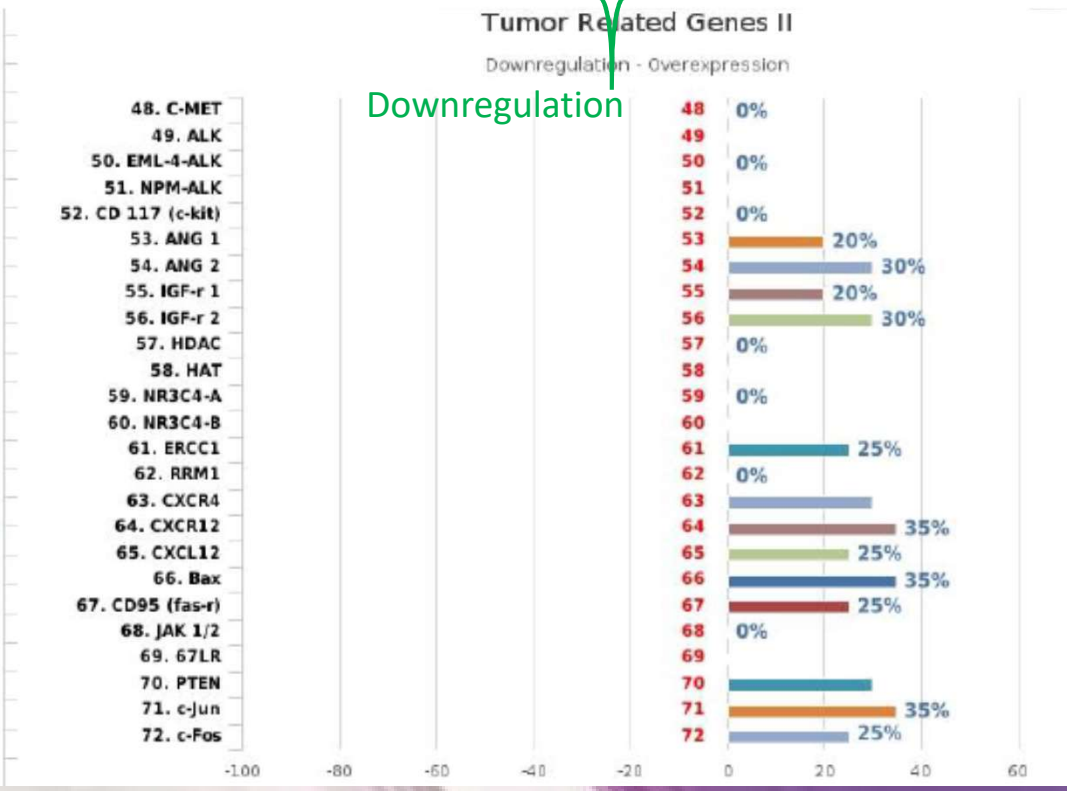
- The markers are meant to answer clinical questions.
  1. Is the cancer fast or slow growing?
  2. Is the cancer resistant in phenotype?
  3. Has the cancer a high risk of metastases?
  - **C-MET:** Increased level means high metastastatic risk
  - **KISS-1, Nm23:** Lowered level means high risk of metatases
  - **MMPs:** Increased level means increase risk of metastases

Why are we focusing on these markers?

- The markers are meant to answer clinical questions.
  1. Is the cancer fast or slow growing?
  2. Is the cancer resistant in phenotype?
  3. Has the cancer a high rate of metastases?
  4. Is the cancer sensitive to radiotherapy/hyperthermia/ablation?
     - **HSP's:** Lower rate is related with sensitivity to radiation/ablation/hyperthermia.


Tumor Related Genes I
Downregulation - Overexpression
1. H-TERT 15%
2. Bcr-abl 0%
3. C-erb-B2 0%
4. C-erb-B1 55%
5. MTor 20%
6. Ras-Raf-MEK-Erk 40%
7. Nm23 0%
8. KISS-1-r 0%
9. Bcl-2 30%
10. Histone-deacetylase-dipeptide 0%
11. CypB1 30%
12. EGF 45%
13. TGF-b 55%
14. RET 0%
15. 06-methyl-DNA-tran 0%
16. DNA-domethylase 0%
17. DNA methyltransferase I 0%
18. Ribonucleoside reductase 0%
19. IkB(a,b,c) 0%
20. NFkB 0%
21. GARFT 0%
22. SHMT 10%
23. DHFR 0%
24. TS 0%
25. MMP 45%
26. 5-LOX 0%
27. COX2 10%
28. PDGF 30%
29. FGF 35%
30. VEGF 25%
31. P16 35%
32. P53 20%
33. Gamma GC 0%
34. TP 0%
35. NP 25%
36. UP 0%
37. DPD 0%
Overexpression


38. P27 10%
39. P180 25%
40. E2F1 15%
41. CES1-2 -10%
42. Estrogen-Receptor 25%
43. Progesterone-Receptor 15%
44. SS-r 0%
45. HSP90 -25%
46. HSP72 -10%
47. HSP27 -15%
-100 -80 -60 -40 -20 0 20 40 60


Tumor Related Genes II
Downregulation - Overexpression
48. C-MET 0%
49. ALK
50. EML-4-ALK 0%
51. NPM-ALK
52. CD 117 (c-kit) 0%
53. ANG 1 20%
54. ANG 2 30%
55. IGF-r 1 20%
56. IGF-r 2 30%
57. HDAC 0%
58. HAT
59. NR3C4-A 0%
60. NR3C4-B
61. ERCC1 25%
62. RRM1 0%
63. CXCR4
64. CXCR12 35%
65. CXCL12 25%
66. Bax 35%
67. CD95 (fas-r) 25%
68. JAK 1/2 0%
69. 67LR
70. PTEN
71. c-Jun 35%
72. c-Fos 25%
-100 -80 -60 -40 -20 0 20 40 60
Downregulation

Presentation of the results

CELL CYCLE REGULATION & IMMORTALIZATION / APOPTOSIS

NAME	RELATED	RESULTS	OUTCOME	FUNCTION	CLINICAL RISK
E2F1	Transcr. Fact of TS & topo I	15%	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	30%	HIGH RISK	Regulation of apoptosis	HIGH RISK
Bax	Apoptosis	35%	HIGH RISK		
CD95 (fas-r)	Apoptosis related receptor	25%	HIGH RISK		
p27	Cell arrest (G0)	10%	LOW RISK	Cell cycle Rate	RAPID
p53	Cell cycle regulator	20%	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	45%	HIGH RISK		

GROWTH FACTORS PROLIFERATION STIMULI

NAME	RELATED	RESULTS	OUTCOME	FUNCTION	CLINICAL RISK
p180	Tyrosin kinase growth f.	25%	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	10%	HIGH RISK	Eicosanoid related protein	HIGH RISK
5-LOX	Tumour Growth	normal	LOW RISK		
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		

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	30%	HIGH RISK		

Chemosensitivity Testing

- Viability (Cytotoxicity) Profile -

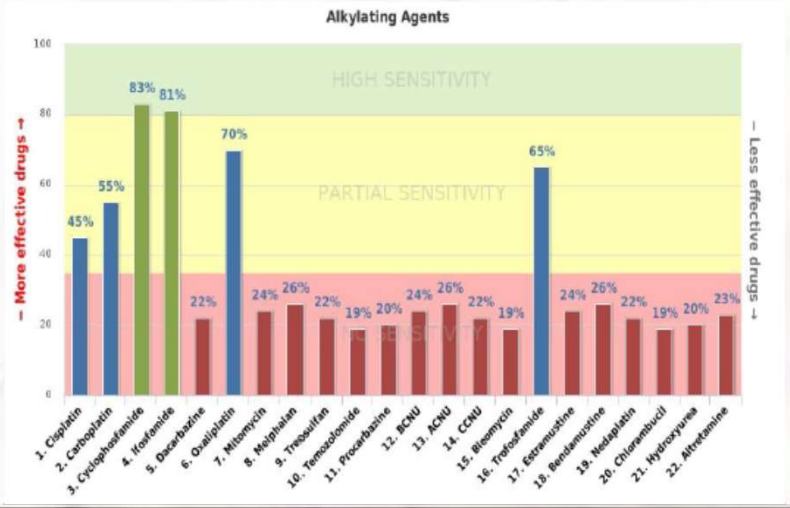
Organising the Viability assays

- The results need to be:
  - Scientifically based and complete
  - Easy to read
  - Easy to understand for a non expert
  - Easy be to interpreted
  - Easy to combine the information

Patient CTCs are cell cultured and thereafter exposed to the active form of a drug to confirm the viability of the cells.

The viability diagram represent the percentage sensitivity of the viable cells to each agent, as compared with the primary unexposed population.

The results table is presented as follows:



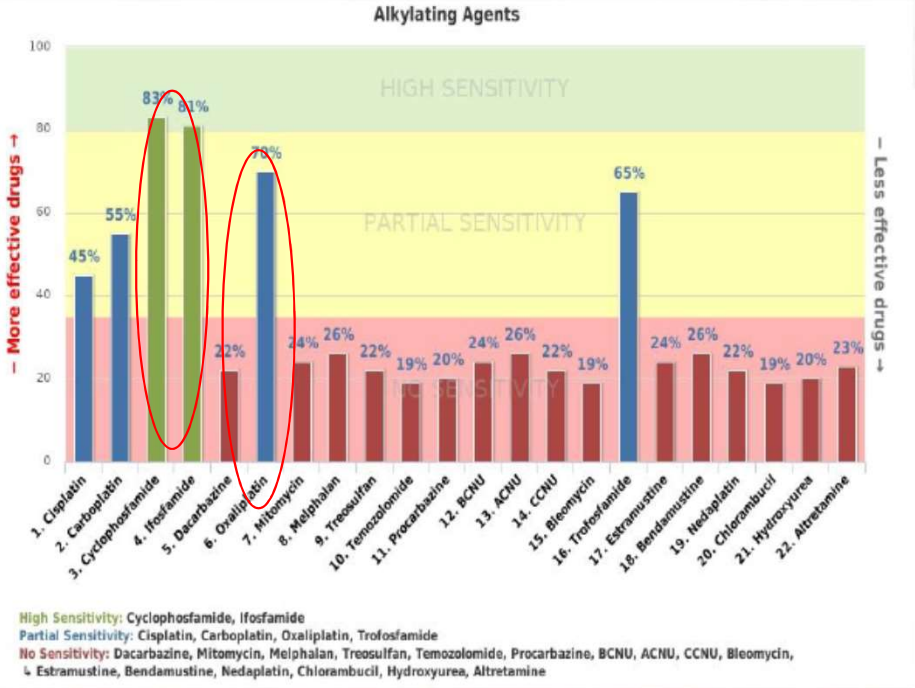
Cut off point:

Over 80% cytotoxicity : Sensitivity to the exposed drug (SENSITIVITY)

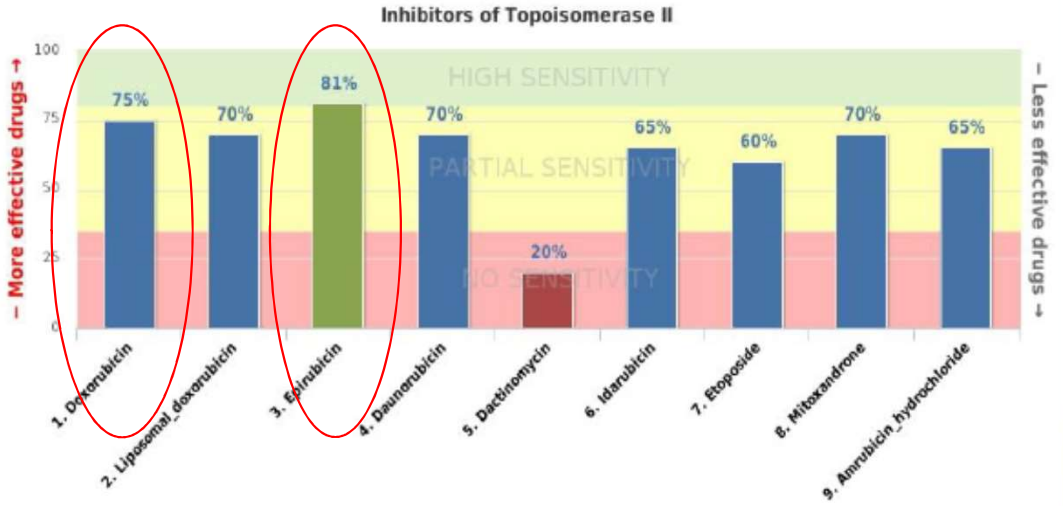
Between 35% to 80%: Moderate sensitivity (PARTIAL SERNSITIVITY)

Below 35%: The substance in ineffective (RESISTANCE)

Example of Chemosensitivity Testing for conventional cytotoxic agents

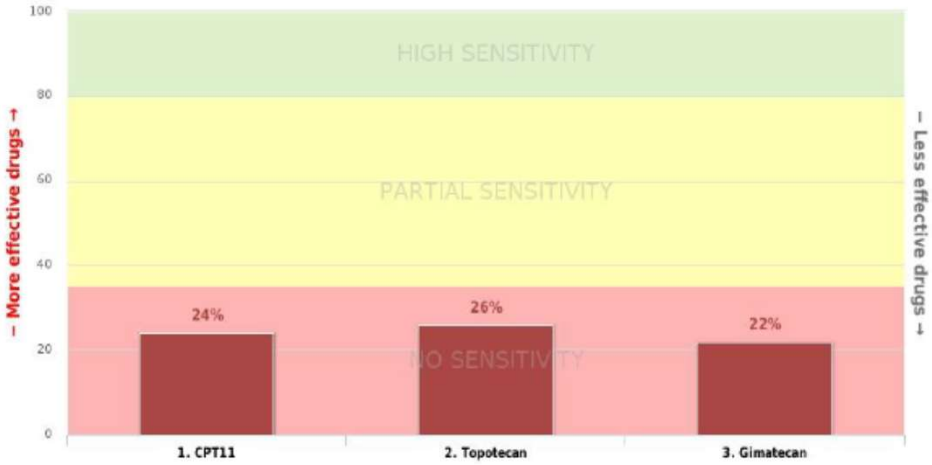


High Sensitivity: Cyclophosfamide, Ifosfamide
Partial Sensitivity: Cisplatin, Carboplatin, Oxaliplatin, Trofosfamide
No Sensitivity: Dacarbazine, Mitomycin, Melphalan, Treosulfan, Temozolomide, Procarbazine, BCNU, ACNU, CCNU, Bleomycin, Estramustine, Bendamustine, Nedaplatin, Chlorambucil, Hydroxyurea,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



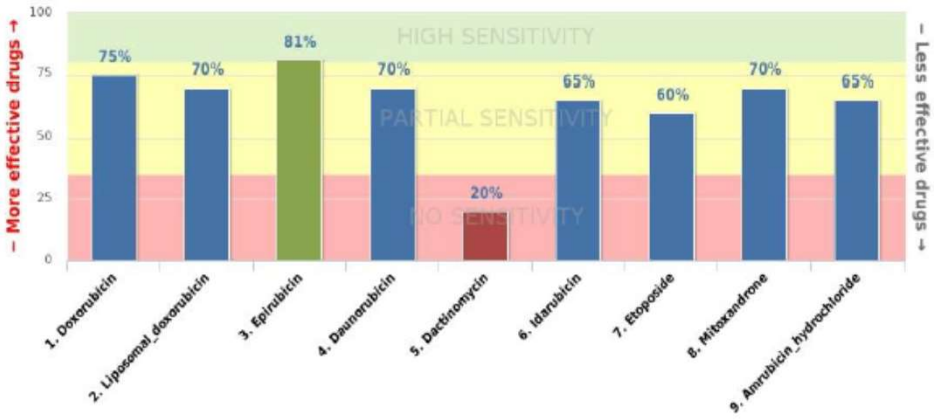
High Sensitivity: Epirubicin
Partial Sensitivity: Doxorubicin, Liposomal_doxorubicin, Daunorubicin, Idarubicin, Etoposide, Mitoxandrone, Amrubicin_hydrochloride
No Sensitivity: Dactinomycin

Inhibitors of Topoisomerase I



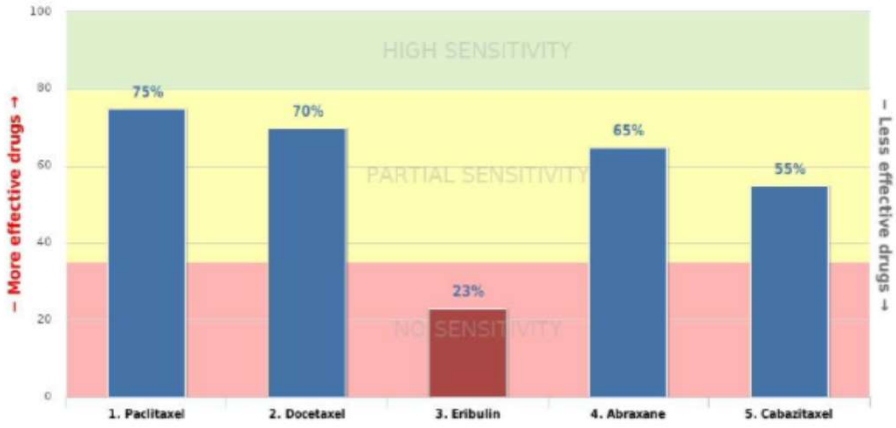
No Sensitivity: CPT11, Topotecan, Gimatecan

Inhibitors of Topoisomerase II



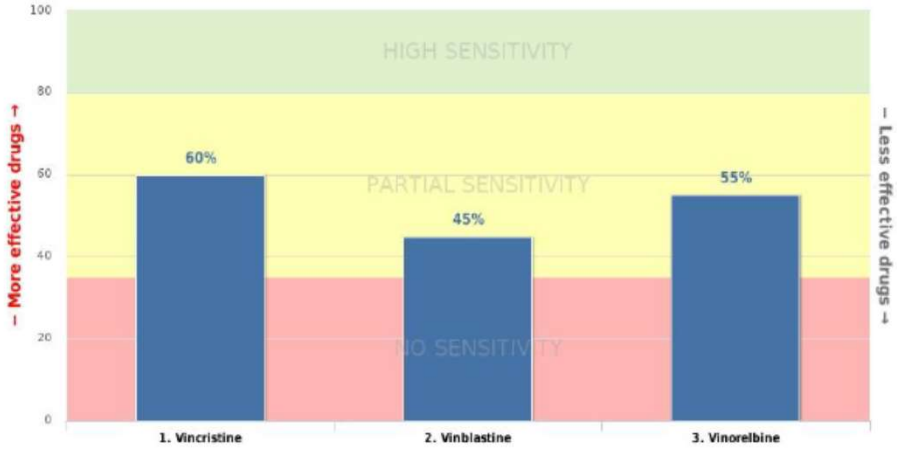
High Sensitivity: Epirubicin
Partial Sensitivity: Doxorubicin, Liposomal_doxorubicin, Daunorubicin, Idarubicin, Etoposide, Mitoxandrone, Amrubicin_hydrochloride
No Sensitivity: Dactinomycin

Nucleus Spindle Stabilizer I

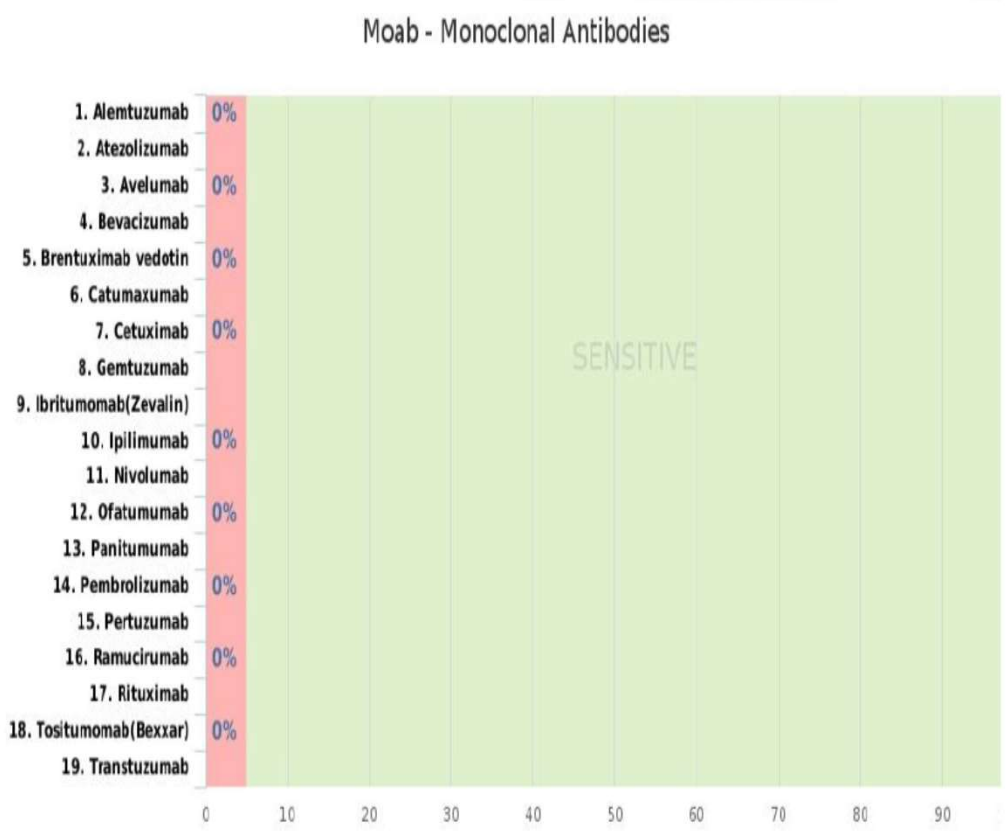


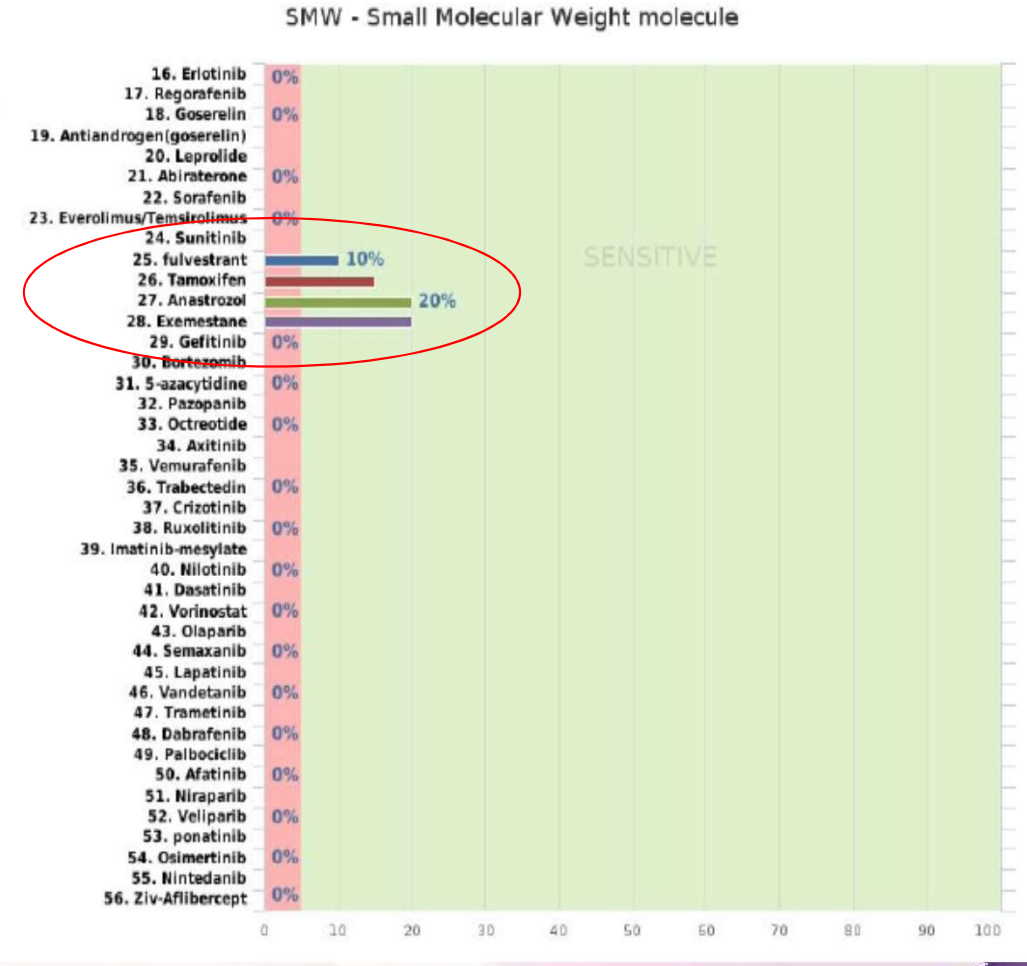
Partial Sensitivity: Paclitaxel, Docetaxel, Abraxane, Cabazitaxel
No Sensitivity: Eribulin

Nucleus Spindle Stabilizer II



Partial Sensitivity: Vincristine, Vinblastine, Vinorelbine


Moab - Monoclonal Antibodies
1. Alemtuzumab 0%
2. Atezolizumab
3. Avelumab 0%
4. Bevacizumab
5. Brentuximab vedotin 0%
6. Catumaxumab
7. Cetuximab 0%
8. Gemtuzumab
9. Ibritumomab(Zevalin)
10. Ipilimumab 0%
11. Nivolumab
12. Ofatumumab 0%
13. Panitumumab
14. Pembrolizumab 0%
15. Pertuzumab
16. Ramucirumab 0%
17. Rituximab
18. Tositumomab(Bexxar) 0%
19. Transtuzumab
SENSITIVE
0 10 20 30 40 50 60 70 80 90


SMW - Small Molecular Weight molecule
16. Erlotinib 0%
17. Regorafenib
18. Goserelin 0%
19. Antiandrogen(goserelin)
20. Leprolide
21. Abiraterone 0%
22. Sorafenib
23. Everolimus/Temsirolimus 0%
24. Sunitinib
25. fulvestrant 10%
26. Tamoxifen
27. Anastrozol 20%
28. Exemestane
29. Gefitinib 0%
30. Bortezomib
31. 5-azacytidine 0%
32. Pazopanib
33. Octreotide 0%
34. Axitinib
35. Vemurafenib
36. Trabectedin 0%
37. Crizotinib
38. Ruxolitinib 0%
39. Imatinib-mesylate
40. Nilotinib 0%
41. Dasatinib
42. Vorinostat 0%
43. Olaparib
44. Semaxanib 0%
45. Lapatinib
46. Vandetanib 0%
47. Trametinib
48. Dabrafenib 0%
49. Palbociclib
50. Afatinib 0%
51. Niraparib
52. Veliparib 0%
53. ponatinib
54. Osimertinib 0%
55. Nintedanib
56. Ziv-Aflibercept 0%
SENSITIVE
0 10 20 30 40 50 60 70 80 90 100

Chemosensitivity Testing

- Natural/Biological Substances -

Chemosensitivity testing – Natural Substances

Natural-biological substances

1. All natural extracts from plants or cells which may have direct or indirect therapeutic (anticancer) activity.
2. The majority of natural substances have an unknown mechanism of action or they have multiple interference in many point and pathways.

Class I
(Direct cytotoxic effect) 28

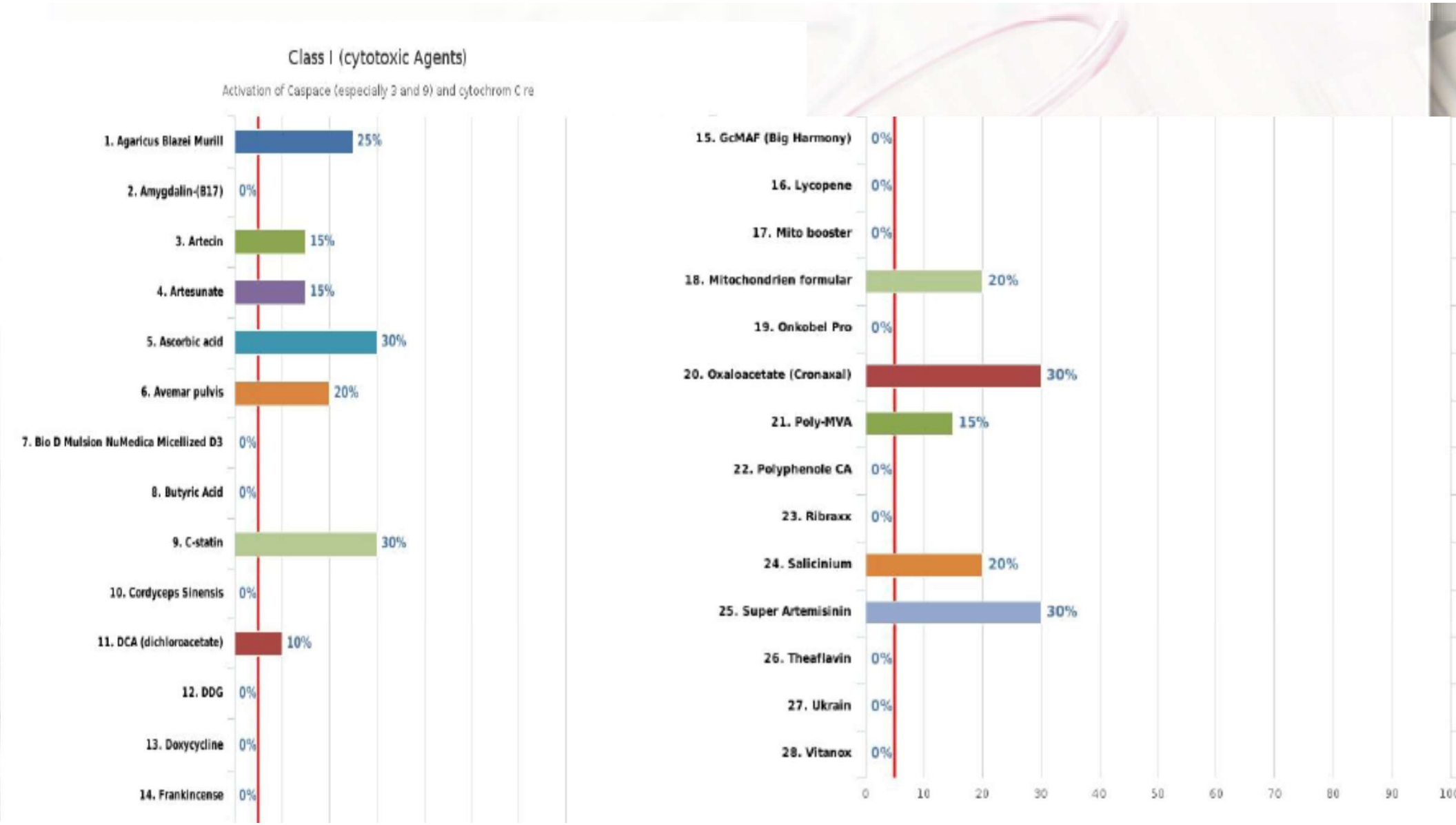
1. Ascorbate
2. Artemisia derivatives
3. Dideoxy-D-Glucose
4. DCA
5. Oxaloacetate

Class II
(Immunomodulatory effect) 6

1. Fucoidan
2. Mistletoe extracts (lectines)
3. GcMAF
4. Boswellia Serrata

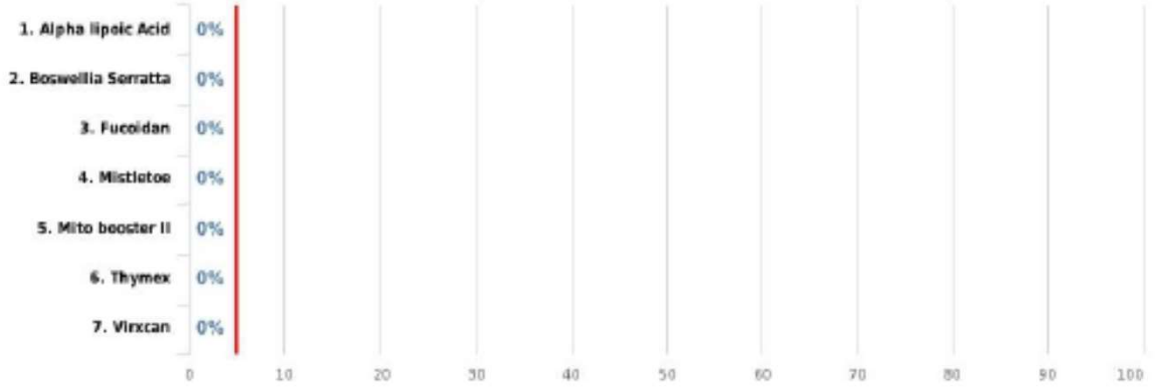
Class III
(Inhibitory effect on kinases GFs) 16

1. Quercetin
2. Genistein
3. Apigenin
4. Isoflavones
5. Resveratrol


Class I (cytotoxic Agents)
Activation of Caspace (especially 3 and 9) and cytochrom C re
1. Agaricus Blazei Murill 25%
2. Amygdalin-(B17) 0%
3. Artecin 15%
4. Artesunate 15%
5. Ascorbic acid 30%
6. Avemar pulvis 20%
7. Bio D Mulsion NuMedica Micellized D3 0%
8. Butyric Acid 0%
9. C-statin 30%
10. Cordyceps Sinensis 0%
11. DCA (dichloroacetate) 10%
12. DDG 0%
13. Doxycycline 0%
14. Frankincense 0%
15. GcMAF (Big Harmony) 0%
16. Lycopene 0%
17. Mito booster 0%
18. Mitochondrien formular 20%
19. Onkobel Pro 0%
20. Oxaloacetate (Cronaxal) 30%
21. Poly-MVA 15%
22. Polyphenole CA 0%
23. Ribraxx 0%
24. Salicinium 20%
25. Super Artemisinin 30%
26. Theaflavin 0%
27. Ukrain 0%
28. Vitanox 0%
0 10 20 30 40 50 60 70 80 90 100

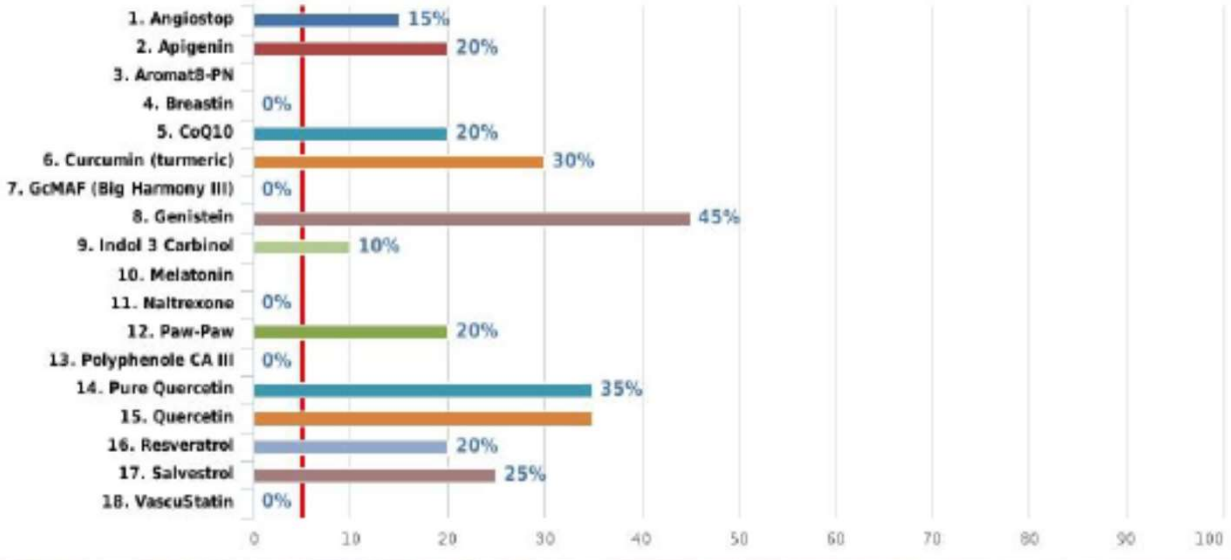
Class II (Immunostimulants/immunomodulators)

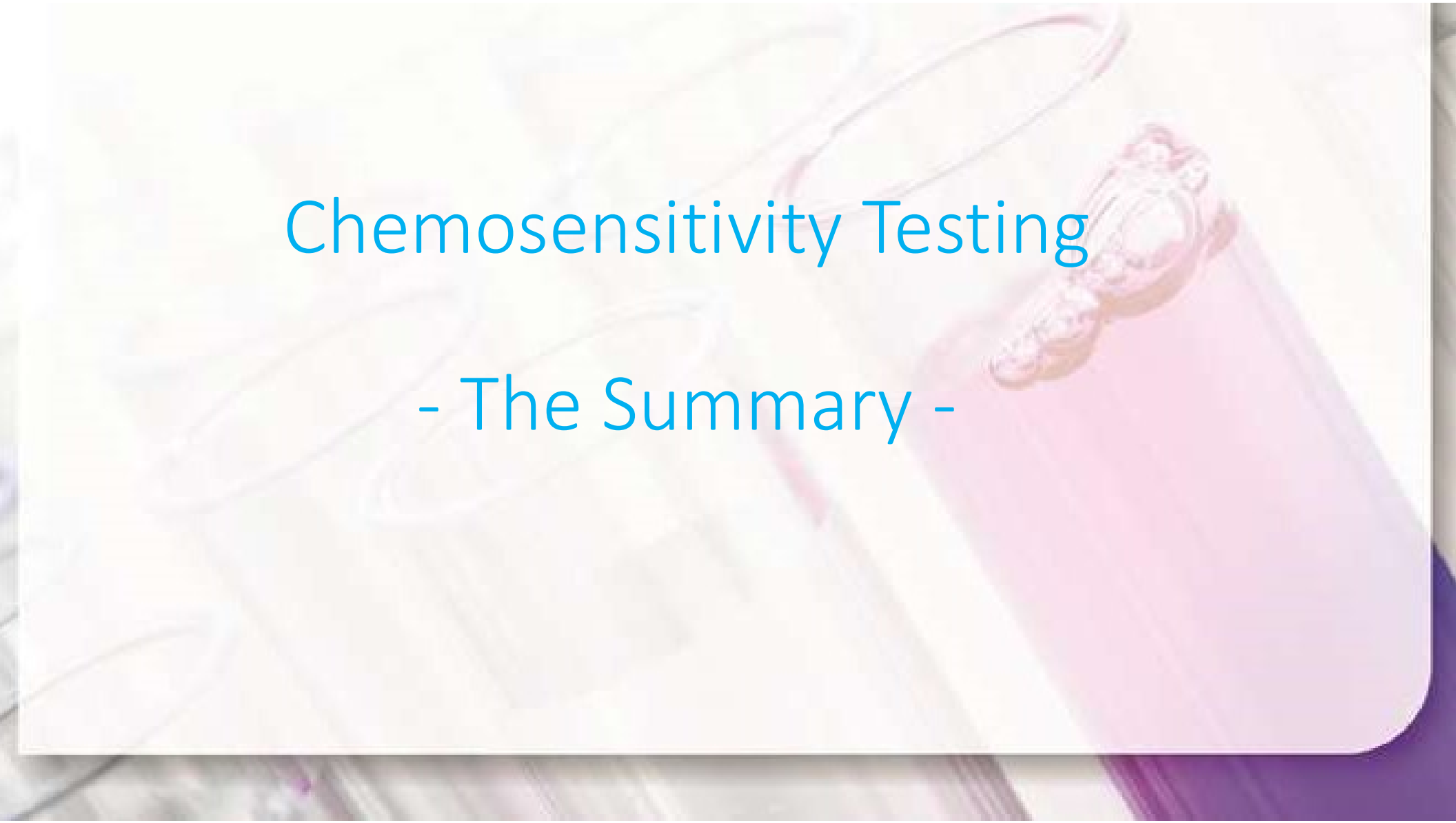
Immunostimulants / Immunomodulators release of Cytokins and Increase of PBMC & NK



Class III (PK inhibitors)

Inhibitors of growth factors receptor inhibitors of EGFr,IGFr,VEGFr,PDGFr, FGFr, signal transduction pathways





Chemosensitivity Testing

- The Summary -

Assessment of the results:

Patient Name:	**Type of cancer:** breast
Physician: Dr [redacted]	**Stage:** II

Risk of relapse:
CTC concentration
Measured: **isolated 4.2cells/7.5ml, SD +/- 0.3cells**
Cut off point <= 5cells/7.5ml

Resistance markers:
MDR1: 55%
MRP: 60%
LRP: 2%
GST: 20%

Metastases/angiogenesis risk related markers

FUNCTION	CLINICAL RISK	MARKERS	RESULTS	OUTCOME
Migration-invasion	HIGH RISK	MMPs	45%	HIGH RISK
		KISS-1-r	normal	LOW RISK
		Nm23	normal	LOW RISK
Angiogenesis	HIGH RISK	VEGFr	25%	HIGH RISK
		FGFr	35%	HIGH RISK
		PDGFr	30%	HIGH RISK

Proliferation related markers:

MECHANISM	CLINICAL RISK	MARKERS	RESULTS	OUTCOME
Signal transduction pathways	HIGH PROLIFERATIVE SIGNAL	Ras/raf/MEK/Erk1-2	40%	HIGH RISK
		mTOR	20%	HIGH RISK
Growth factor receptors	HIGH PROLIFERATIVE SIGNAL	EGFr	45%	HIGH RISK
		TGF-β1/2	55%	HIGH RISK
		c-erb-B2	normal	LOW RISK
Hormone receptors	HORMONE INDEPENDENT	Estrogen Receptor	25%	HIGH RISK
		Progesterone Receptor	15%	HIGH RISK
		NC3R4-A	normal	LOW RISK
		NC3R4-B	normal	LOW RISK
Cell cycle rate	RAPID	P27	10%	LOW RISK
		P16	35%	HIGH RISK
		P53	20%	HIGH RISK

Resistance phenotype markers:

MARKERS	RESULTS	OUTCOME	PHENOTYPE
Dnmt1	normal	LOW RISK	NON RESISTANT
06-methyl-DNA-tran.	normal	LOW RISK	
HAT	normal	LOW RISK	
Histone deacetylase	normal	LOW RISK	

Therapeutic options

Conventional cytostatics:

Non cell cycle depended	S phase of cell cycle				Metaphases
Alkylating agents	*Inhibitors of topoisomerase I*	*Inhibitors of topoisomerase II*	*antimetabolites*	*Inhibitors of tubulin polymerization*	*Spindle poisoning agents*
Cyclophosfamide Ifosfamide		Epirubicin	Capecitabine		

Targeted therapies

Moab (Monoclonal Antibodies)	SMW (Small Molecular Weight molecule)
	Fulvestrant as inhibitor of estrogen positive proliferative signal. **Tamoxifen** as inhibitor of estrogen positive feedback. **Anastrozol** as inhibitor of estrogen synthesis. **Exemestane** as inhibitor of aromatase enzyme.

Biological/natural substances:

Class I (cytotoxic agents)	Class II (immuno-modulatory effect)	Class III (growth factors inhibitors)
Agaricus Blazei Murill Artecin Artesunate Ascorbic acid Avemar pulvis C-statin DCA (dichloroacetate) Mitochondrien formular Oxaloacetate (Cronaxal) Poly-MVA Salicinium Super Artemisinin		Angiostop Apigenin CoQ10 Curcumin (turmeric) Genistein Indol 3 Carbinol Paw-Paw Pure Quercetin Quercetin Resveratrol Salvestrol

It is recommended to use in a monthly base one agent from each class and then switch them after a month with the next potent agent from the same class in order to avoid secondary resistance.

Radiotherapy/Hyperthermia sensitivity:

Marker	Result (%)	Clinical outcome per marker	Clinical outcome
HSP90	-25%	SENSITIVE	SENSITIVE
HSP72	-10%	SENSITIVE	
HSP27	-15%	SENSITIVE	

Follow-up options:

YES	✓
NO	

Time interval (when)

After 3 months	After 6 months	After 12 months
✓		

Test for follow-up

ONCOTRAILS							ONCOTRACE	ONCOCOUNT
Breast	Lung	Sarcoma	Colon	GI	Prostate	melanoma		
✓								

Incorporating the Pharmacokinetics

- **Chemosensitivity Assays** **Pharmacodynamics** **"Which cytostatic agents have the best effect"**
- **Detoxification Genomics** **Pharmacokinetics** **"How well the body utilizes the cytostatic agents"**
- Looks at each individual's ability to activate and metabolize each therapeutic agent to its effective form in a normal rate.
- **Thereafter apply these genomic results to each category of cytotoxic agent**
    - **Alkylating Agents**
    - **Topoisomerase I Inhibitors**
    - **Topoisomerase II Inhibitors**
    - **Antimetabolites**
    - **Spindle Poisons**

Basic-Phase I

Polymorphism	Outcome
CYP2D6*2	Normal Metabolizer
CYP2D6*3A	Poor Metabolizer
CYP2D6*3B	Possible Poor Metabolizer
CYP2D6*6	Poor Metabolizer
CYP2D6*9	Normal Metabolizer
CYP2D6*10	Poor Metabolizer
CYP2C19*2	Normal Metabolizer
CYP2C19*3	Normal Metabolizer
CYP2C19*17	Ultra-Fast Metabolizer
CYP1A2*1F	Normal Metabolizer
CYP1A2*1K	Normal Metabolizer
CYP2C9*2	Normal Metabolizer
CYP2C9*3	Normal Metabolizer
CYP3A4*1B	Poor Metabolizer
CYP3A4*20	Normal Metabolizer
CYP1B1	Possible Normal Metabolizer

Basic-Phase II

Polymorphism	Outcome
GSTP1*Ala114Val	Possible Normal Metabolizer
GSTP1*Ile105Val	Possible Normal Metabolizer
EPHX1* His139Arg	Possible Normal Metabolizer
EPHX1*Tyr113His	Possible Normal Metabolizer
NAT2*5	Possible Slow Metabolizer
NAT2*6	Normal Metabolizer
NAT2*7	Normal Metabolizer
NAT2*14	Normal Metabolizer
NAT2*11A	Possible Normal Metabolizer
TPMT*4A	Normal Metabolizer
TPMT*2	Normal Metabolizer
ABCB1*Ile1145Ile	Possible Normal Metabolizer
ABCB1*Ser893Ala	Possible Normal Metabolizer
ABCG2*Gln141Lys	Normal Metabolizer

Classification of patients

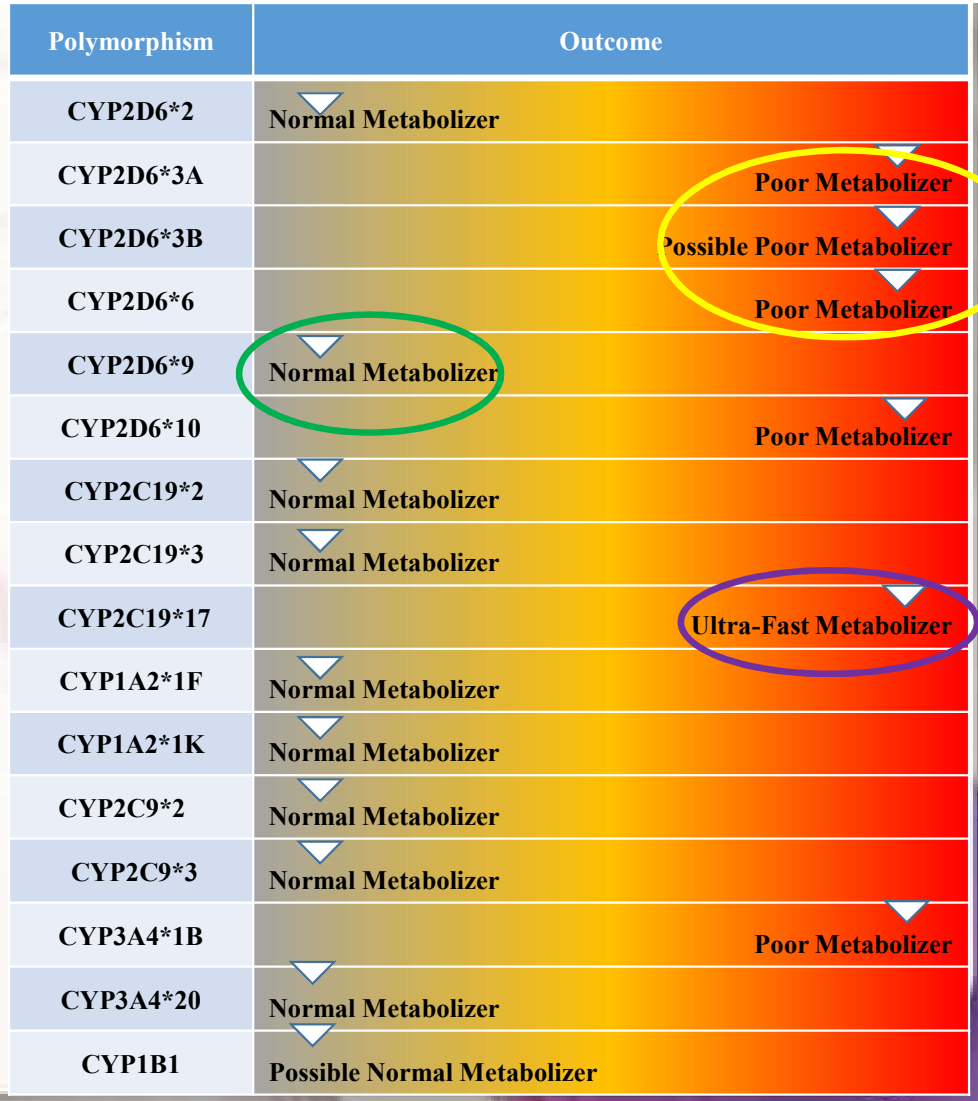
Normal Metabolizers

Rapid Metabolizers

Accumulators (Slow or Non-Metabolizers)

With this information, we will AVOID using agents where the literature indicates adverse events due to polymorphisms of

NO ACTIVATION,
SLOW/POOR METABOLISM or
RAPID METABOLISM



Therapeutic options

Conventional cytostatics:

Non cell cycle depended	S phase of cell cycle				Metaphases
Alkylating agents	*Inhibitors of topoisomerase I*	*Inhibitors of topoisomerase II*	*antimetabolites*	*Inhibitors of tubulin polymerization*	*Spindle poisoning agents*
Cyclophosfamide Ifosfamide		Epirubicin	Capecitabine		

Targeted therapies

Moab (Monoclonal Antibodies)	SMW (Small Molecular Weight molecule)
	Fulvestrant as inhibitor of estrogen positive proliferative signal. **Tamoxifen** as inhibitor of estrogen positive feedback. **Anastrozol** as inhibitor of estrogen synthesis. **Exemestane** as inhibitor of aromatase enzyme.

Biological/natural substances:

Class I (cytotoxic agents)	Class II (immuno-modulatory effect)	Class III (growth factors inhibitors)
Agaricus Blazei Murill Artecin Artesunate Ascorbic acid Avemar pulvis C-statin DCA (dichloroacetate) Mitochondrien formular Oxaloacetate (Cronaxal) Poly-MVA Salicinium Super Artemisinin		Angiostop Apigenin CoQ10 Curcumin (turmeric) Genistein Indol 3 Carbinol Paw-Paw Pure Quercetin Quercetin Resveratrol Salvestrol

It is recommended to use in a monthly base one agent from each class and then switch them after a month with the next potent agent from the same class in order to avoid secondary resistance.

Radiotherapy/Hyperthermia sensitivity:

Marker	Result (%)	Clinical outcome per marker	Clinical outcome
HSP90	-25%	SENSITIVE	SENSITIVE
HSP72	-10%	SENSITIVE	
HSP27	-15%	SENSITIVE	

Follow-up options:

YES	✓
NO	

Time interval (when)

After 3 months	After 6 months	After 12 months
✓		

Test for follow-up

ONCOTRAILS							ONCOTRACE	ONCOCOUNT
Breast	Lung	Sarcoma	Colon	GI	Prostate	melanoma		
✓								

ALKYLATING AGENTS

Drug	Polymorphism	Outcome
Cisplatin	ERCC1*Asn118Asn	Increased likelihood of nephrotoxicity
	LRP2*Lys4094Glu	Increased risk of Ototoxicity
	ERCC1*Gln504Lys	Increased likelihood of nephrotoxicity
	COMT*19955692C>T	Decreased risk of Deafness
Cyclopshosphamide	TP53*Pro72Arg	Decreased likelihood of Drug Toxicity (cyclophosphamide and fluorouracil)
	GSTP1*Ile105Val	Increased response to cyclophosphamide, epirubicin and fluorouracil
	GSTP1*Ile105Val	Decreased severity of toxicity (cyclophosphamide-epirubicin)
	NOS3*Asp298Glu	Decreased disease free survival (cyclophosphamide, doxorubicin, fluorouracil, methotrexate)
	MTHFR*Ala222Val	Increased likelihood of Drug Toxicity (cyclophosphamid-fluorouracil)
	ABCB1*Ser893Ala	Increased survival (cyclophosphamide-doxorubicin)
	ALDH3A1*Pro329Ala	Increased likelihood of Cystitis (carboplatin, cyclophosphamide,thiotepa)
	CYP3A4*1B	Increased disease free survival (cyclophosphamide, doxorubicin ,fluorouracil)
	CYP2B6*Gln172His	Decreased likelihood of dose reduction (cyclophosphamide-doxorubicin)
	SOD2*Val16Ala	Increased survival
	CYP2B6*Arg22Cys	Decreased likelihood of dose delay (cyclophosphamide,doxorubicin)
	CYP2B6*Arg22Cys	Increased time to progression (cyclophosphamide,doxorubicin)
	ABCC4*8803391G>T	Decreased risk of gastrointestinal toxicity Decreased severity of Neutropenia
	ABCC4*8803391G>T	Decreased risk of ADR (cyclophosphamide, doxorubicin,fluorouracil)

Therapeutic options

Conventional cytostatics:

Non cell cycle depended	S phase of cell cycle				Metaphases
Alkylating agents	*Inhibitors of topoisomerase I*	*Inhibitors of topoisomerase II*	*antimetabolites*	*Inhibitors of tubulin polymerization*	*Spindle poisoning agents*
Cyclophosfamide Ifosfamide		Epirubicin	Capecitabine		

Targeted therapies

Moab (Monoclonal Antibodies)	SMW (Small Molecular Weight molecule)
	Fulvestrant as inhibitor of estrogen positive proliferative signal. **Tamoxifen** as inhibitor of estrogen positive feedback. **Anastrozol** as inhibitor of estrogen synthesis. **Exemestane** as inhibitor of aromatase enzyme.

Biological/natural substances:

Class I (cytotoxic agents)	Class II (immuno-modulatory effect)	Class III (growth factors inhibitors)
Agaricus Blazei Murill Artecin Artesunate Ascorbic acid Avemar pulvis C-statin DCA (dichloroacetate) Mitochondrien formular Oxaloacetate (Cronaxal) Poly-MVA Salicinium Super Artemisinin		Angiostop Apigenin CoQ10 Curcumin (turmeric) Genistein Indol 3 Carbinol Paw-Paw Pure Quercetin Quercetin Resveratrol Salvestrol

It is recommended to use in a monthly base one agent from each class and then switch them after a month with the next potent agent from the same class in order to avoid secondary resistance.

Radiotherapy/Hyperthermia sensitivity:

Marker	Result (%)	Clinical outcome per marker	Clinical outcome
HSP90	-25%	SENSITIVE	SENSITIVE
HSP72	-10%	SENSITIVE	
HSP27	-15%	SENSITIVE	

Follow-up options:

YES	✓
NO	

Time interval (when)

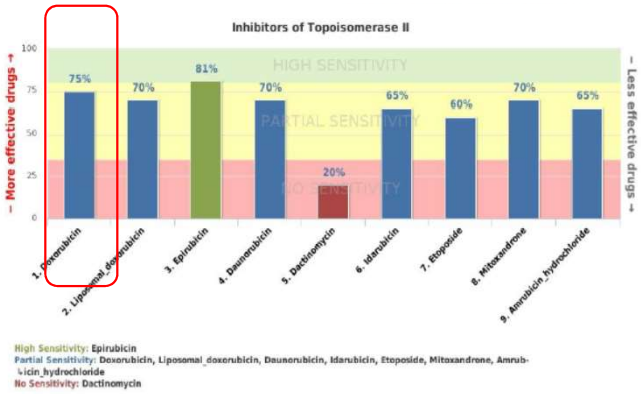
After 3 months	After 6 months	After 12 months
✓		

Test for follow-up

ONCOTRAILS							ONCOTRACE	ONCOCOUNT
Breast	Lung	Sarcoma	Colon	GI	Prostate	melanoma		
✓								

TOPO I Inhibitors

Drug	Polymorphism	Outcome
Irinotecan	UGT1A1*Gly71Arg	Increased metabolism of irinotecan



Drug	Polymorphism	Outcome
		Decreased likelihood of complete response (anthracyclines and related substances ,taxanes)
		Increased time to progression (anthracyclines and related substances, doxorubicin, epirubicin)
		Increased risk of Heart Failure (anthracyclines and related substances)
		Increased risk of resistance (anthracyclines and related substances)
		Increased risk of Cardiomyopathies (anthracyclines and related substances)
Doxorubicin	ABCB1*Ile1145Ile	Increased metabolism of doxorubicin
	NOS3*Asp298Glu	Decreased disease free survival (cyclophosphamide, doxorubicin, fluorouracil, methotrexate
	ABCB1*Ser893Ala	Increased metabolism of doxorubicin Increased survival (cyclophosphamide-doxorubicin)
	CBR1*Ala209Ala	Increased clearance of doxorubicin
	CYB2B6*Gln1*2His	Decreased likelihood of dose reduction (cyclophosphamide, doxorubicin)
	SLCC22A16*His49Arg	Increased likelihood of dose delay (cyclophosphamide,doxorubicin)
	CBR1*133G>A	Increased clearance of doxorubicin
	ABCC4*8803391G>T	Decreased risk of ADR (cyclophosphamide, doxorubicin,fluorouracil)
Epirubicin	ABCB1*Ile1145Ile	Increased time to progression (anthracyclines and related substances, doxorubicin ,epirubicin)
	GSTP1*Ile105Val	Increased response to cyclophosphamide, epirubicin and fluorouracil
	GSTP1*Ile105Val	Decreased severity of toxicity (cyclophosphamide-epirubicin)

Therapeutic options

Conventional cytostatics:

Non cell cycle depended	S phase of cell cycle				Metaphases
Alkylating agents	*Inhibitors of topoisomerase I*	*Inhibitors of topoisomerase II*	*antimetabolites*	*Inhibitors of tubulin polymerization*	*Spindle poisoning agents*
Cyclophosfamide Ifosfamide		Epirubicin	Capecitabine		

Targeted therapies

Moab (Monoclonal Antibodies)	SMW (Small Molecular Weight molecule)
	Fulvestrant as inhibitor of estrogen positive proliferative signal. **Tamoxifen** as inhibitor of estrogen positive feedback. **Anastrozol** as inhibitor of estrogen synthesis. **Exemestane** as inhibitor of aromatase enzyme.

Biological/natural substances:

Class I (cytotoxic agents)	Class II (immuno-modulatory effect)	Class III (growth factors inhibitors)
Agaricus Blazei Murill Artecin Artesunate Ascorbic acid Avemar pulvis C-statin DCA (dichloroacetate) Mitochondrien formular Oxaloacetate (Cronaxal) Poly-MVA Salicinium Super Artemisinin		Angiostop Apigenin CoQ10 Curcumin (turmeric) Genistein Indol 3 Carbinol Paw-Paw Pure Quercetin Quercetin Resveratrol Salvestrol

It is recommended to use in a monthly base one agent from each class and then switch them after a month with the next potent agent from the same class in order to avoid secondary resistance.

Radiotherapy/Hyperthermia sensitivity:

Marker	Result (%)	Clinical outcome per marker	Clinical outcome
HSP90	-25%	SENSITIVE	SENSITIVE
HSP72	-10%	SENSITIVE	
HSP27	-15%	SENSITIVE	

Follow-up options:

YES	✓
NO	

Time interval (when)

After 3 months	After 6 months	After 12 months
✓		

Test for follow-up

ONCOTRAILS							ONCOTRACE	ONCOCOUNT
Breast	Lung	Sarcoma	Colon	GI	Prostate	melanoma		
✓								

ANTIMETABOLITES

Drug	Polymorphism	Outcome
5-Fluorouracil	TP53*Pro72Arg	Decreased likelihood of Drug Toxicity (cyclophosphamide and fluorouracil)
	GSTP1*Ile105Val	Increased response to cyclophosphamide, epirubicin and fluorouracil
	NOS3*Asp298Glu	Decreased disease free survival (cyclophosphamide, doxorubicin, fluorouracil, methotrexate)
	MTHFR*Ala222Val	Increased likelihood of Drug Toxicity (cyclophosphamid-fluorouracil)
	DPYD*Ile543Val	Decreased likelihood of middle-severe nausea and vomiting
	DPYD*Ile543Val	Increased clearance of fluorouracil.
	DPYD*Cys29Arg	Increased likelihood of overall gastrointestinal toxicity
	DPYD*Met166Val	Increased likelihood of Neutropenia
	DPYD*Met166Val	Decreased risk of toxicity of fluoropyrimidine-based chemotherapy , (capecitabine,fluorouracil)
	DPYD*1905+1G>A	Decreased likelihood of mucositis, thrombocytopenia
	DPYD*1905+1G>A	Decreased severity of drug toxicity
	DPYD*Asp949Val	Decreased severity of drug toxicity
	ABCC4*8803391G>T	Decreased risk of ADR (cyclophosphamide, doxorubicin,fluorouracil)
Capecitabine	DPYD*Met166Val	Decreased risk of toxicity of fluoropyrimidine-based chemotherapy , (capecitabine,fluorouracil)
Methotrexate	NOS3*Asp298Glu	Decreased disease free survival (cyclophosphamide, doxorubicin, fluorouracil, methotrexate)
Cytarabine	CDA*Lys27Gln	Increased toxicity
	CDA*20915590delC	Increased toxicity
	CDA*-92A>G	Increased toxicity
Gemcitabine	CDA*Ala70Thr	Increased metabolism of gemcitabine
	RRM1*Thr*41Thr	Increased risk of Neutropenia

Designing of Treatment Protocol

Pharmacodynamic analysis
Viability & Gene Expression
(Summary Report)

Conventional Cytostatic agents
- X
- Y
- Z

Natural substances
1. Class I (cytotoxic affect)
   - F
   - G
   - H
2. Class II (growth factor inhib.)
   - R
   - M
3. Class III (immunomodulators)
   - Q
   - N

Pharmacokinetic analysis
Detoxification Genomics

Conventional Cytostatic agents
- X (Normal metabolizer)
- Y (Normal metabolizer)
- Z (Rapid metabolizer)

Natural substances
1. Class I (cytotoxic affect)
   - F (Normal metabolizer)
   - G (Normal metabolizer)
   - H (Rapid metabolizer)
2. Class II (growth factor inhib.)
   - R (Normal metabolizer)
   - M (Accumulator)
3. Class III (immunomodulators)
   - Q (Normal metabolizer)
   - N (Rapid metabolizer)

Therapy options for clinical use

Conventional Cytostatic agents
- X
- Y

Natural substances
1. Class I (cytotoxic affect)
   - F
   - G
2. Class II (growth factor inhib.)
   - R
3. Class III (immunomodulators)
   - Q

SUMMARY

- CTCs can be successfully transported “within a time window” without altering their genotype and phenotype.
- CTCs can be effectively isolated (with high integrity) using combination of selection assays
- CTCs can be cultured (with high rate of success) without altering their profile “within a time “window”.
- CTCs may also have markers which may predict the site of metastases.
- Use methods with low LoD and high rates of Sensitivity, Specificity, PPV and NPV.
- CTCs can be used for making clinical decision for cancer patients.
- Decisions should be based not only on from data profiling the cancer (PK), but also from the patients capacity to metabolize the therapeutic agents (PD).
- The treatment approach should also include the performance status of a patient (ECOG Score)

THANK YOU

THANK YOU FOR YOUR TIME AND PATIENCE

QUESTIONS OR ENQUIRIES

peter@nutripath.com.au