Biofocus‘ Molecular Diagnostic Panel

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www.biofocus.de

- Molecular detection of infectious diseases
- Human & veterinary hereditary diseases / genetic predisposition
- **Molecular Oncology**

Aims of Molecular Oncology

- Detection von Circulating Tumor Cells in blood (CTCs) → „residual disease“
- Determination of „drug target“-genes and resistance-markes

→ **Goal**: personalized therapy

Typical Genetic Alterations in Tumor Cells

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Overexpression / mutation of growth factor receptors

Erb-b2, EGFR, c-Kit, K-ras

Genes associated with cell replication

Telomerase, c-myc

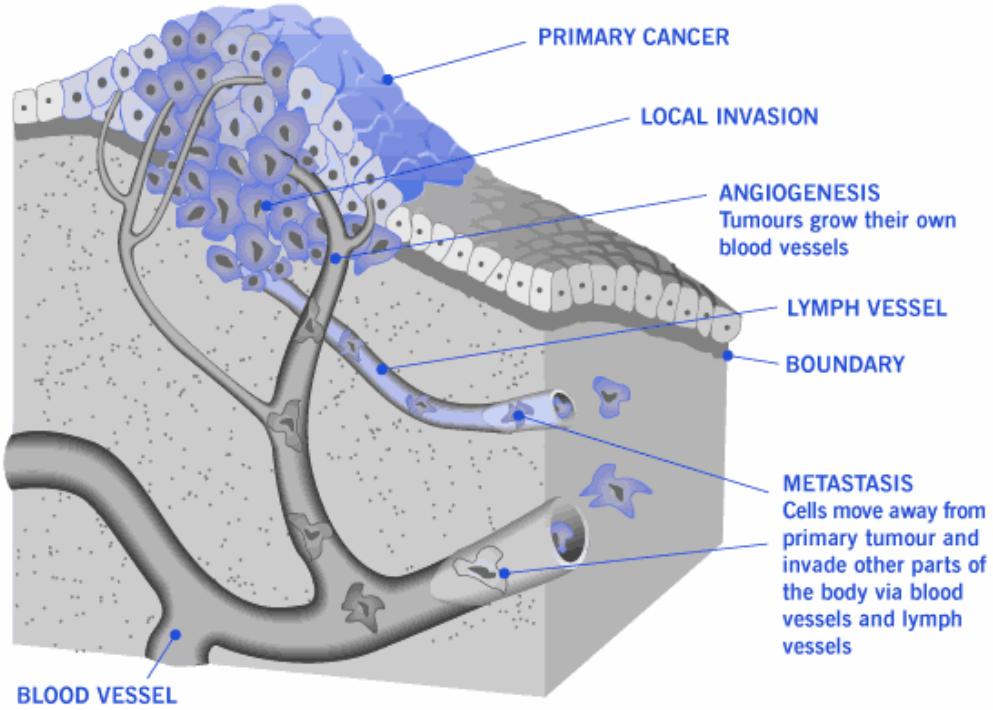
Angiogenesis factors

VEGF, bFGF

Apoptosis-Genes (= programed cell death)

Bcl-2, p53, Survivin

Dissemination - Metastasis



CTCs:

rare: 100 – 1000 per ml blood

sparely: 1 CTC in 10^6 to 10^7 WBCs

Isolation of CTCs is challenging

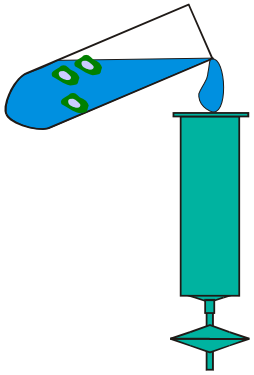
Isolation of CTCs from blood by positive selection

blood sample

CTC isolation

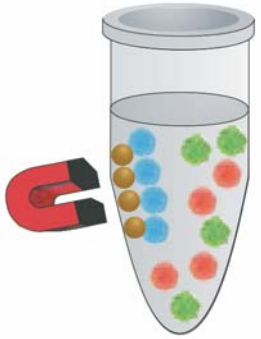
Size differentiation

(eg filtration)



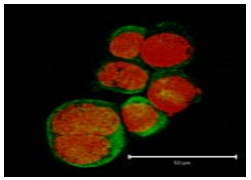
Immuno absorption

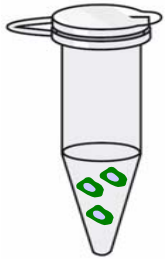
(eg. magnetic beads)

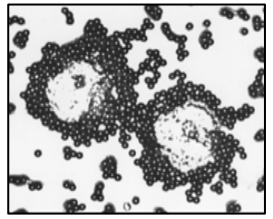


Wash

Molecular characterisation





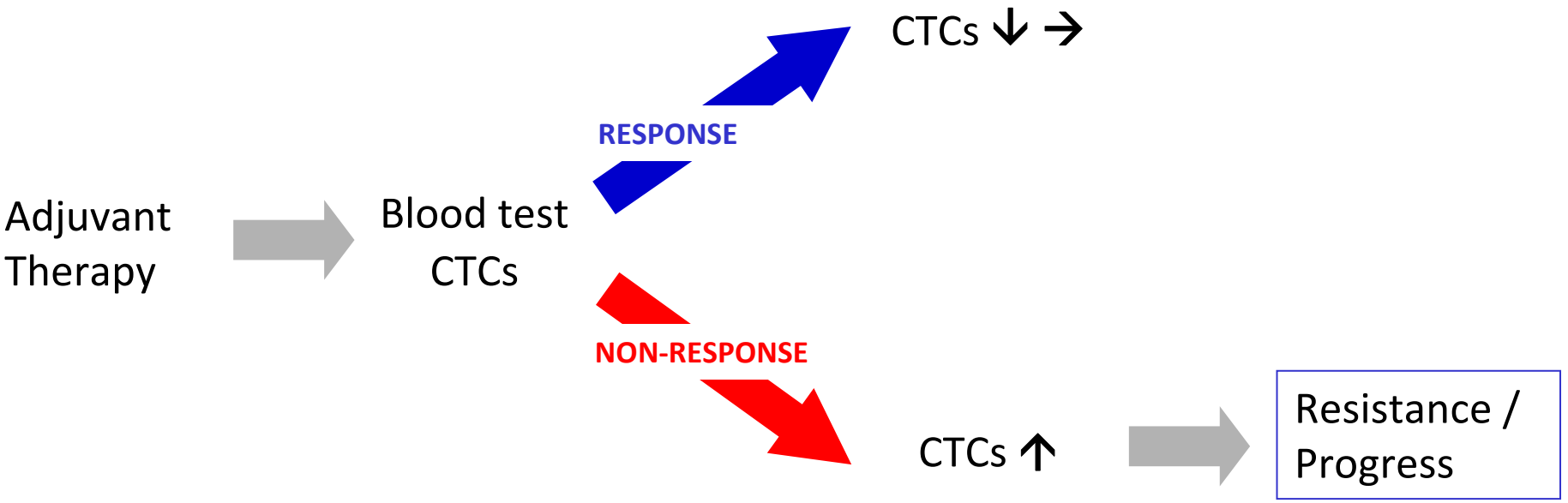


Molecular identification of captured cells as CTCs

- **Isolation of RNA from captured CTCs**
- **Differential Gene expression CTCs ⇔ Blood**
- **Molecular Tumor markers by quantitative real-time PCR:**
  - Cytokeratins (CK19, CK20)
  - Cell cycle genes (c-myc, erbb2, telomerase, survivin)
  - tissue specific genes: PSA (prostate), G250 (renal), MART (melanoma)

Therapy Monitoring

- **Adjuvant therapy** (after removal of primary tumor)

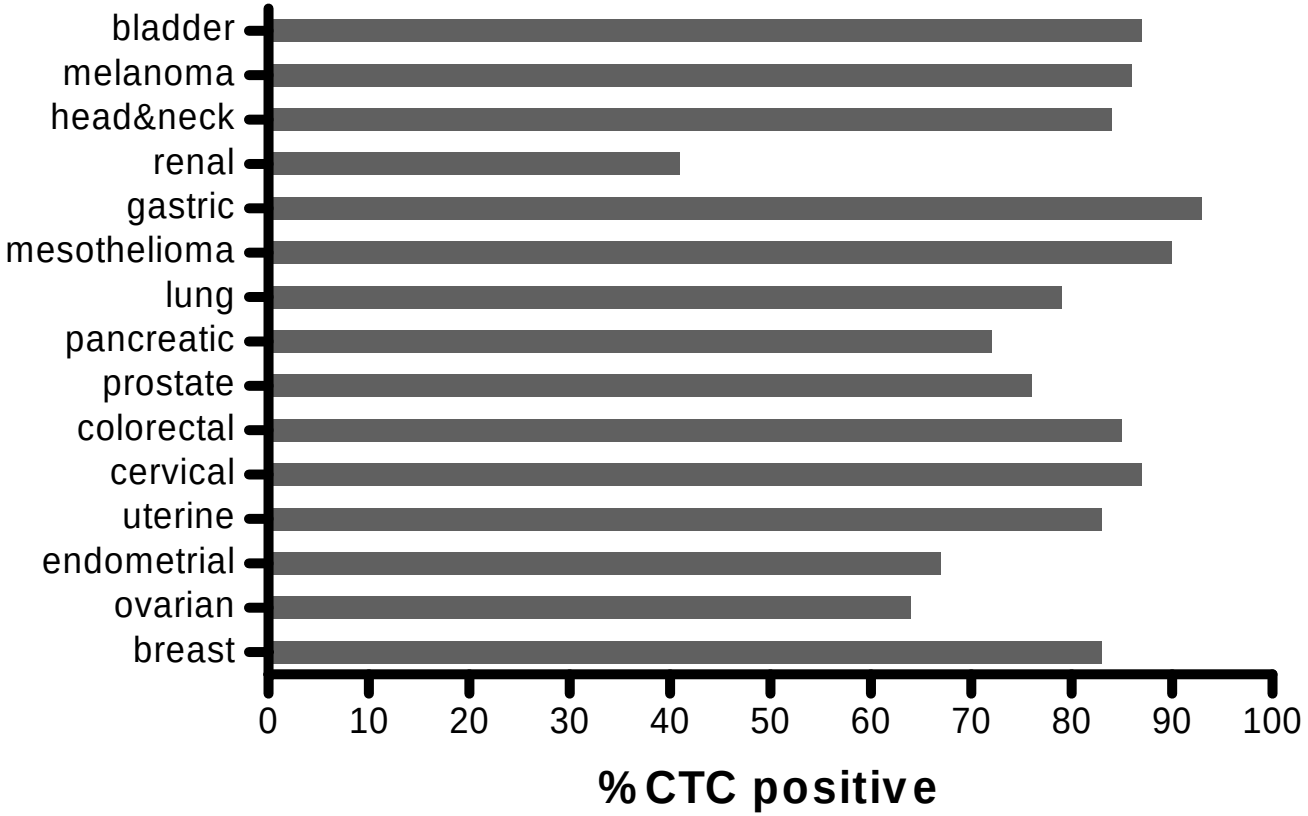


Genetic Detection of CTCs from blood

- **Four-Marker assay; Carcinoma** (e.g. CK19, ERBB2, C-MYC, Telomerase):

	≥1 Marker positive	≥ 2 Marker positive
Normal-patients n = 70	3/70 4.3 %	1/70 1.4 %
Tumor CA Patients n = 200	159/200 80 %	121/200 60 %

Detection rate of CTCs



→ **Average ca. 80 % in advanced tumors**

Tumor cell dormancy hypothesis

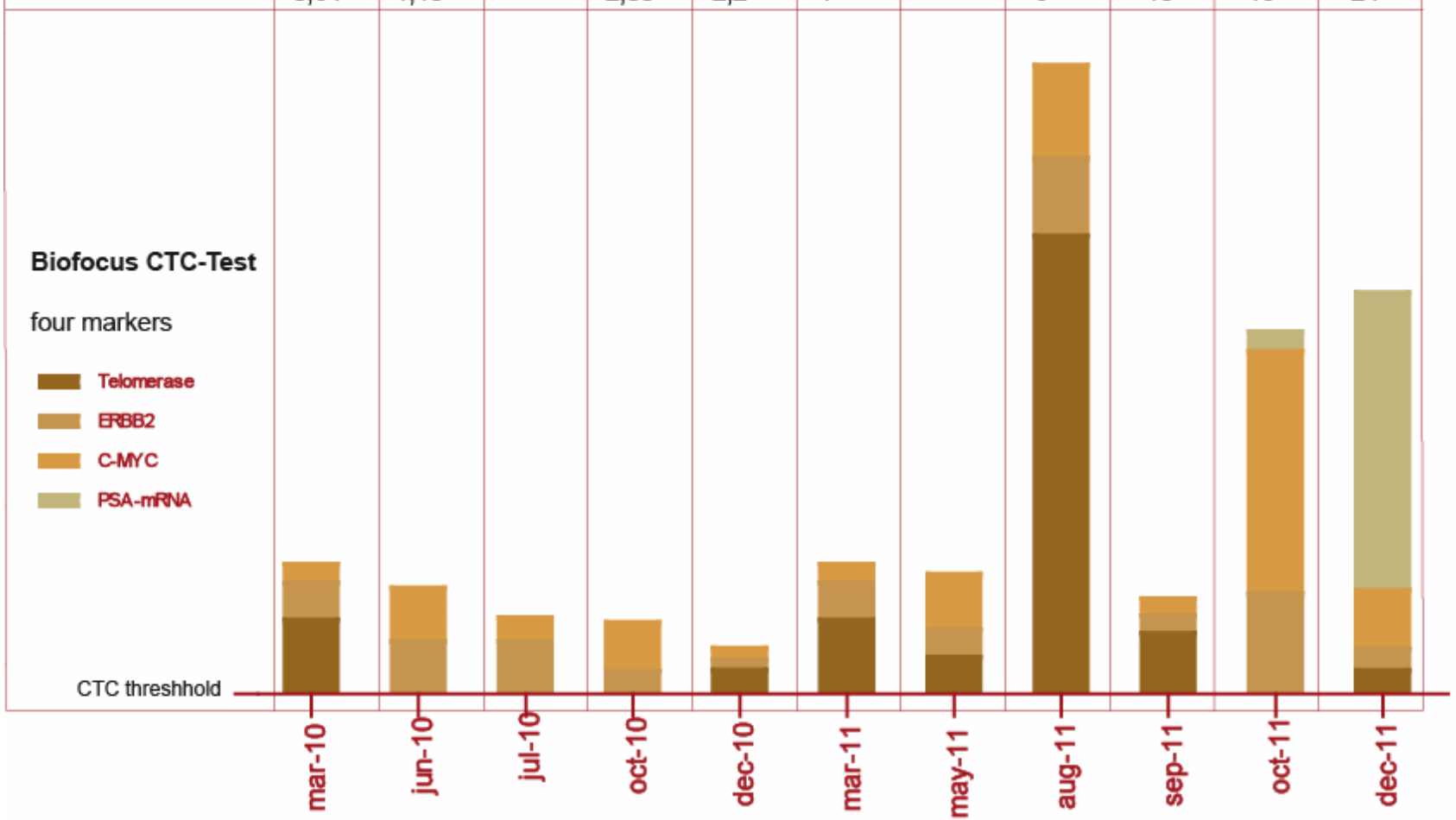
- Recurrence after long intervals of remission (decades)
- Remaining tiny sources of tumor cells in the body („occult metastasis“)
- More patients in remission have detectable CTCs than are at statistical risk for recurrence
- Only a portion of patients with CTCs will develop recurrence
- Limited life span of CTCs, process of constant cell death and replication
- CTCs can be repeatedly detected in dormancy patients at steady low levels, (may at some point negative)

→ **Increase of CTC-markers may indicate relapse**

Case report

Patient: Prostate CA, ED 01/2009 T3N1M0, Prostate ectomia Mar-2009 -> serum tPSA 0,069

TKTL1 score				pos	pos	pos	neg	neg		neg	
imaging CT / MRT					N0						N+
serum PSA	0,64	1,15		2,03	2,2	4		9	15	19	24



Molecular characterization of CTCs

→ **Gene expression analysis in CTCs for prediction of therapy resistance:**

Drug Metabolizing Genes

Activation, Degradation, Detoxification

„Drug-Target“ Genes

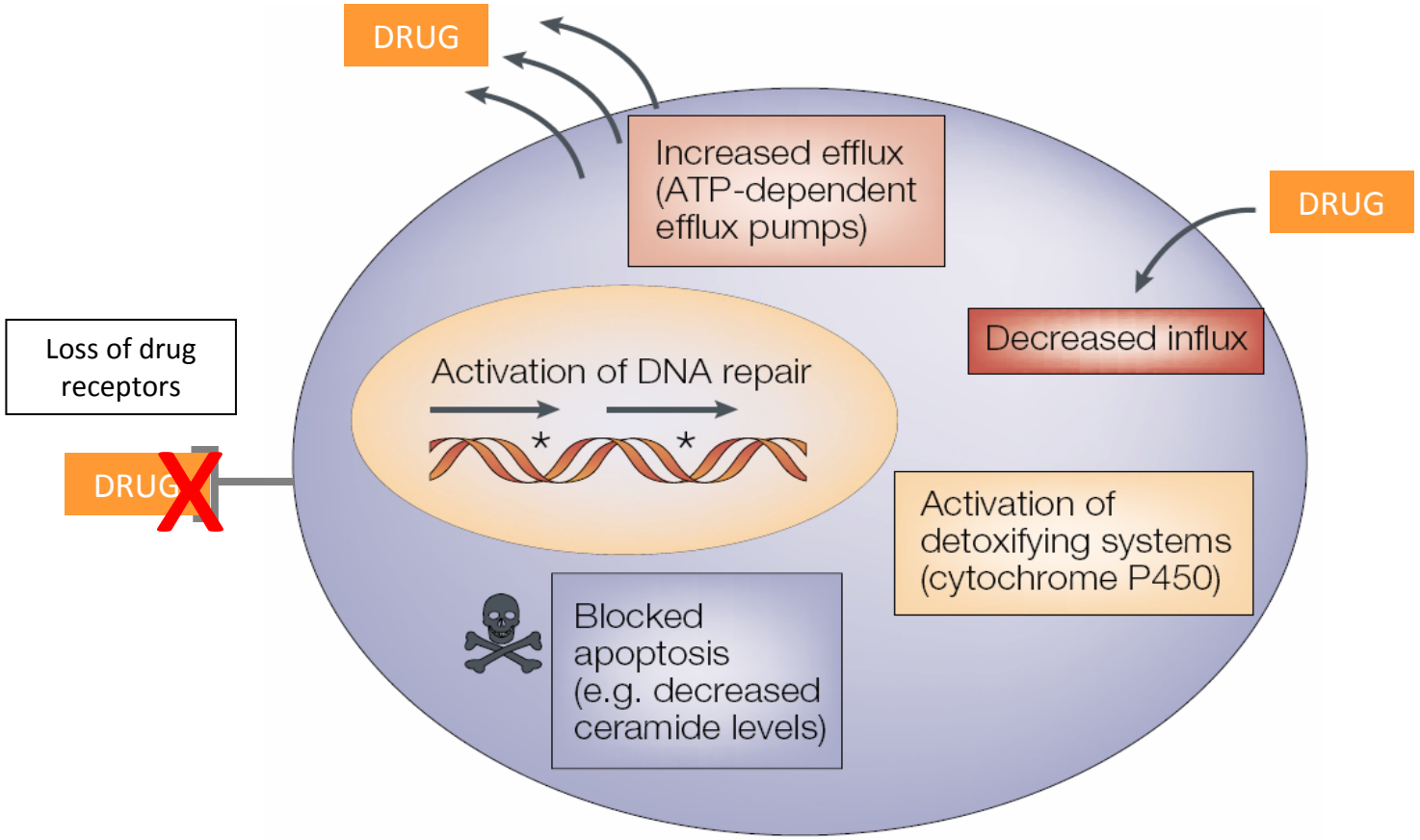
Cellular function inhibited by the drug

Genetic factors of Chemo-Resistance

- **Different drug-targets and metabolizing genes depending on drug**

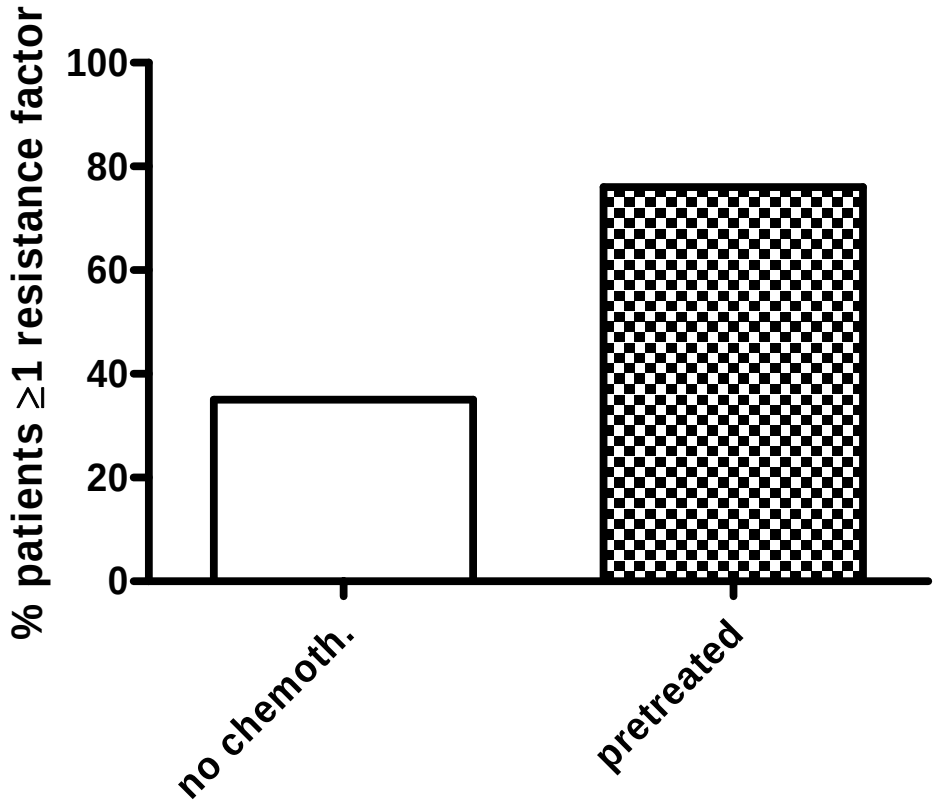
Drugs	Target	Resistance
Anthracyclines	Topoisomerase II	Topo II downregulation
Irinotecan	Topoisomerase I	Topo I downregulation
Platinum comp.	DNA	induction of ERCC1 repair
Nitroso-Ureas	DNA	induction of MGMT repair
Methotrexate	Folate metabolism	overexpression of DHFR
5-Fluorouracil	Nucleotide/DNA synthesis	overexpression of TS / DPD

Mechanims of Chemo-Resistance

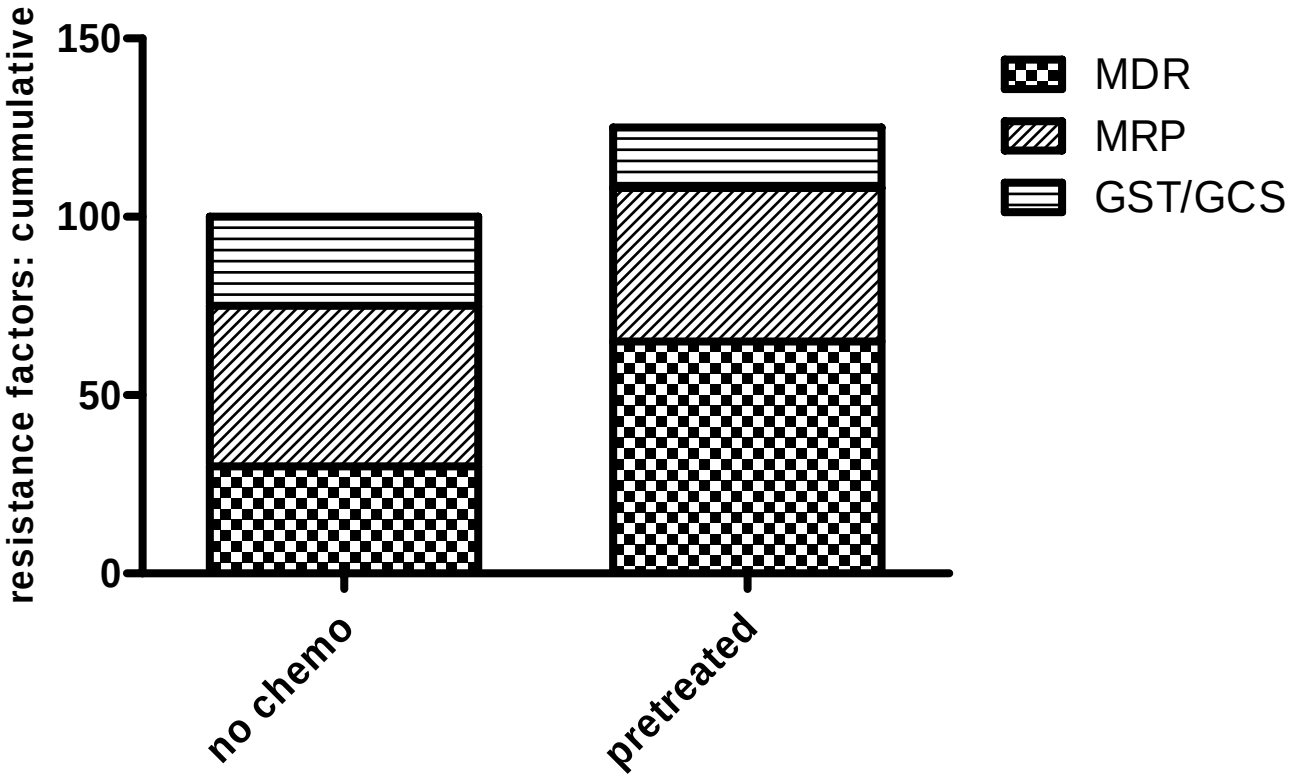


Multi-resistance in chemo-treated vs. non-treated patients

multidrug resistance factors: MDR1, MRP1, GST/GCS



Involved multidrug-resistance factors

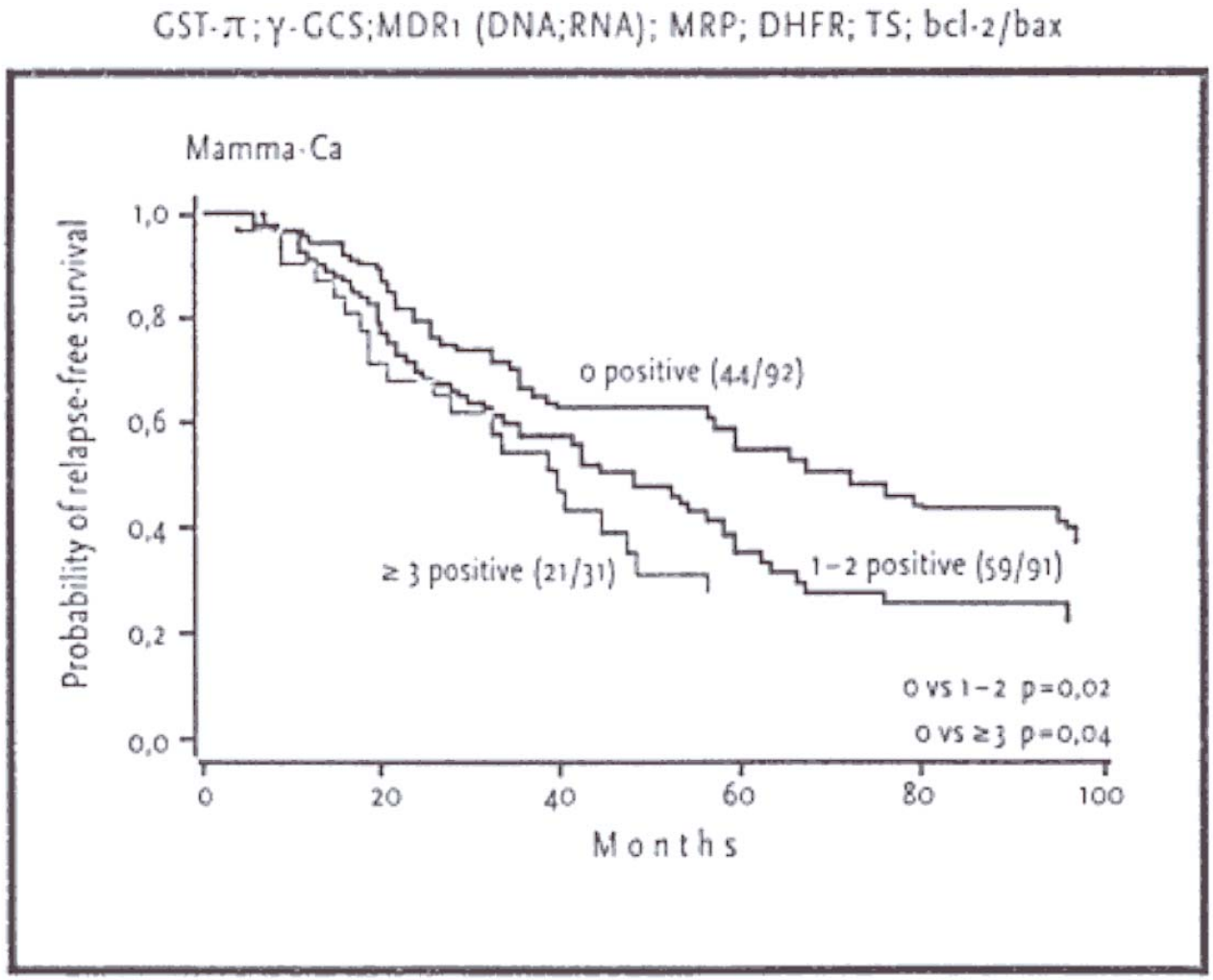


MDR1: Taxane, Anthracycline, Mitomycin, Irinotecan, Vinca-Alkaloides

MRP1: Anthracycline, Vinca-Alkaloide, Methotrexat

GST/GCS: Anthracycline, Mitomycin, Vinca-Alkaloides, Platin

Impact of resistance factors on relapse-free-survival



Therapy: Prediction and Outcome

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Tumor type	Therapy	Test-Result	clinical outcome
Mamma	Mitoxantron	resistent	Progress
Colon	5-FU	resistent	Progress
Stomach	Mitomycin + 5-FU + Cyclophpos.	Mitomycin: resistent Cyclophpos.: resistent 5-FU: intermediate	Progress / death
Ovar	Gemcitabine + Cyclophpos.	Gemcitabine: resistent Cyclophpos: sensitiv	Progress / death
Mamma	MTX + Cisplatin	MTX: resistent Cisplatin.: resistent	Partial response
Colon	Oxaliplatin	sensitiv	Partial response
Mamma	MTX + Gemcitabin	MTX: sensitiv Gemcitabin: sensitiv	Partial response
Mamma	MTX + 5-FU	MTX: sensitiv 5-FU: intermediate	Partial response
Mamma	Herceptin	sensitiv	Partial response
Pancreas	Herceptin	sensitiv	Partial response
Thymoma	Epirubicin + Mitomycin + 5-FU	Mitomycin: sensitiv 5-FU: sensitiv Epirubicin: intermediate	Complete response
Melanoma	Sorafenib	sensitiv	Complete response

Limitations of the prediction model

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Resistance ⇔ Sensitivity:

- Generally it is easier to predict resistance than response
- Focus on major resistance pathways only

Alternative Therapies:

Clinical response is observed despite positive resistance marker

→ Modulation of the resistance genes by alternative agents

Modulation of Resistance Factors

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Alternative agents can modulate resistance genes:

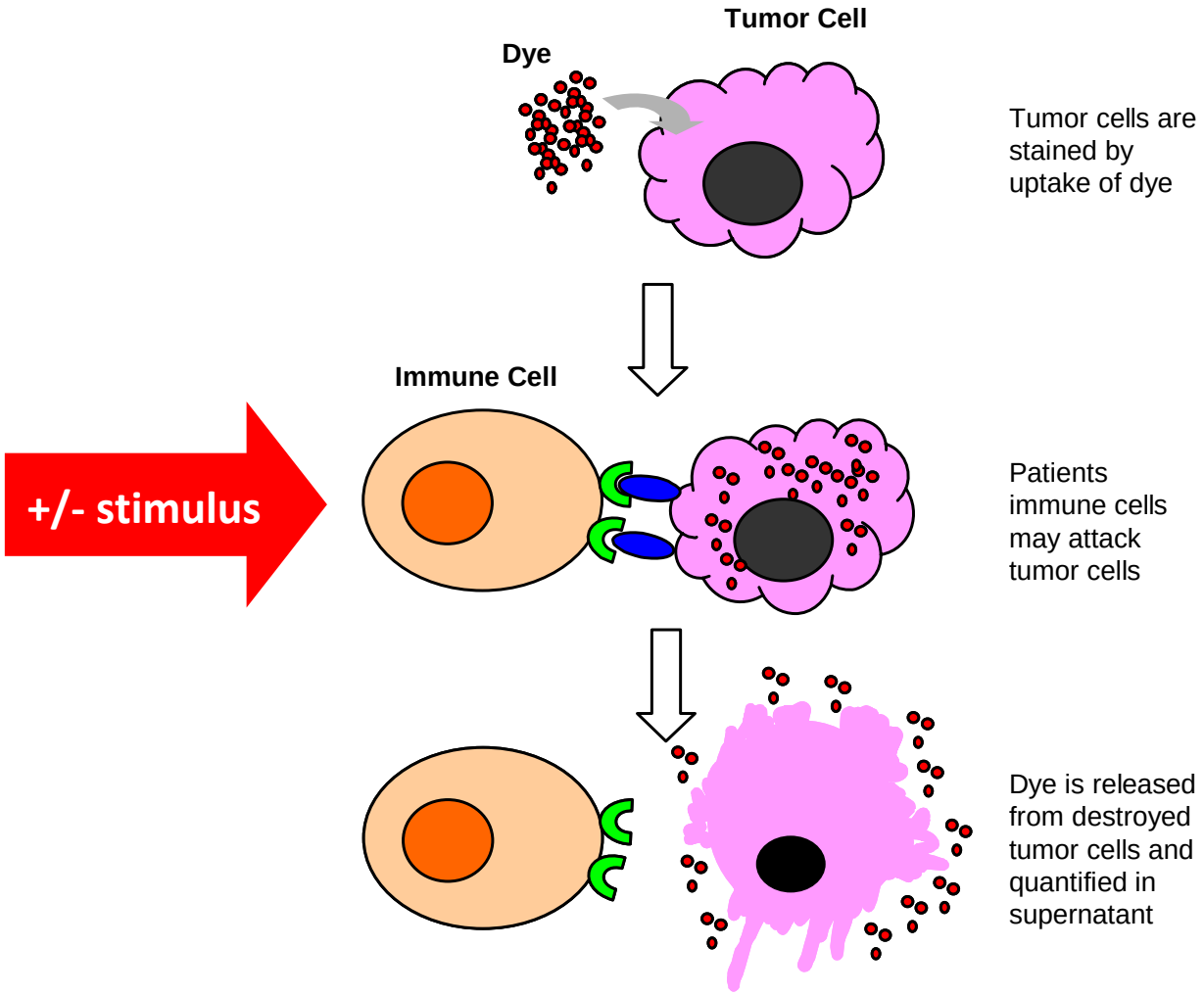
MDR → Curcumin, Acetogenin, Haelan

MRP → Artemisinin, Haelan

GST → Ellagic acid, Curcumin

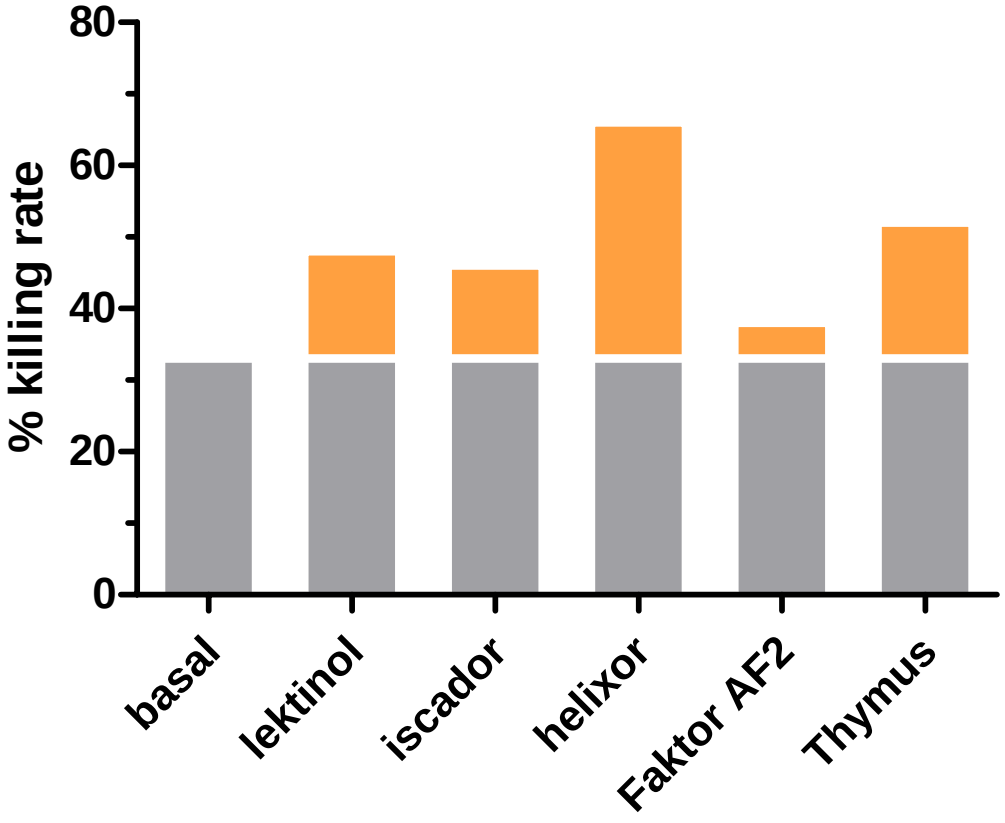
Immune function testing by Cellular NK-Test

Assay principle



Cellular NK-test

Testing of immune stimulative agents



Agent	Included in the assay
Thymus-extract	routinely
Factor AF2	routinely
Iscador mistletoe-extract	routinely
Lektinol mistletoe-extract	routinely
Helixor mistletoe-extract	upon request
Eurixor mistletoe-extract	upon request
Fraxini mistletoe-extract	upon request
Carnivora	upon request
Interleukin 2	upon request

Testing for alternative agents

Testing is possible for alternative agents with known genetic basis of action:

Agent	
Quercetin	Artemsinin derivatives
IP6 (Inositol-6-Phosphate)	Amygdalin B17
C-statin	Vitamin C
Dammarane sapogenins	Indol-3-carbinol (I3C)
Acetogenin, Graviola	Taurolidine
Haelan951	Ellagic Acid
Curcumin	Arglabin, Laetrile

Testing for Amygdalin B17

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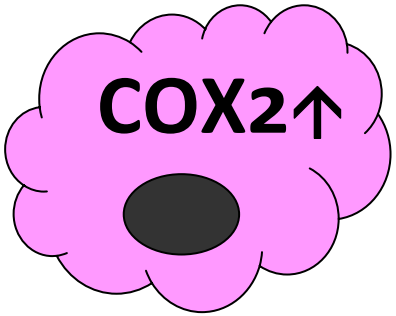
Measuring expression of Rhodanese and COX2 expression in tumor cells aids in selection of Amygdalin therapy

Testing for alternative agents e.g. Amygdalin B17

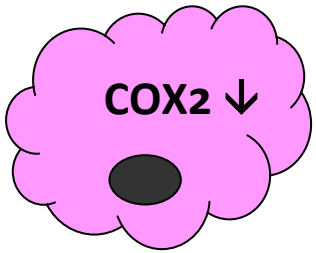
1. Suppressing expression of COX2 (Cyclooxigenase 2)

COX2:
- **Inflammation**
- **Tumor promotion**



CANCER CELL
high levels of COX2

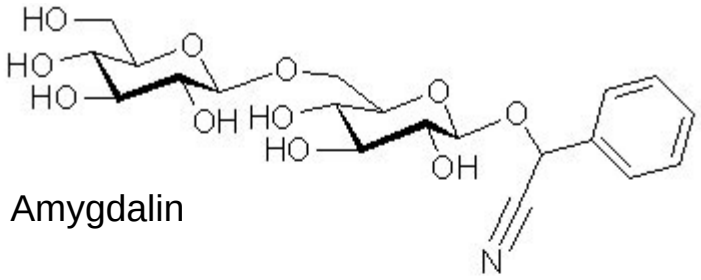
Amygdalin B17



Amygdalin B17 suppresses COX2 expression

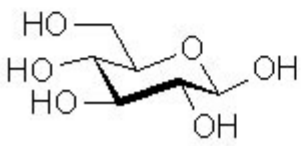
Testing for alternative agents e.g. Amygdalin B17

2. Detoxification by Rhodanese

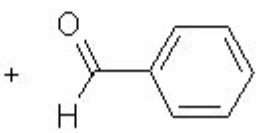


Amygdalin

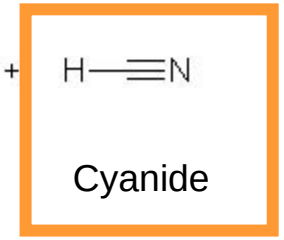
beta-glucosidase
beta-glucuronidase



Glucose



Benzaldehyde



Rhodanese

Detoxification of Cyanide → Resistance to B17

Poisening of cancer cells

Rhodanese expression in CTCs

Overexpression of rhodanese in CTC is obviously rare

Rhodanese level	Observed in % of CTCs (n=45)
underexpressed	43 %
base-level (equal to normal cells)	51 %
overexpressed	6 %