CANCER TARGETS AND BIOMARKERS, PERSONAL THERAPY, RESISTANCE

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MOLECULAR TARGETS OF CANCER THERAPY: A BRIEF HISTORY

→ Introduction of **hormonal therapy** in 1939: Charles Huggins, based on an early observation on the effect of estrogens on breast cancer made by Beatson in 1896, treated men with prostate cancer with hormones and was able to show responses by decreases in **acid phosphatase levels**.

→ Using **a nitrogen mustard**, Alfred Gilman and Louis Goodman observed regressions in mice bearing a transplanted lymphoid tumor. In 1943, they convinced their colleague Gustaf Lindskog, a thoracic surgeon, to administer it to a patient with non-Hodgkin's lymphoma and severe airway obstruction. Marked regression was observed in this and other lymphoma patients. These results boosted the synthesis and testing of several related alkylating compounds, including oral derivatives such as **chlorambucil** and ultimately **cyclophosphamide.**

→ Nutritional research before and during WWII had identified a factor present in green leafy vegetables that was important for bone marrow function. This factor turned out to be **folic acid**, and folate deficiency could produce a bone marrow picture reminiscent of the effects of nitrogen mustard. A series of folic acid analogues, which were in fact folate antagonists, were tested, and these compounds included aminopterin and amethopterin, now better known as **methotrexate**.

De Vita and Chu, Cancer Res., 2008, 68: 8643.

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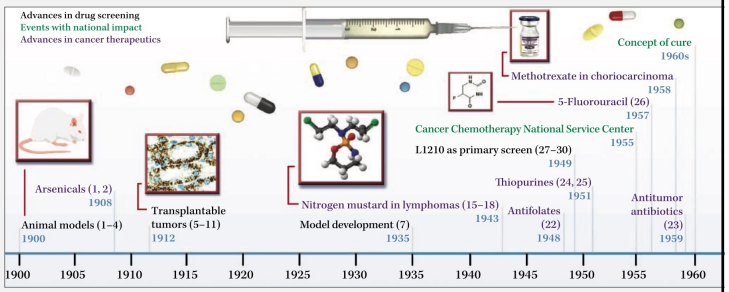
→ Another WWII-related program was the large-scale screening of fermentation products by the pharmaceutical industry to isolate and produce antibiotics. **Actinomycin D,** came from this program, and this effort yielded a series of active antitumor antibiotics in common use today.



→ The schedule of administration of drugs was proving to be important, and **combinations of drugs** were found to be superior to single agents.
In children with leukemia, taking advantage of the newly discovered **Vinca alkaloid, vincristine**, a program known as "VAMP" (vincristine, amethopterin, 6-mercapto-purine, and prednisone) was the first of a series of cyclically administered treatment programs that increased the remission rate and duration.

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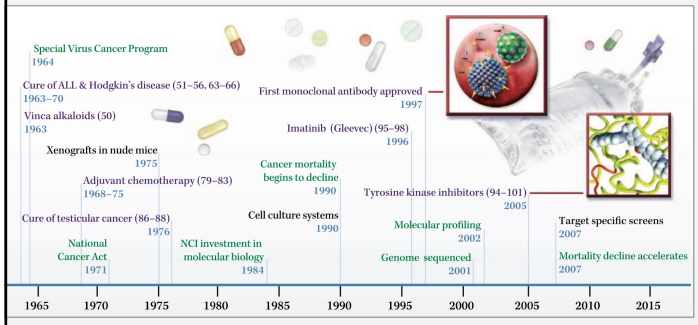
→ As of 1975, the Developmental Therapeutics Program screened ~10,000 drugs per year and developed a new series of screening systems. The mouse L1210 leukemia model was abandoned as the primary screen in favor of a panel of tumors (human xenografts in nude mice). The **taxanes** had their antitumor effects identified in this panel.

→ The advent of monoclonal antibodies has enhanced the effects of chemotherapy. Hybridomas were described in 1975, and **monoclonal antibodies** were proven clinically useful starting in the mid- 1990s.



→ Chemotherapy has transitioned to the age of "**targeted therapy**." The first and best example is the development of the tyrosine kinase inhibitor *imatinib* for the treatment of chronic myelocytic leukemia. Design of a drug that fits into the ATP-binding site of the Bcr-Abl protein created by the « Philadelphia chromosome » translocation and inhibits the function of this aberrant kinase. CML may be unique in that a single molecular abnormality drives the disease, whereas in most cancers there are multiple abnormalities that must be targeted.

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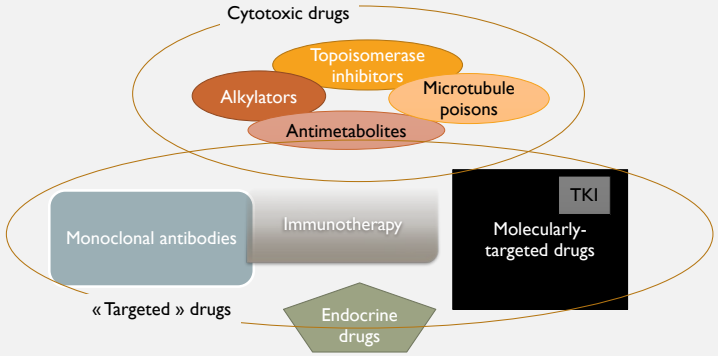
Anticancer Drugs



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CANCER BIOMARKERS

A biomarker is a unique mutated nucleic acid sequence, protein, glycoprotein or group of proteins, expressed by the tumour cells but not normally by healthy cells

Four main types of biomarkers:

- → **Pre-disposition**
  Likelihood of developping a disease
- → **Diagnostic**
  To confirm a patient has a particular cancer
- → **Predictive**
  Determining which cohort of patients may benefit from a particular drug therapy
- → **Prognostic**
  Suggesting how the cancer may develop in the individual

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BLOOD STREAM TUMOR MARKERS

A tumor marker can be defined as a substance of any type produced either by a tumor or by the body in response to a tumor and that aids cancer detection and/or monitoring

Assessment by immuno-assay

Types of Molecules That Have Been Used Traditionally as Cancer Markers

Type of Molecule	Typical Example	Cancer(s) where used
Enzymes	LDH	NSGCT
	Acid phosphatase	Prostate
	Alkaline phosphatase	Osteosarcoma
	PSA	Prostate
Immunoglobulins	Paraproteins	B-cell malignancy
	Bence-Jones proteins	B-cell malignancy
Fetal-placental proteins	CEA	Colorectal, other adenocarcinomas
	AFP	Hepatocellular, NSGCT
	HCG	NSGCT, choriocarcinoma
Cytokeratins	TPA	Multiple adenocarcinomas
	TPS	Multiple adenocarcinomas
	CYFRA 21-1	Lung (non-small cell)
Hormones	Calcitonin	Medullary thyroid
	VMA, HVA	Neuroblastoma
	5-HIAA	Carcinoid
Mucins	CA 19-9	Pancreatic
	CA 15-3, BR 27.29	Breast
	CA 72-4	Gastric

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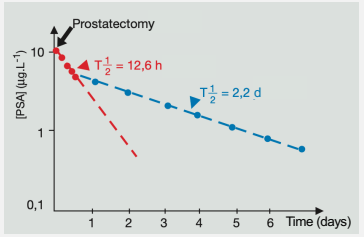
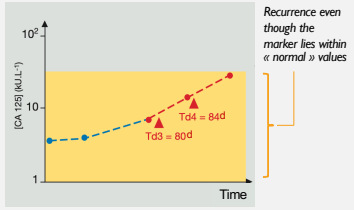
→ **Not for diagnosis.**
Generally cannot discriminate between healthy and diseased subjects due to inter-individual variations, to the age (example of PSA, which increases after 50y in men), to the hormonal status or to non-cancerous diseases (CA125 and menopause).

→ Suitable for patient's follow-up according to appropriate pharmacokinetic models. Best response with organ-specific markers.



→ Previous evolution curve needed to interpret any recurrence/relapse. Gives access to an estimate of the tumor doubling time.



Bioforma. N°32. Les marqueurs tumoraux sériques des tumeurs solides

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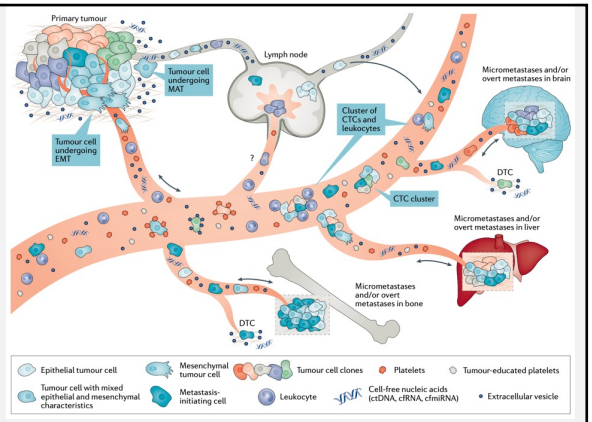
EMT = Epithelial to Mesenchymal Transition

MAT = Mesenchymal to Amoeboid Transition

DTCs = Dissociated Tumour Cells



Liquid biopsy:

→ Circulating Tumor Cells (CTCs)
→ Circulating Tumor DNA
→ Cell-free miRNA and other ncRNA
→ Extracellular vesicles (exosomes, microvesicles,...)
→ Tumor-educated platelets

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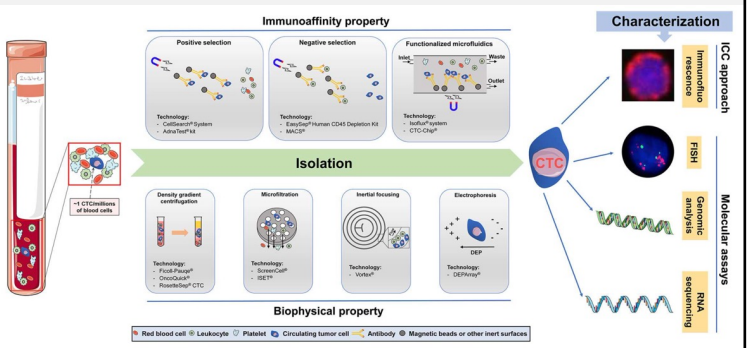
CTCs: isolation and characterization



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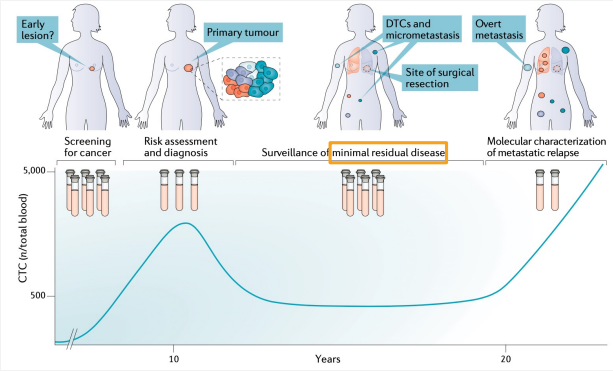
Residual disease



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CELLULAR/TISSULAR CANCER MARKERS

→ Genotypic + Phenotypic levels
→ Variable according to the cancer type
→ **Relevance as a diagnostic, predictive and/or prognostic factor**
→ **Contribute to define the appropriate treatment**
→ ***Relevance as a therapeutic target***

Genetic level

Mostly in somatic cells

Oncogenes and Anti-oncogenes
Chromatin modifiers

Mutations
Ex: BCR-ABL in leukemia

Copy number variation
Ex: Her-2/ERBB2 in breast cancer

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Table 1
Genes, mutation type and biochemical product active in breast cancer

Symbol	Name	Location	Mutation Type	Product Type
BRCA1	breast cancer 1	17q21.31	germinal/somatic	protein coding
TP53	tumor protein 53	17p13.1	somatic/germinal	protein coding
BRCA2	breast cancer 2	13q13.1	germline/somatic	protein coding
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha	3q26.32	somatic	protein coding
MYC	Myc protoncogene	8q24.21	somatic	protein coding
PTEN	Phosphatase and tensin homolog	10q23.31	Somatic/germinal	protein coding
CCND1	Cyclin D1	11q13.3	somatic	protein coding
ERBB2	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2	17q12	somatic	protein coding
ERBB3	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 3	12q13.2	somatic	protein coding
FGFR1	Fibroblast growth factor receptor.1	8p11.23	somatic	protein coding
FGFR2	fibroblast growth factor receptor 2	10q26.13	somatic/germinal?	protein coding
GATA3	Gata-binding protein 3	10p14	somatic	protein coding
AKT2	AKT serine/threonine kinase 2	19q13.2	somatic	protein coding
ARID1B	AT rich interactive domain 1B (SWI1-like)	6q25,3	somatic	protein coding
CASP8	caspase 8, apoptosis-related cysteine peptidase	2q33.1	somatic/germinal?	protein coding
CDKN1B	cyclin-dependent kinase inhibitor 1B (p27, Kip1)	12p13.1	somatic	protein coding
MAP3K1	mitogen-activated protein kinase kinase kinase 1, E3 ubiquitin protein ligase	5q11.2	somatic/germinal?	protein coding
MAP3K13	mitogen-activated protein kinase kinase kinase 13	3q27.2	somatic	protein coding
NCOR1	nuclear receptor corepressor 1	17p12-p11	somatic	protein coding
SMARCD1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 1	12q13.12	Somatic	protein coding
TBX3	T-box 3	12q24.21	somatic	protein coding
RB1	retinoblastoma 1	13q14.2	somatic	protein coding
ESR1	estrogen receptor 1	6q25.1-q25.2	somatic	protein coding
FOXA1	forkhead box A1	14q21.1	somatic	protein coding
CDH1	cadherin 1, type 1, E-cadherin (epithelial)	16q22.1	somatic/germinal	protein coding
APOBEC3B	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B	22q13.1	somatic	protein coding
PALB2	partner and localizer of BRCA2	16p12.2	germinal/somatic?	protein coding
ATM	ataxia telangiectasia mutated	11q22.3	germinal/somatic	protein coding
CHEK2	checkpoint kinase 2	22q12.1	germinal/somatic	protein coding
RAD51	RAD51 recombinase	15q15.1	germinal	protein coding
RAD51C	RAD51 paralog C	17q22	germinal/somatic?	protein coding
MSH2	mutS homolog 2	2p21-p16	germinal/somatic	protein coding
BARD1	BRCA1 associated RING domain 1	2q35	germinal/somatic?	protein coding
STK11	serine/threonine kinase 11	19p13.3	germinal/somatic	protein coding
BRIP1	BRCA1 interacting protein C-terminal helicase 1	17q23.2	germinal/somatic	protein coding
MALAT1	metastasis associated lung adenocarcinoma transcript 1	11q13.1	somatic	non-protein coding
HOTAIR	HOX transcript antisense RNA	12q13.13	somatic	non-protein coding
MEG3	maternally expressed 3	14q32.2	somatic	non-protein coding
H19	H19, imprinted maternally expressed transcript	11p15.5	somatic	non-protein coding

Biancolella, et al., Seminars in Cancer Biology https://doi.org/10.1016/j.semcancer.2020.03.013

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mRNA / Protein level

Expression

Ex: Estrogen and Progesterone Receptors in breast cancers
Also Her-2 expression

→ Specific therapeutic targeting
→ Triple-Negative BC

Ex: Immunophenotyping of leukemias

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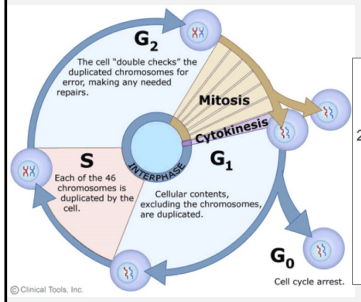
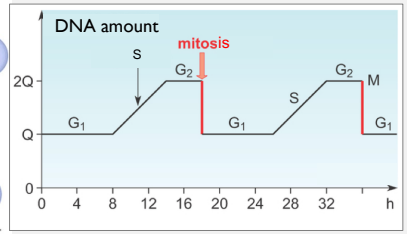
Assessment

Qualitative and quantitative

Genetic level

→ Genome sequencing
→ Measurement of DNA content





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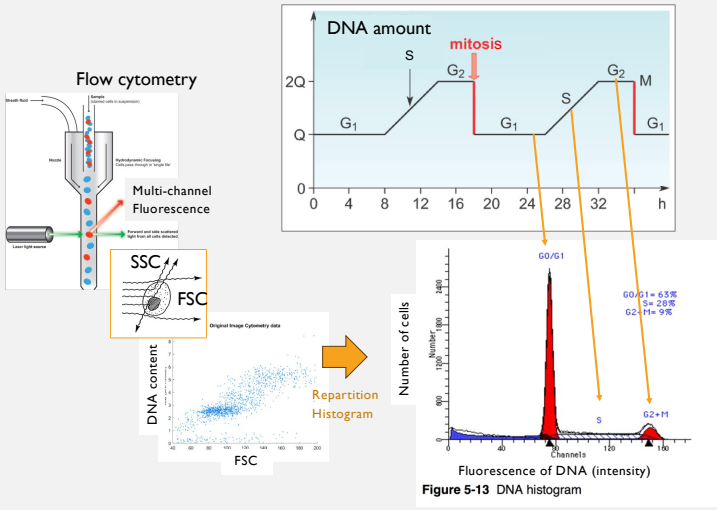

Figure 5-13 DNA histogram

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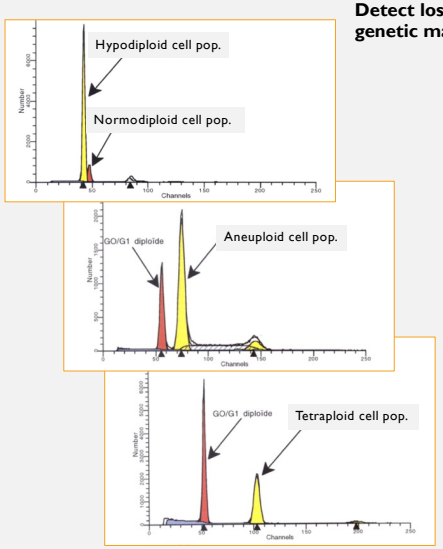
Detect loss or gain of genetic material



← Chromosome duplication

← Whole genome duplication

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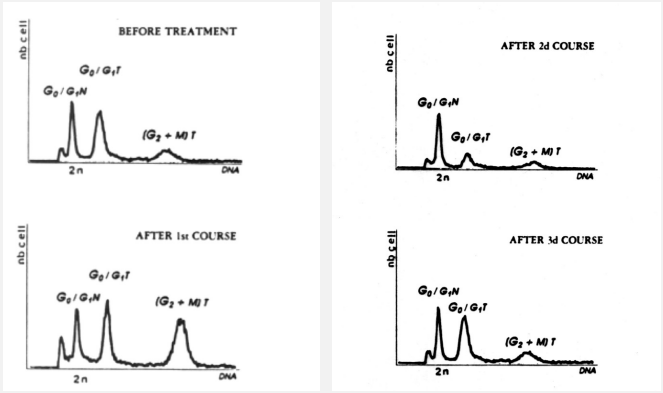
Efficacy of chemotherapy / emergence of resistance



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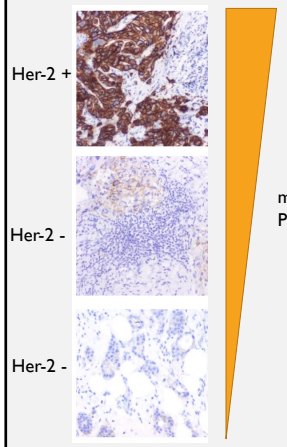
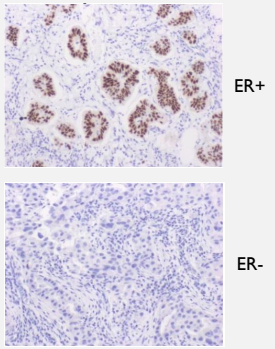
Qualitative and quantititive assesments

mRNA / Protein level

→ Tumor cell phenotyping
- Immuno histochemistry
- RT-qPCR
- Protein Immuno-assay



Her-2
mRNA level
Protein amount



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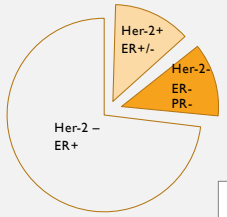
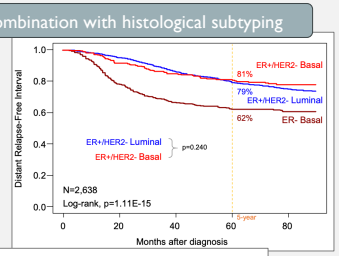
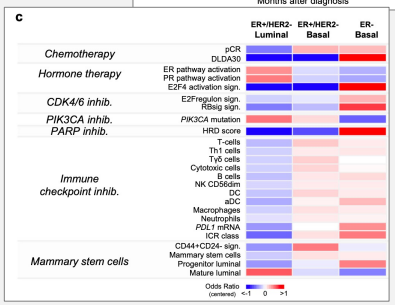
Traget-based classification



Combination with other molecular / cellular phenotypes

Combination with histological subtyping





Bertucci et al., Breast Cancer, 2020

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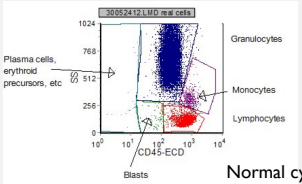
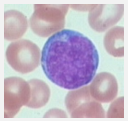
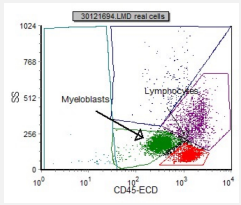
Immuno-phenotyping



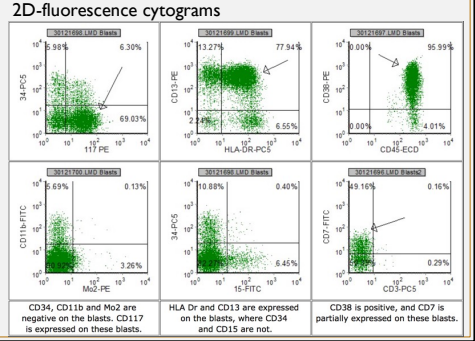
Normal cytogram

Myeloblastic leukemia M1





2D-fluorescence cytograms



CD34, CD11b and Mo2 are negative on the blasts. CD117 is expressed on these blasts.

HLA Dr and CD13 are expressed on the blasts, where CD34 and CD15 are not.

CD38 is positive, and CD7 is partially expressed on these blasts.

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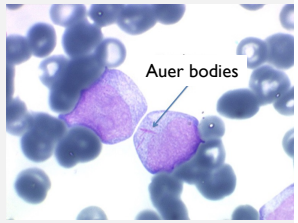
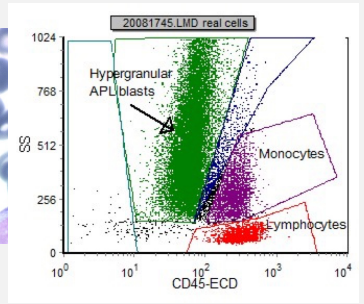
Cell-specific phenotypes

Promyelocyte leukemia M3





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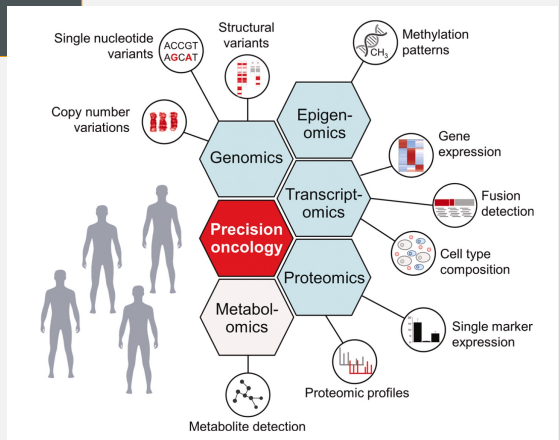
Cancer biomarkers & Precision oncology



Walter et al. , Semin. Cancer Biol. 2020, in press.
https://doi.org/10.1016/j.semcancer.2020.10.015

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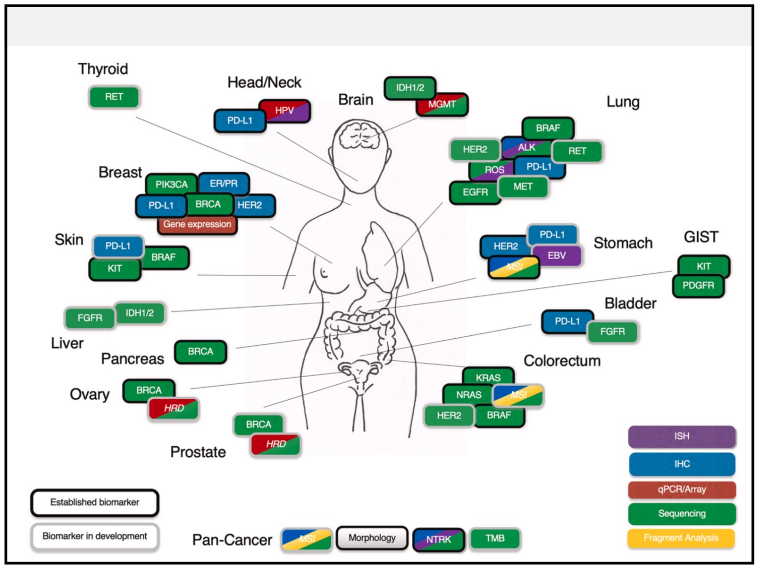

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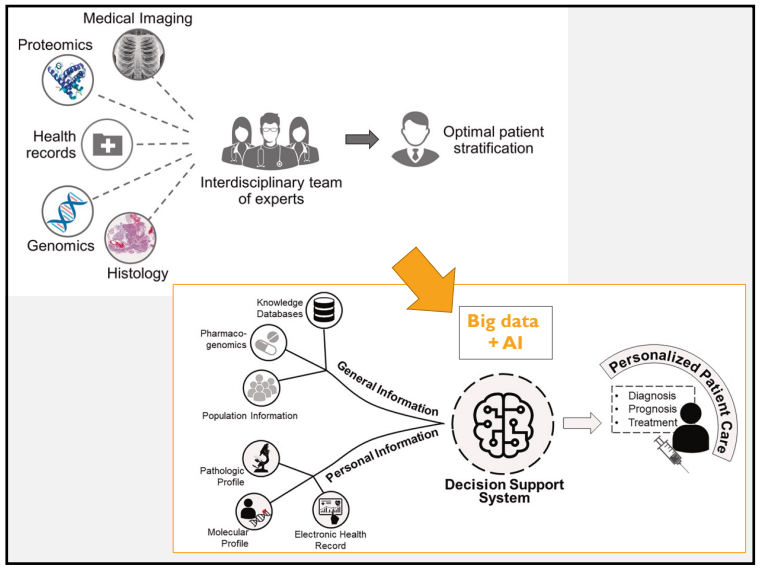

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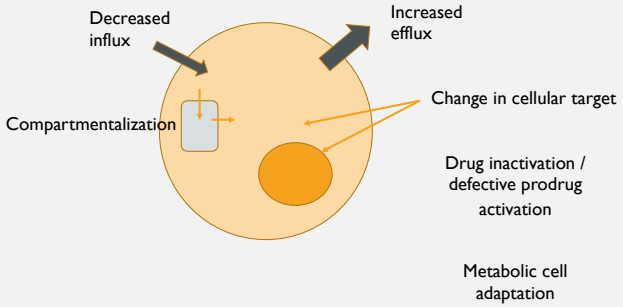
DRUG RESISTANCE

→ Instrinsic
→ Acquired

→ By clonal selection of resistant cells
→ Through cancer stem cells



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→ **Tumor heterogeneity & microenvironment**

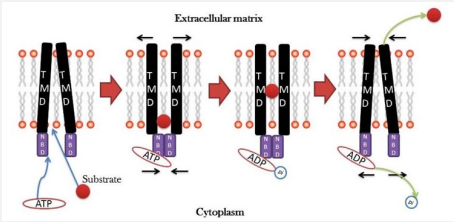
Growth factor local production, pH decrease, Hypoxia, ...

→ **Cancer cell stemness**

Dormancy in niches, MDR overexpression, long lifetime, stress-resistance

→ **Increased efflux by Multidrug Resistance (MDR) mechanism**

ATP-Binding Cassette (ABC) transporters, mostly ABCB1 (=P-gP) and AGBG2



→ **Decreased influx** (down-regulation or change in the affinity of Solute Carrier Transporters – SLC)

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→ **Inactivation / defective activation / alteration of the metabolism of anticancer drugs**

Ex: cytarabine (AraC) used in the treatment of acute leukemias. Functions as an anti-nucleotide after phosphorylation. Down-regulation of the kinase → chemoresistance

Ex: increased expression of CYP genes (that encode Cytochromes P-450) by cancer cells

→ **Modification of drug targets**

Secondary mutations that alter the main target of the drug:
- *Mutation of topolsomerase II = target of anthracyclines*
- *Mutations of β-tubulin = target of taxanes*
- *Mutations of Bcr-Abl = target of imatinib (up to 70 mutations reported, not all with the same impact i.e. some of them can be overcome by increased drug concentration, while others require the use of alternative, second-generation drugs (dalatinib, nilotinib)*
- *Increased DNA-repair mechanisms that couteract DNA-alkylating agents*

→ **Gene amplification of drug targets**

Dihydrofolate reductase gene can be amplified up to more than 100 times = target of Methotrexate

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→ **Epigenetic alterations**

DNA methylation of CpG islands. Frequent for MDR1
Histone modifications

→ **Transcription level**

miRNA
lncRNA

Table 2. miRNAs involved in cancer drug resistance

miRNA	Target	Tumor	Chemotherapy agent
miR-7	MDR1	SCLC	Anthracyclines
miR-9	MDR1/ABCG2	Glioblastoma	Temozolomide
miR-17-5p	PTEN	Ovary	Paclitaxel
miR-21	PTEN, PDCD4	Breast	Trastuzumab
miR-25	ABCG2	Breast	Epirubicin
miR-103/107	P-gp	Gastric	Doxorubicin
miR-127	MDR1/MRP1	Glioma	Adriamycin
miR-129-5p	ABCB1	Gastric	Vincristinecisplatin 5-fluorouracil
miR-134	MRP1/ABCC1	Breast	Doxorubicin
miR-145	P-gp/ABCB1	Ovarian	Paclitaxel
miR-181a	PTEN	NSCLC	Paclitaxel, Cisplatin
miR-196a	MDR1/MRP1	NSCLC	Cisplatin
miR-200c	P-gp/ABCB1	Colorectal	Vincristineoxaliplatincisplatin 5-fluorouracilmitomycin C
miR-202	BAFF	Multiple myeloma	Bortezomib, Thalidomide, Dexamethasone
miR-217	PTEN	Breast	Tamoxifen, Etoposide, Lapatinib
miR-221/222	MRP1/ABCC1	Multiple myeloma	Melphalan
miR-508-5p	P-gp/ABCB1	Gastric	Vincristineadriamycincisplatin 5-fluorouracil
miR-519c	ABCG2	Colorectal	5-fluorouracil
miR-634	CCND1, GRB2, ERK2, RSK1, RSK2	Ovary	Cisplatin
miR-4689	KRAS, AKT1	NSCLC	EGFR inhibitors

Mansoori et al. , Adv. Pharm. Bull. 2017, 7:339.

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FUTURE PROSPECTS

→ Therapeutics: combination of treatments
- *Chemotherapy, adjuvant chemotherapy, neoadjuvant chemotherapy (prior to surgery)*
- *Immunotherapy, Radiotherapy*

→ Better stratification of patients and personalized medicine
- *Integration of mutiple big data sources*
- *Development of AI*

- *Cost*
- *Benefit/risk balance*

→ Better understand resistances & cell adaptation processes to develop new molecules or therapeutic strategies
- *Intrinsic vs acquired resistance*
- *Efforts to make new targets, to devise new adjuvant strategies*

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