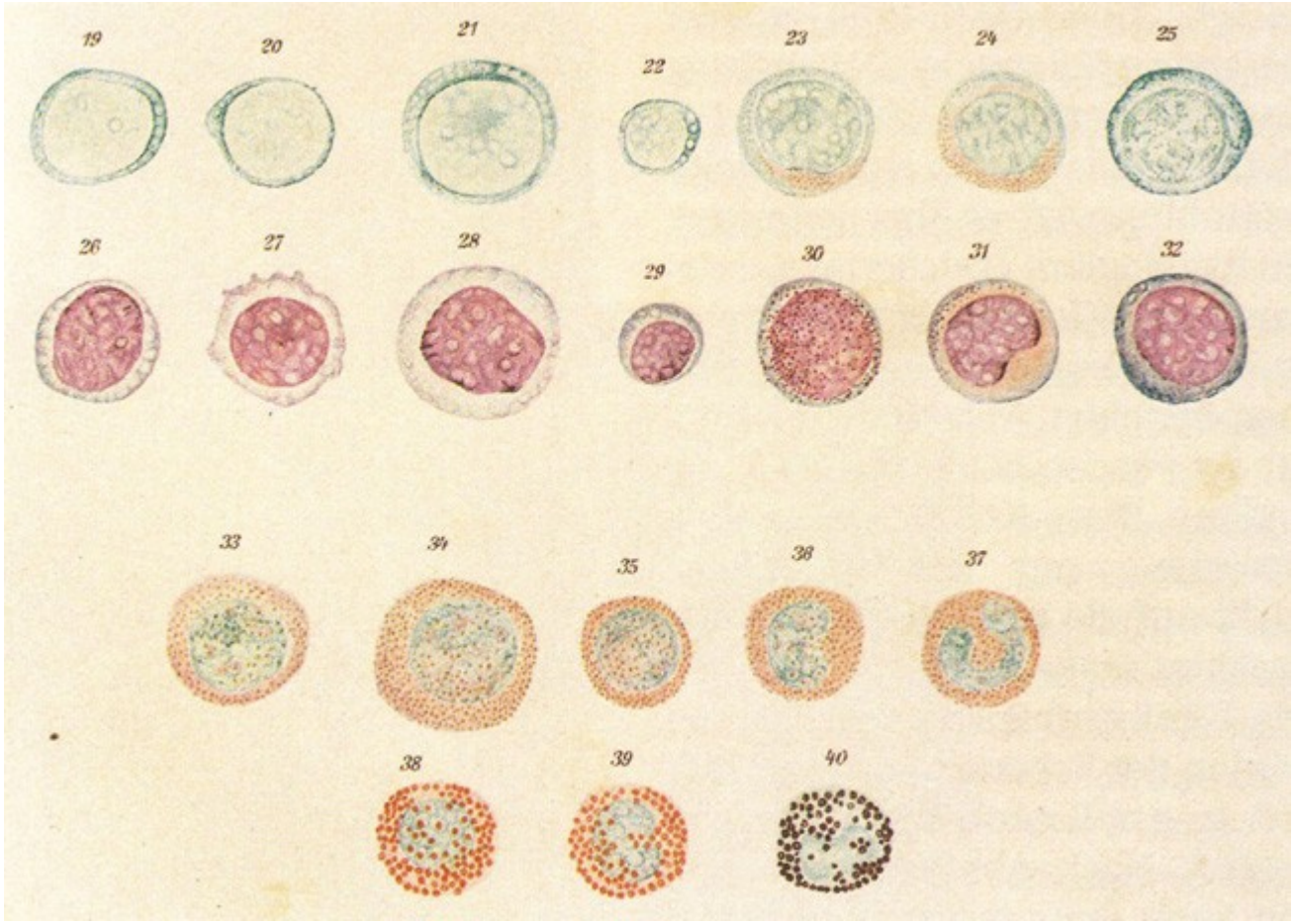
Development of Hematology

Development of Cancer Therapy

A R Zander

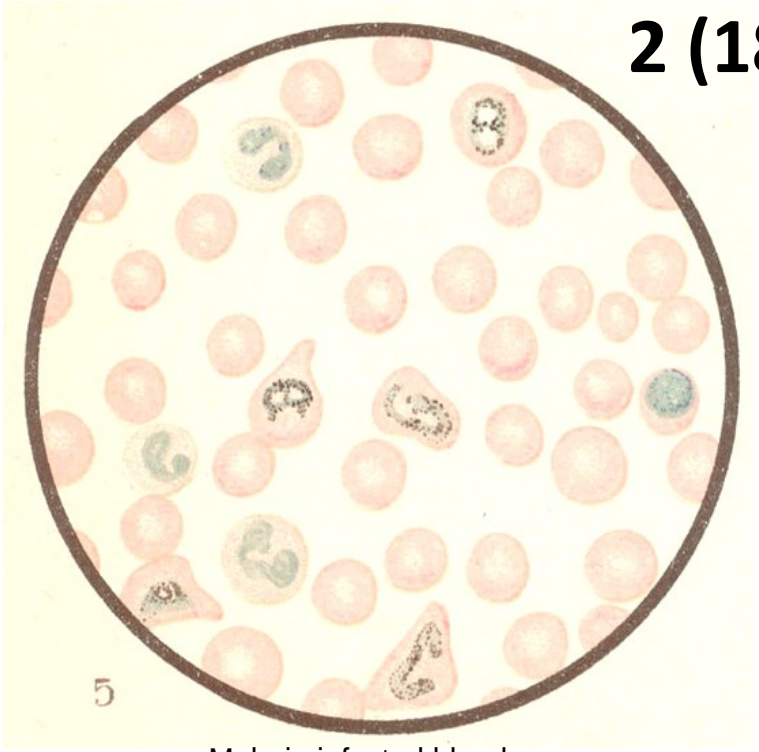
April 2014

1878: Medical student Paul Ehrlich earns his doctorate with “Contributions to theory and practice of histological staining” („Beiträge zur Theorie und Praxis der histologischen Färbung“)



Von Ehrlich eingefärbte Blutzellen

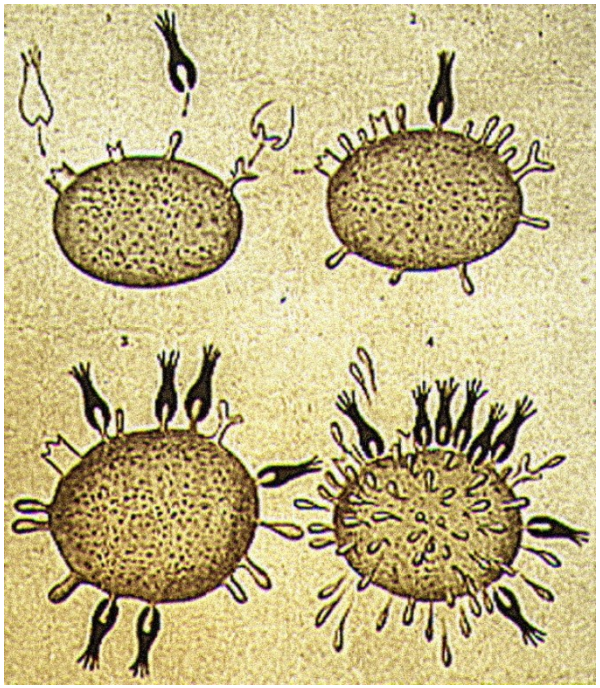
“On methylene blue and its clinical-bacterioscopic usage“ („Ueber das Methylenblau und seine klinisch-bakterioskopische Verwerthung“) Zeitschrift für klinische Medizin 2 (1882), 710-713



Malaria infected blood,
Methylene blue-eosin staining, 1897

1891: Paul Ehrlich and Paul Guttmann use methylen blue for the treatment of malaria – first steps towards the development of chemotherapy

1897: Paul Ehrlich proposes the **side-chain theory**



Wertbestimmung des Diphtherieserums, 1897, Klinisches Jahrbuch 6: 299-326

"The imperative to assume the toxin and antitoxin to be two such matched groups (such as a keys to a lock), should also give an indication as to how one could figure out this so puzzling origin of the antitoxins easiest. Most researchers probably accept Behring´s view that anti-bodies are reactive products of the living organism and not metabolites of the incorporated toxin.“

„Die zwingende Notwendigkeit, im Toxin und Antitoxin zwei derartig aufeinander abgepasste Gruppen (wie Schlüssel zu Schloss) anzunehmen, dürfte auch einen Hinweis darauf geben, wie man sich die so rätselhafte Entstehung der Antitoxine am leichtesten denken könnte. Es ist wohl von der Mehrzahl der Forscher die Ansicht Behring‘s acceptiert, dass die Antikörper Reaktionsprodukte des lebenden Organismus, nicht aber Umwandlungsprodukte des eingeführten Giftes darstellen.“

Theory about anti-bodies formation. Every cell posses side chains (receptors) that react and bind with specific chemical structures for metabolites or intruding toxins (antigens). This contact will induce the cell to produce and release copies of the binding side-chain in to the blood. Ehrlich described this reaction with “key-lock-principle” alike the one used first by Emil Fischer 1894 for enzyme/receptor binding.

►**1908 Nobel prize in medicine**.

Weapons against cancer:

Surgery

Radiotherapy

Chemotherapy

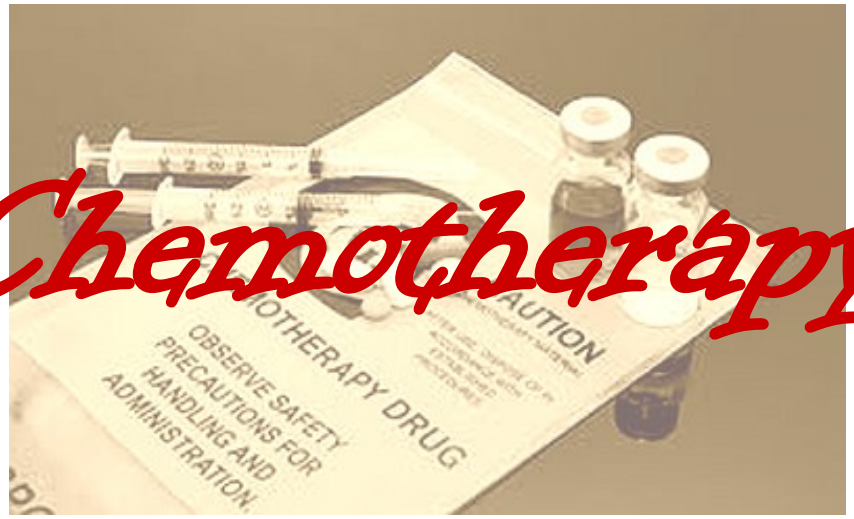
Stem cell transplantation

Immunotherapy

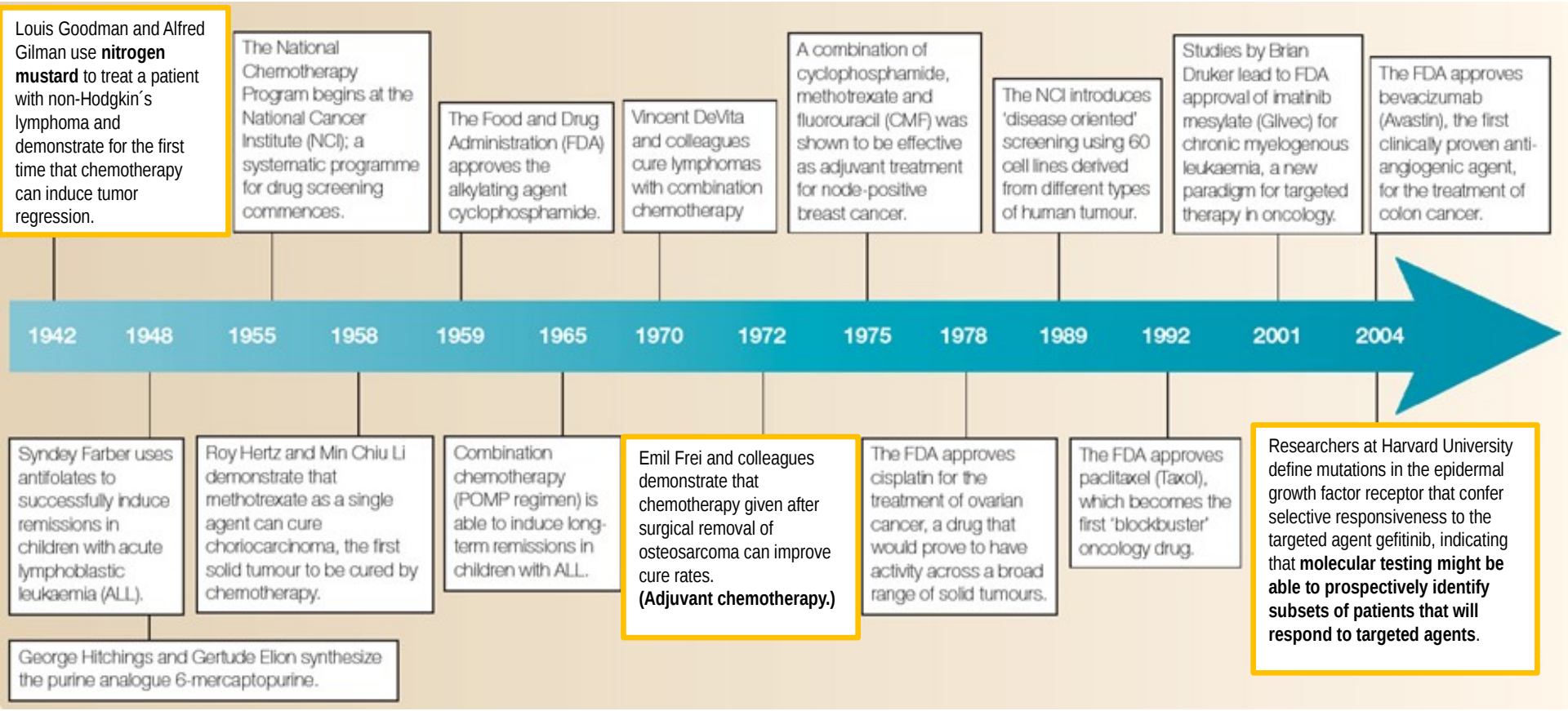
Chemotherapy

Stem cell transplantation

Chemotherapy

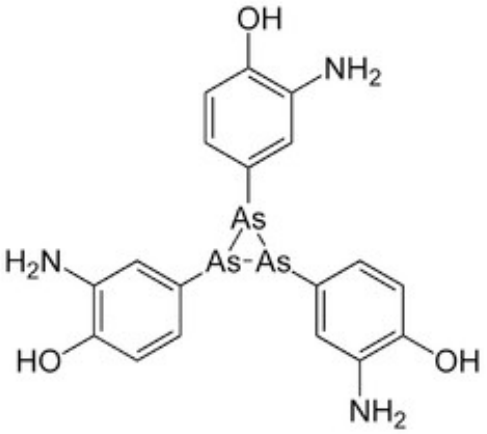


The history of chemotherapy



1909, Paul Ehrlich in Berlin seeks a cure for sleeping sickness and finds with his new colleagues Sahachiro Hata a cure for **syphilis**.

The **organic arsenic compound** arsphenamine cures the disease in three weeks and thus becomes the origin of chemotherapy.



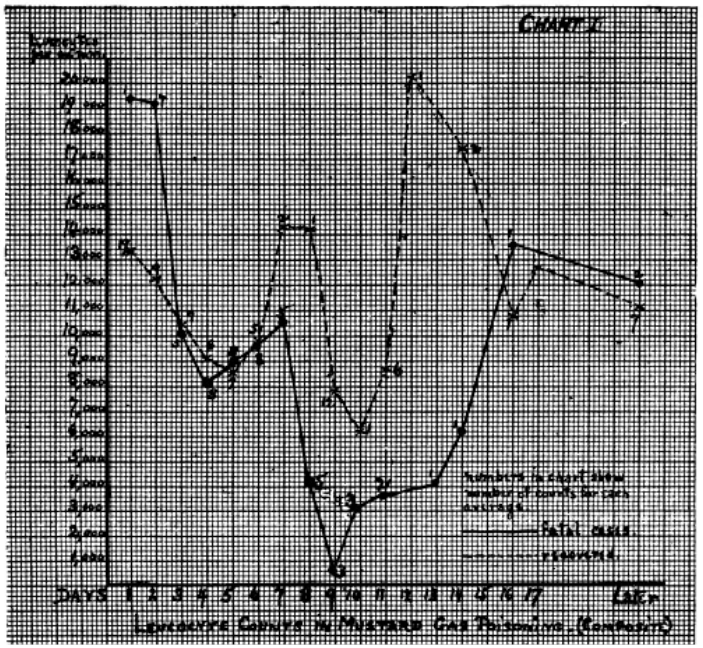
In the night of July 12, 1917, German troops used mustard gas at **Ypern** in Belgium for the first time.

1919: The American pathologist-couple **Krumbhaar** studies long-term effects. **Normal hematopoietic cells and the bone marrow were dehydrated**.

The victims were **anemic**, in need for blood transfusions and susceptible to infections.

The white cell count was below normal.

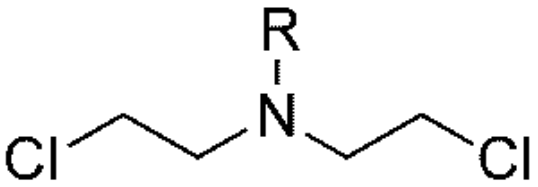
Due to post-war chaos and publication in second-rate journals, no attention was paid to this discovery.



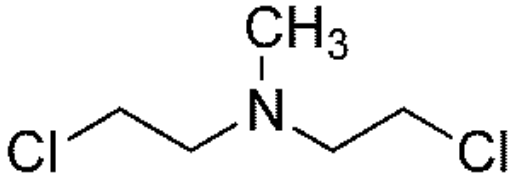
"Average leucocytic counts of fatal and non-fatal cases of yellow cross gas poisoning."
THE BLOOD AND BONE MARROW IN YELLOW CROSS GAS (MUSTARD GAS) POISONING.
Journal of Medical Research 1919

Nitrogen mustard gas/ yperite

After research into the symptoms and effects of the chemical warfare agent **mustard gas** development of cytostatics based on the less toxic nitrogen mustard



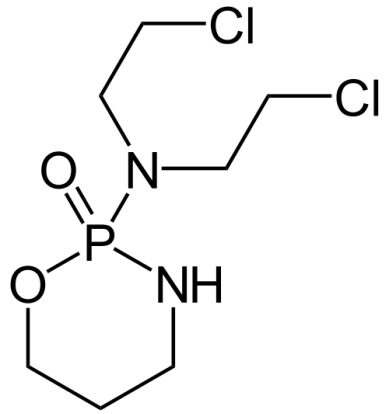
nitrogen mustard



mechlorethamine

was started after **World War 1**.

In 1942 during World War 2 **mechlorethamine** was used as first cytostatic eventually was used as first cytostatic agent against malignant lymphomas in the US. Less reactive drugs such as **cyclophosphamide** and **chlorambucil** are based on it.



cyclophosphamide

How mechlorethamine development started...

Alexander noticed during his tests that **white blood cells were killed** by the mustard gas. He recommended in his report the further study of mustard gas or its less toxic derivates as treatment against cancer.

This report reached the scientists **Luis Goodman und Alfred Gilman**, who observed this during studies for the US Army but did not pursued this seriously until then.

Liberty class freighter in World War 2.

After the *Harvey*-report, Goodman and Gilman started intensive research in 1943 and discovered together with Thomas Dougherty the efficacy of mechlorethamine against lymphomas in men and animal.

(S. Mukherjee : The Emperor of All Maladies, Harpercollins UK, 2011)

They suggested their discoveries to the surgeon Gustaf Lindskog as possible therapy – he in turn offered it to his patient J.D. as last hope treatment.
10 o'clock, August 27, 1942: First dose in a chemotherapy.
After initial response and interim remission JD died on day 96 of his treatment.
(Christakis P. The birth of chemotherapy at Yale. Bicentennial lecture series. Yale J Biol Med. 2011 Jun;84(2):169-72.

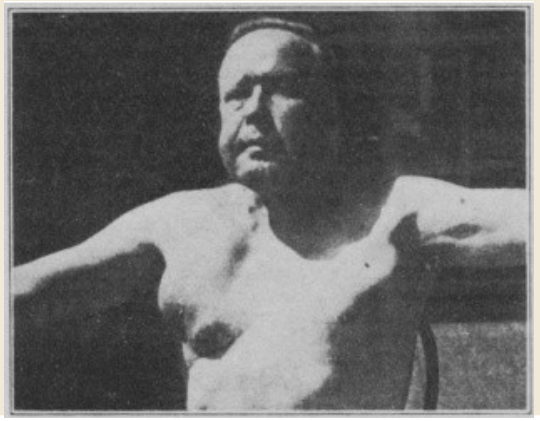
This therapy allowed treatment and healing of diseases like acute lymphoblastic leukemia (ALL), which had a mortality of 100% until then.

Development time < 1 year

Patient J.D., 48 years, silversmith.

Hospitalized in August 1942 at the **terminal stage of a lymphosarcoma**.

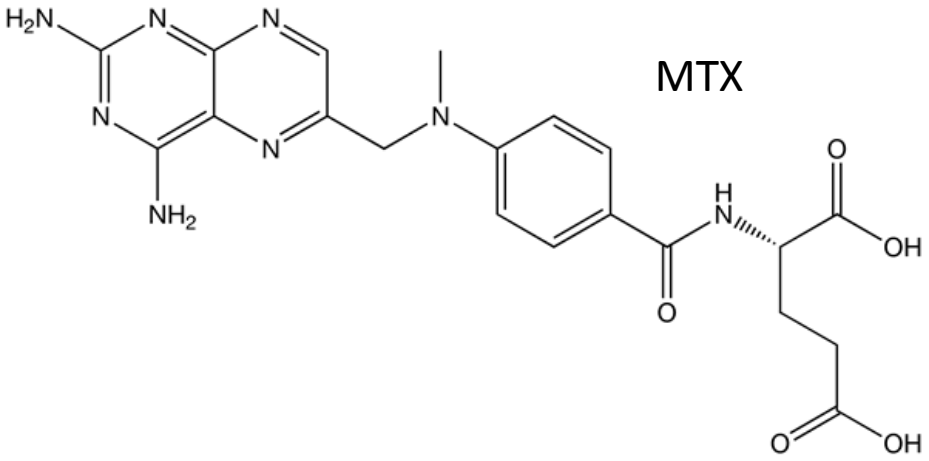
Anamnesis:
Swelling in the right neck.
Radiotherapy (March 41) and palliative local resection (June 41).
Relapse December 41.New RT..
Since May 42 rapid growth and dissemination to axilla, mediastinum and face. No response to treatment.



Folic acid antagonists

After WW2 the pathologist Syndney Farber studied the effect of folic acid on leukemia patients and noticed that it promotes the progression of acute lymphocytic leukemia (ALL). In a first example for drug design, Farber, Harriett Kilts and Yellapragada Subbarao developed substances that interfered with the folic acid metabolism by inhibiting DNA- and RNA-synthesis respectively: Folic acid antagonists.

Methotrexat (MTX),
is an example for this class
of substance.



Combination- or polychemo-therapies

1965: James Holland, Emil Freireich & Emil Frei suggest to combine chemotherapeutic drugs with different mode of actions within a therapy. Thereby making it harder for the cancer cells to develop resistances.

Inspiration:
combination therapy for tuberculosis.

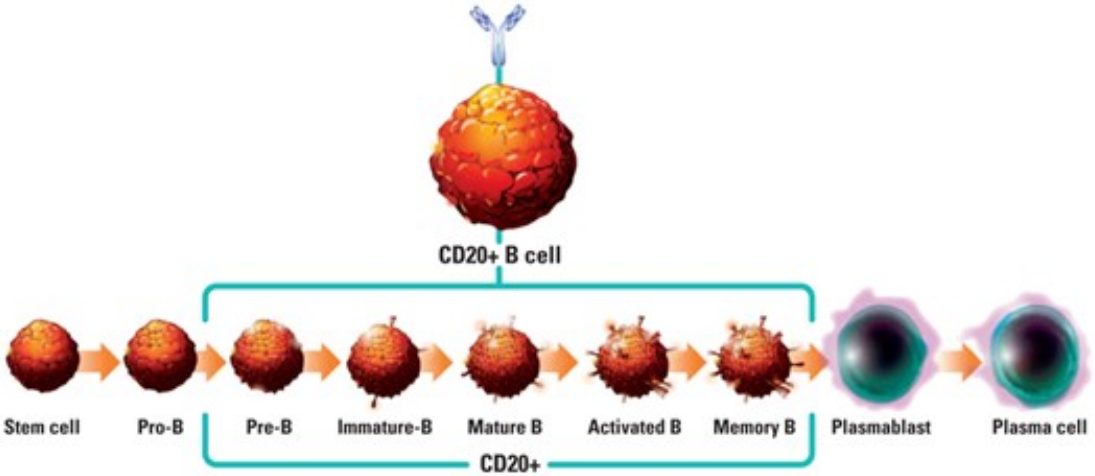
Example: COPP-regimen for T- and B-cell-lymphoma		
Cyclophosphamide	650 mg/m² i.v.	Day 1 + 8
Vincristine	1,4 mg/m2 i.v.	Day 1 + 8
Procarbazine	100 mg/m² p.o.	Day 1-14
Prednisolone	40 mg/m² p.o.	Day 1-14

Targeted Therapies

These therapies attack specific molecules responsible for growth and development within the cancer cells. Their “targeted” approach preserves normal body cells.

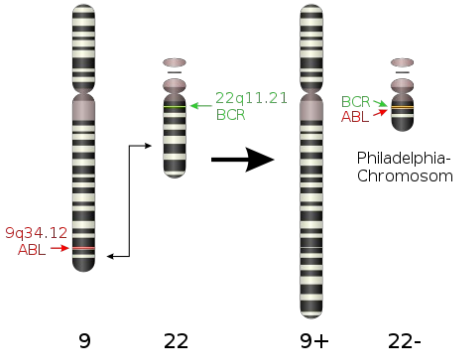
Mostly monoclonal antibodies are used (names ending with “mab“) or small molecules (ending on “mib” or “nib“).

In 1997 Rituximab was approved as the first cancer drug of this kind. It is used for example against Non-Hodgkins Lymphomas.



Example: Chronic Myeloid Leukemia (CML) – the Imatinib-story

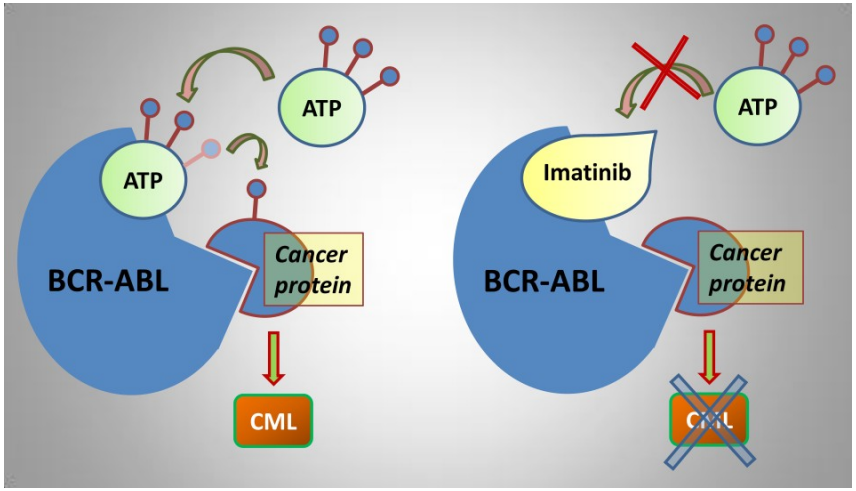
A chromosome break causes a translocation and creates the fusion genes BCR-ABL and ABL-BCR.
ABL codes for tyrosine kinase (TK). The fusion BCR-ABL is permanently activated (oncogene).
Affected cells proliferate rapidly and uncontrolled.
More than 90% of CML-Patients possess the BCR-ABL-translocation.



Although there are many TK-enzymes, **Imatinib** is a specific **TK-inhibitor** for Abl, c-kit and PDGF-R. It occupies their active site for ATP. Normal cells can compensate this blockade through alternative metabolic path ways, cancer calls for the most part not.

Resistance

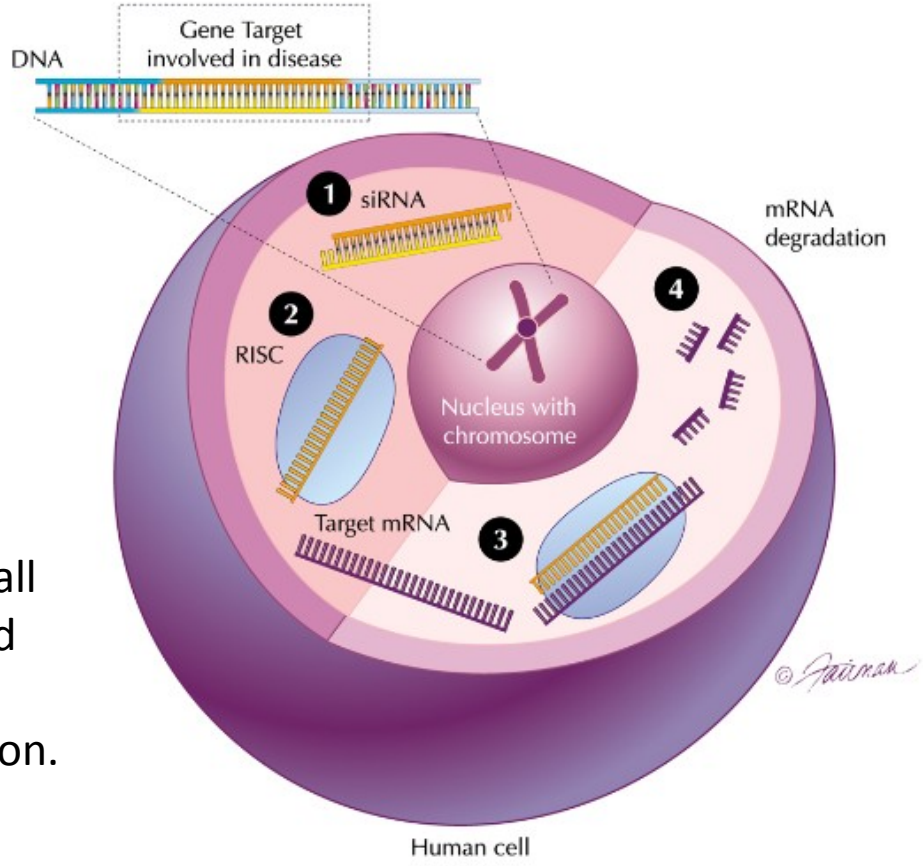
BCR-ABL-mutations can interfere with the binding of Imatinib to the active site for ATP.
TK-inhibitors of the 2nd generation like **Nilotinib** and **Dasatinib** can help such imatinib-resistant patients.



New developments

RNA-Interference (RNAi, RNA-Silencing)

This natural mechanism of eukaryotic cells allows for the **factual deactivation of specific genes** by destruction of their mRNA. Thus the production of the corresponding proteins is stopped.



For this targeted mRNA is intercepted by small interfering RNA (siRNA) and the RNA-induced silencing complex (RISC) or by Micro-RNA (miRNA). Both ways lead to mRNA degradation.

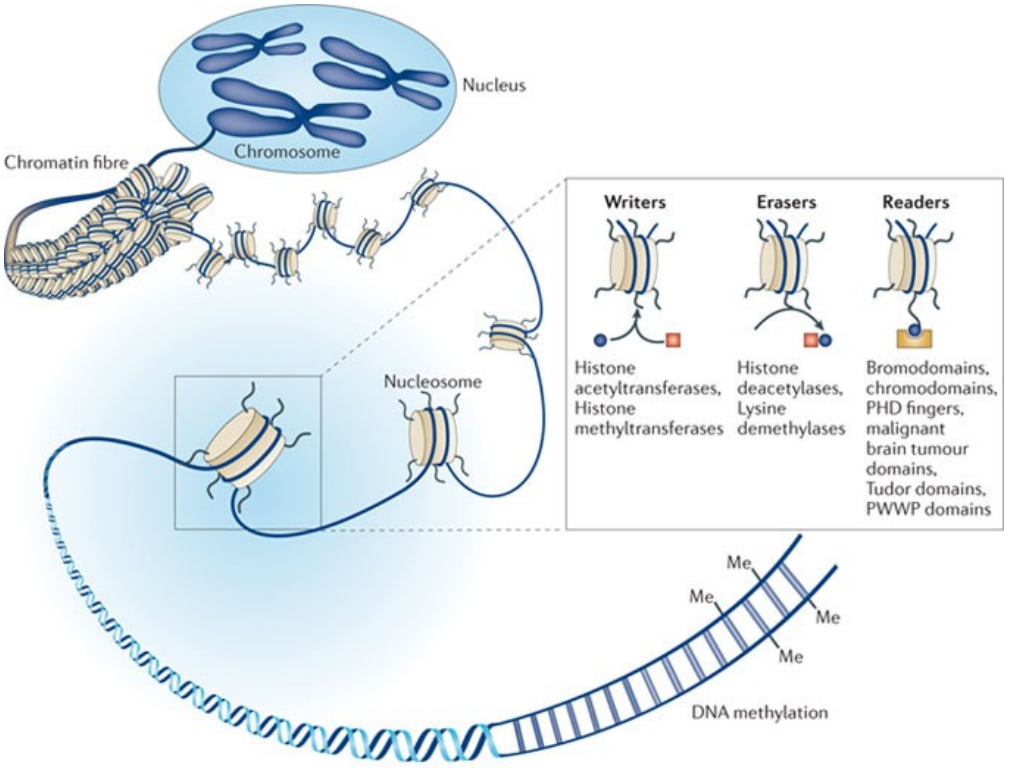
Its accuracy makes this mechanism an important tool for research and target for drug development. Even with setbacks like the failure of **Bevasiranib** in phase III, which caused Hoffman-La Roche and other major pharmaceutical companies to abandon research into siRNA, this remains a promising vantage point for new therapies.

New developments

Epigenetic drugs

Epigenetic regulation affects the phenoptype of the cell, which can also cause disease. On a molecular level cell characteristics are **modified by proteins such as histones**. For instance, an acetylation of a histone will make it more compact and less accessible for RNA. Methylation can increase or inhibit their activity depending on circumstances .

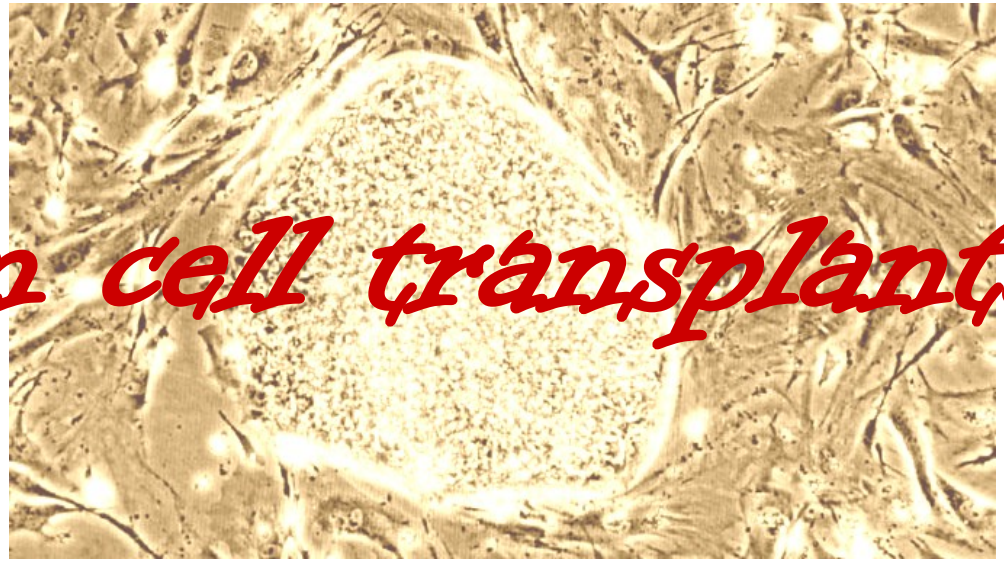
This approach to therapy aims to fight diseases by modulation of such molecular factors. First examples are already approved inhibitors for DNA-methylation or hystone-deactylase (HDAC).



Substance group of cytostatic drugs

- **Antimetabolites**
  Replace natural molecules in metabolic pathways within cancer cells, in this way interfering with the metabolic process. Causes malfunction and blockade of essential enzymes.
  Example: Methotrexate (MTX).
- **Alkylating Agents**
  Agents, or their reactive intermediates, form covalent bonds with DNA to form an adduct in which a methyl or ethyl group is added. This makes the duplication of the genetic material impossible and thus stops proliferation of the cancer cells.
  Examples: Cyclophosphamide und Cisplatin.
- **Topoisomerase-inhibitors (enzyme)**
  Bind with topoisomerase enzymes and interrupt DNA replication in cancer cells.
  Example: Topotecan.
- **Mitotic-inhibitors**
  Interfere with cell division by disrupting microtubules.
  Examples: Vinca alkaloids from the hallucinogenic plant Madagascar Periwinkle (*catharanthus roseus* and taxanes from yew-trees (taxus).
- **Antibiotics**
  Examples: Epirubicin and Mitoxantrone.
- **Biological cytostatic drugs**
  Fight cancer similar to the immune system. Mainly anti-bodies and cytokines.
  Examples: Rituximab (anti-bodies) or tumor necrosis factor (cytokine).
- **Other cytostatic drugs**
  For example Gefitinib or Imatinib

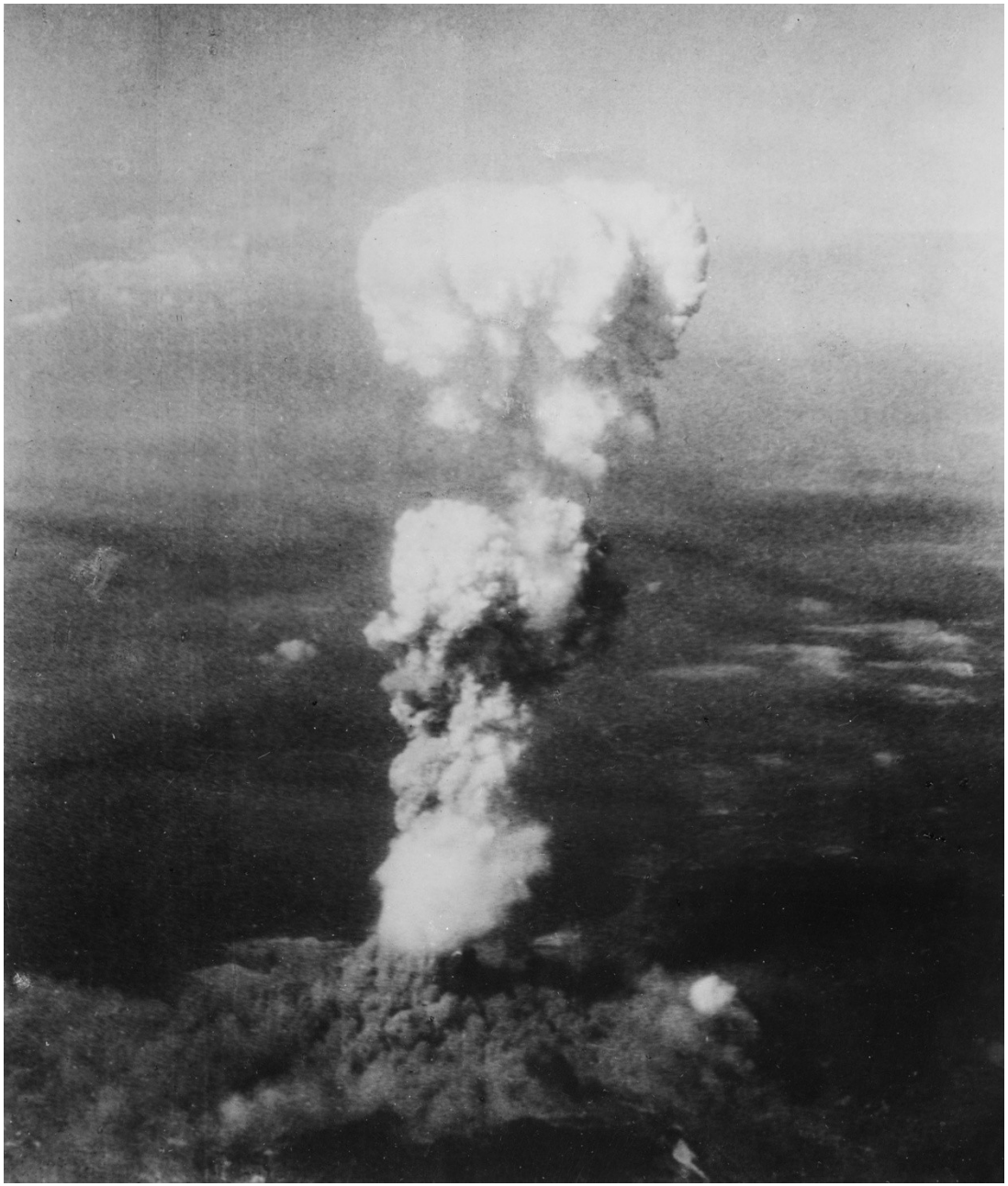
Stem cell transplantation



“The Lymphocyte as a stem cell common to different blood elements in embryonic development and during the post-fetal life of mammals”

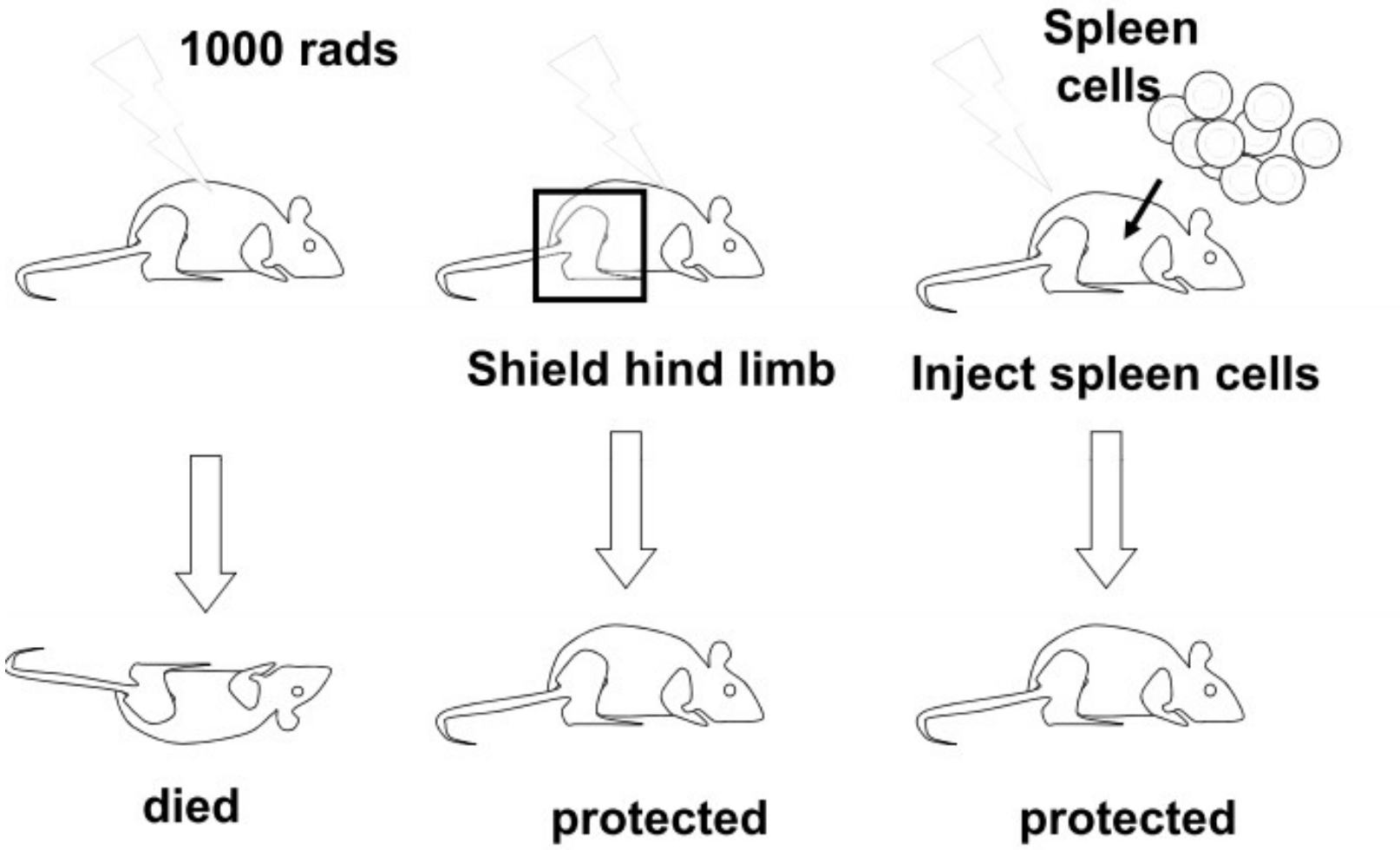
„Der Lymphozyt als gemeinsame Stammzelleder verschiedenen Blutelemente in der embryonalen Entwicklung und im postfetalen Leben der Säugetiere“

Folia Haematologica
8.1909, 125-134.



Hiroshima, 1945

Leon Jacobsons´s experiments on radio protection

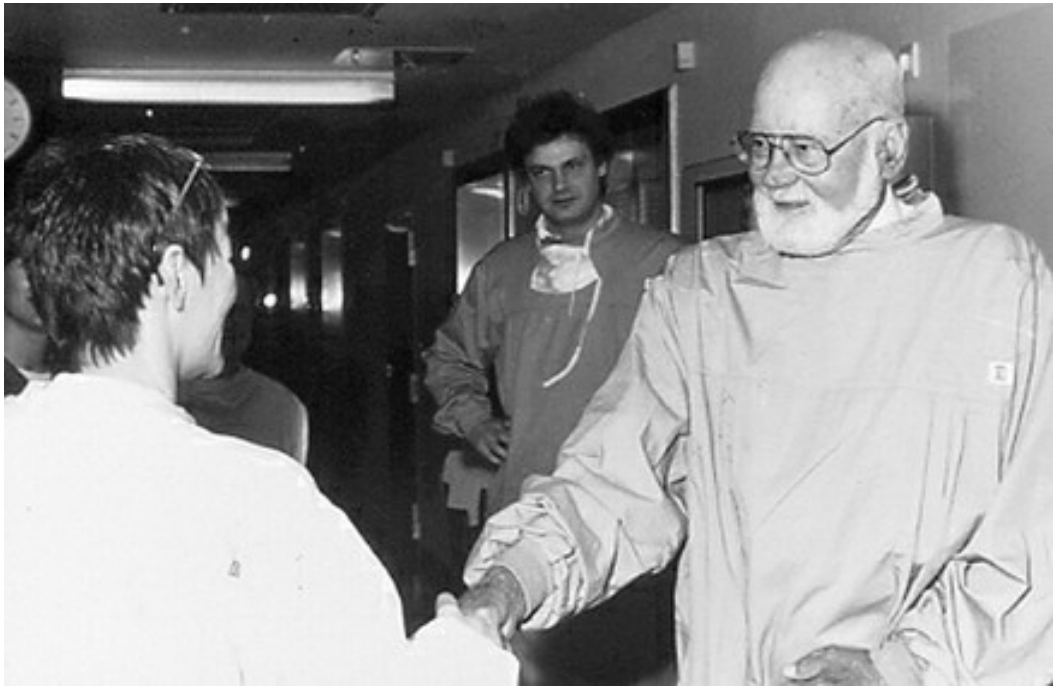


Jacobson, L.O., Marks, E.K., Robson, M.J., Gaston, E.O. & Zirkle, R.E.
Effect of spleen protection on mortality following x-irradiation.
Journal of Laboratory and Clinical Medicine, 34 (1949), 1538–1543.

Stem cell transplantation milestones

1956: E. Donnall Thomas performed first stem cell transplantation in a patient with progressed leukemia.

1969: Thomas performed first successful allogeneic stem cell transplantation.



Don Thomas visits the University Hospital in Hamburg (UKE), January 1990

Stem cell transplanation milestone

1980 Autologous transplantation

1985 Allogeneic transplantation

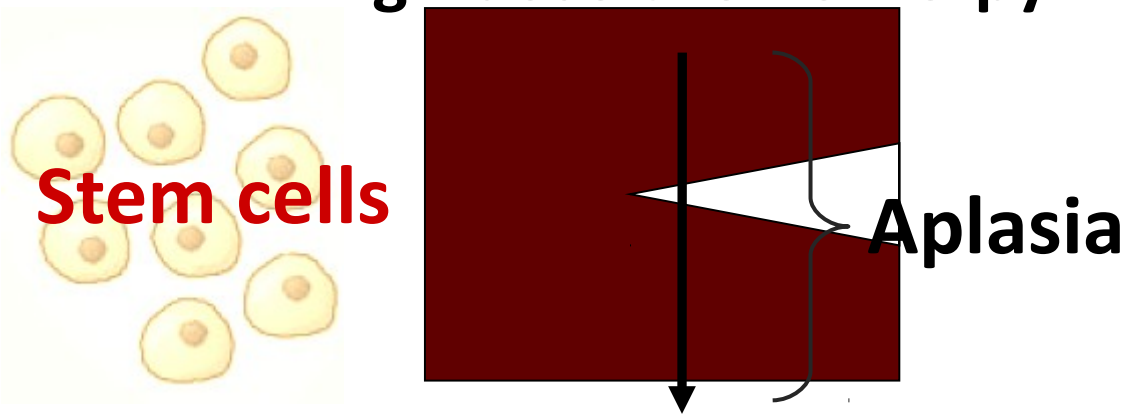
1987 Transplantation of hematopoietic stem cells

1990 Stem cell transplantation from cord blood

1997 Transplantation of human stem cells after reduced-intensity conditioning (RIC)

Stem cell transplantation

Conditioning with
high-dose chemotherpy



Engraftment

Formation of a new immune system from the donated stem cells

Stem cell therapy for…

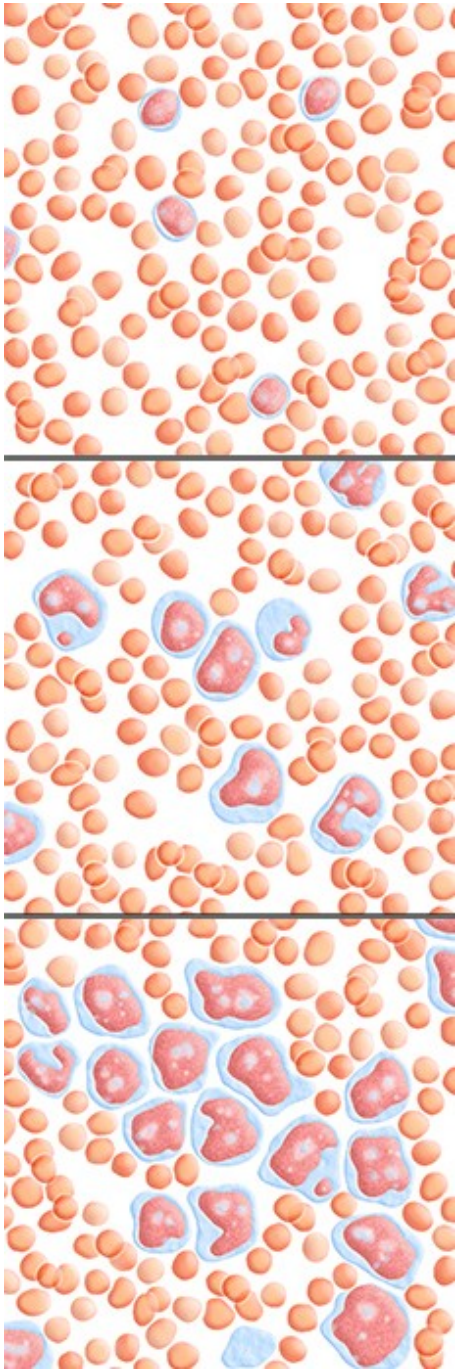
- Acute Myeloid Leukemia (AML)
- Acute Lymphoblastic Leukemia (ALL)
- Chronic Myeloid Leukemia (CML)
- Chronic Lymphocytic Leukemia (CLL)
- Osteomyelofibrosis (OMF)
- Non-Hodgkin-Lymphoma (NHL)
- Hodgkin-Lymphoma (Hodgkin's Disease – HD)
- Severe Aplastic Anemia (SAA)

CML

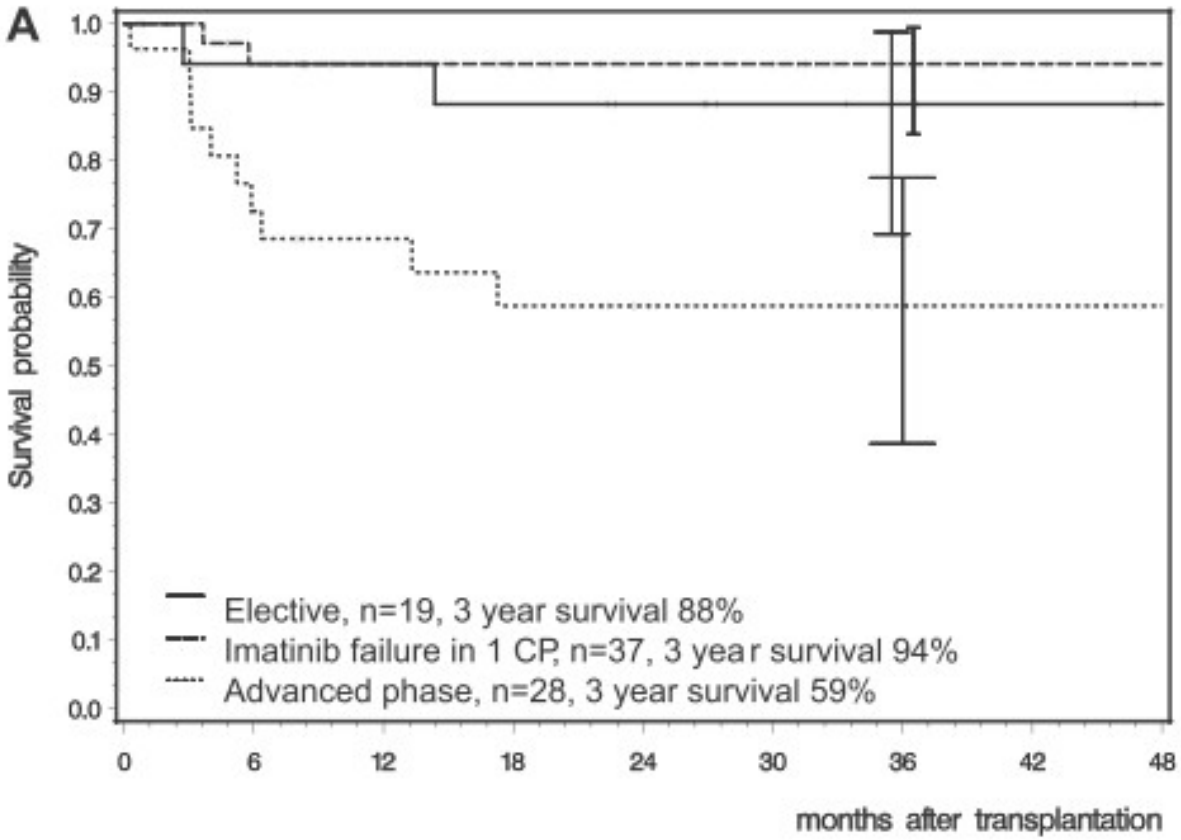
Typical Progress

- Chronic phase
- Accelerated phase
- Blast crisis

Chronic phase	Advanced phases	
	Accelerated phase	Blast crisis
Median duration 5-6 years	Median duration 6-9 months	Median survival 3-6 months



CML



Patients at risk (n)

Month	0	12	24	36	48
Elective	19	16	13	9	5
Imatinib failure	37	27	17	10	3
Advanced phase	28	14	8	5	2

(A) After allo-SCT.
Patients with **elective SCT** in first CP (n =20; group I) and patients who with SCT **after imatinib** failure in first CP (n = 36; group II) had a 3-year survival probability of 88% and 94% (CI: 69.3-98.7 and 83.9-99.4), respectively; patients with SCT in advanced disease (n = 28; group III) had a 3-year survival probability of 59% (CI: 38.6-77.5).
Tick marks indicate last observation of living patients.

Saussele S, Lauseker M, Gratwohl A *et al*. Allogeneic hematopoietic stem cell transplantation (allo SCT) for chronic myeloid leukemia in the imatinib era: evaluation of its impact within a subgroup of the randomized German CML Study IV. Blood 2010; 115: 1880–1885.

CML

As new, non-curative drugs, TK-inhibitors have mostly replaced curative therapies like allogeneic STC.

„Window of opportunity“.

“In view of the curative potential of allo-SCT and survival results that were equally good as with imatinib treatment, allo-SCT could become the preferred second-line option after failure of first-line TKI therapy for suitable patients with a donor.”

LETTER TO THE EDITOR “Outcome of patients with CML after SCT in the era of tyrosine kinase Inhibitors”
S Saussele, R Hehlmann, A Gratwohl and A Hochhaus
Bone Marrow Transplantation (2012) 47, 304; doi:10.1038/bmt.2011.70; published online 28 March 2011

CML

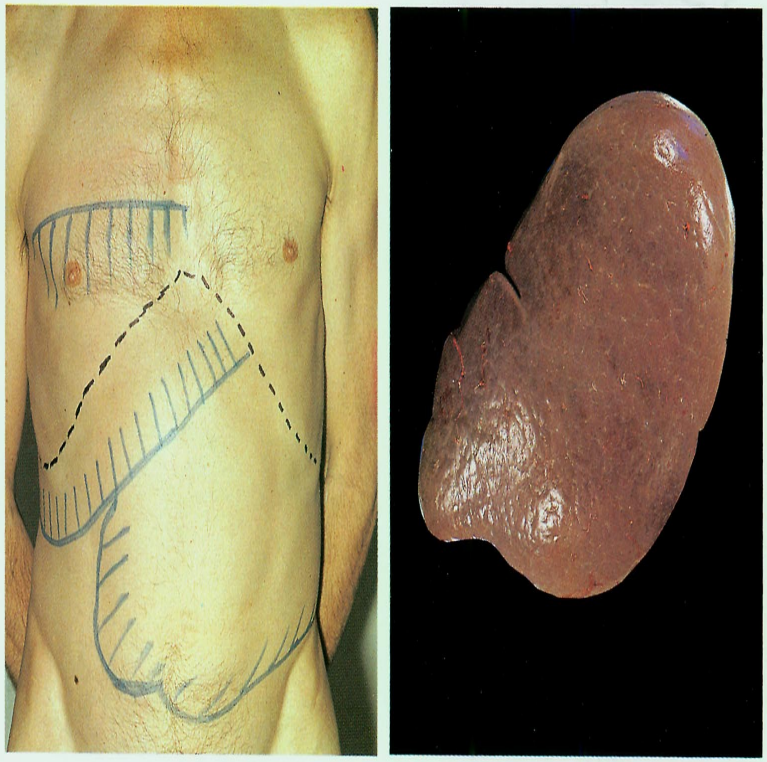
What next?

Target:
Optimal interaction of all possible therapies.

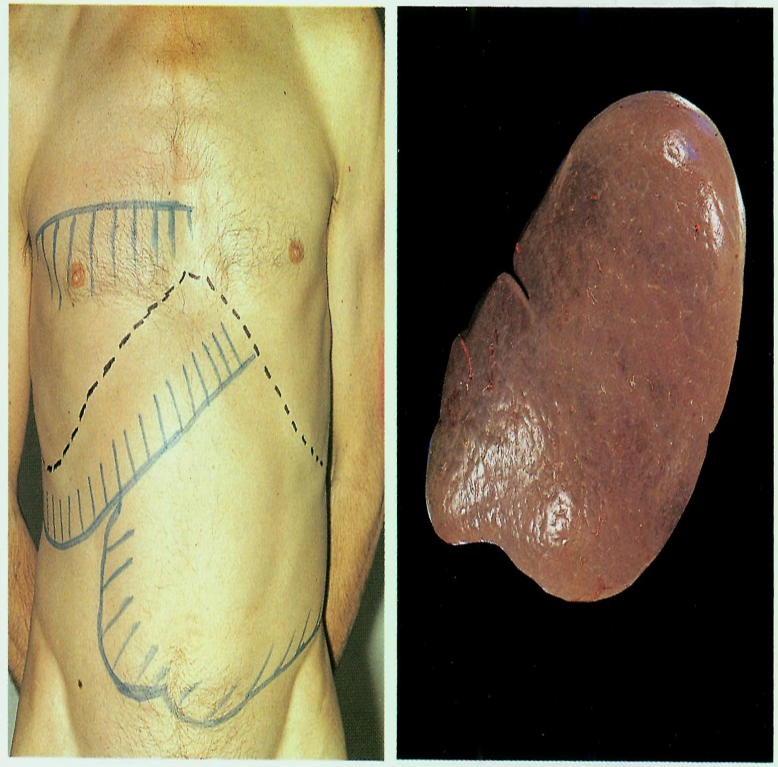
(Drugs, stem cell transplantation, cellular therapy)

Requirements:
Good clinical diagnostics
Cytology
FACS
Molecular diagnostics

OMF



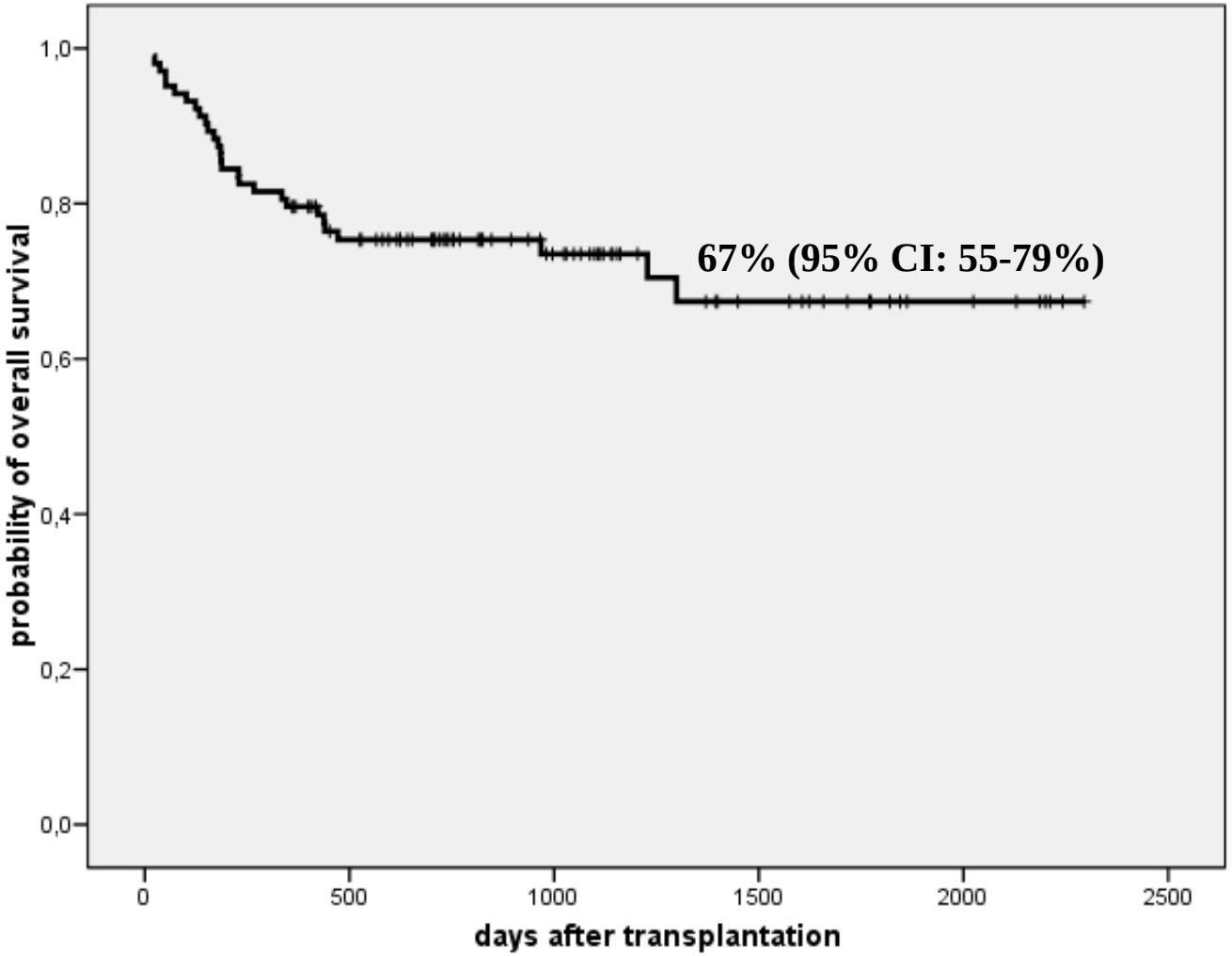
Osteomyelofibrose: (Links) Splenohepatomegalie; (rechts) am oberen Rand weist die Milz eine umschriebene Einkerbung auf. Die tiefe Einziehung am unteren Rand war bei der klinischen Untersuchung tastbar.



Osteomyelofibrose: (Links) Splenohepatomegalie; (rechts) am oberen Rand weist die Milz eine umschriebene Einkerbung auf. Die tiefe Einziehung am unteren Rand war bei der klinischen Untersuchung tastbar.

OMF

Survival after a median follow-up of 3 years after stem cell transplantation



OMF

JAK2 Inhibitor: Ruxolitinib

- COMFORT – 1 Study
- Double blind, placebo-controlled trial
- IPSS intermit 2, +, 3
  - N = 309
  - R = 155
  - Placebo = 154
- Results:

Symptoms ↓

Spleen Size ↓

Survival ↑

→8.4% vs 15.6% death

COMFORT
Controlled Myelofibrosis Study with Oral JAK Inhibitor Treatment

S. Verstovsek NEJM 2012

OMF

Stem cell transplantation and molecular therapy

Stem cell therapy

- Curative
- Early transplantation→ Better results
- HLA identical (MRD, MUD) → Best result
- TRM ~ 10% -13%

Molecular therapy

- Most likely not curative
- Mid-level toxicity
- Better survival compared to placebo control group

OMF

Combined approach: Middle and high risk OMF

- Induction with Molecular Therapy
- HLA identical donor → transplant
- No identical donor
- 2nd or 3rd Molecular Therapy
- Alternate donor
- And MUD, Haplo identical, Cord

OMF

Comparatve study

High and Intermediate Risk OMF

Induction: Molecular Therapy

HLA Identical MRD, MUD → SCT

No HLA identical Donor → Sequential Molecular Therapy → Alternate Donor SCT

(off study)

Stem cell transplantation

versus

molecular therapy

The problem

For many hemtological diseases a allogeneic stem cell transplantion is the only curative therapy.
For example:
CML, OMF, myelodysplastic syndrom (MDS) andd multiple myeloma (MM).

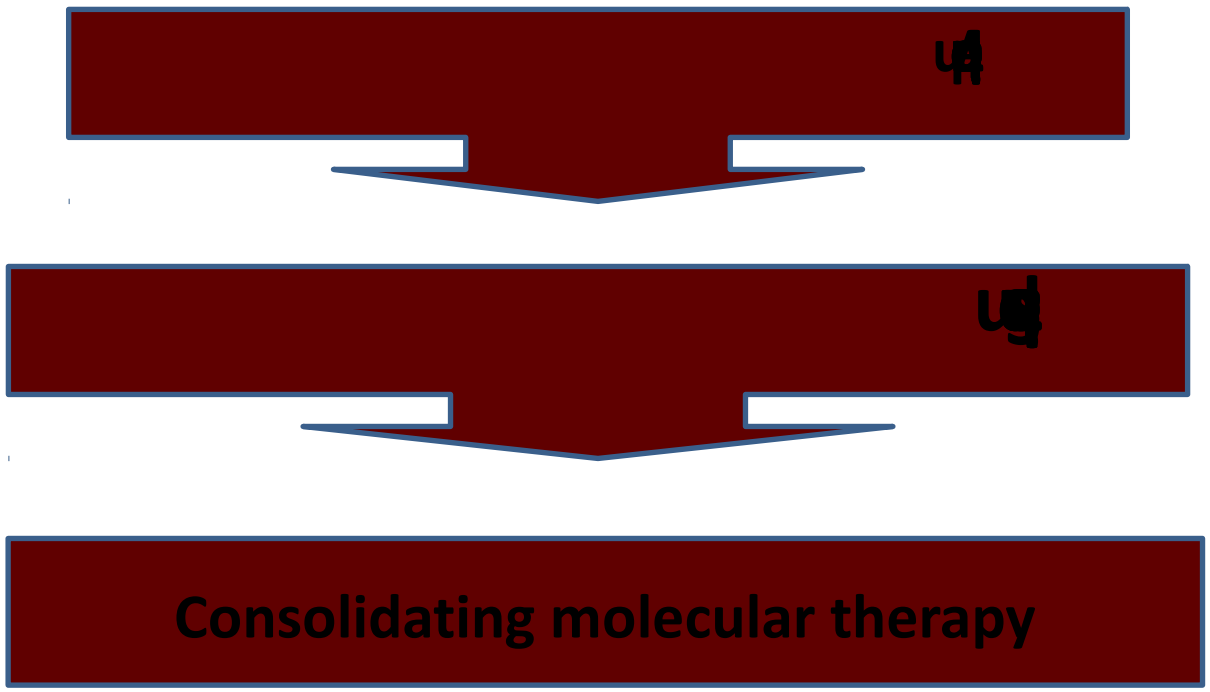
Allogeneic transplantation have a significant morbidity and mortality.

New molecular therapies are less toxic and more efficient with all these diseases.

CML: TKI-inhibitors.
OMF: Jak2-inhibitors et and others.
MDS: Revlimide, VIDAZA, and others.
MM: Revlimide, Velicade, and others.

The solution

Combination of molecular therapy and stem cell transplantation



New Ways: Research Blockbuster-Style

Drug development: Old school

Development time:

12 – 20 years

Costs:

1,000,000,000 $ or more for *one single drug* to become market-ready!

99% of the initially studied substances are not suitable as therapeutic drug.

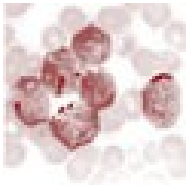
European LeukemiaNet

194 centers in 39 countries with over 1,000 researchers.

Aims to bring the 108 leading research groups (CML, AML, ALL, CLL, MDS, CMPD), their 105 partners from other areas (e.g. for diagnostics) , major pharmaceutical companies and small and medium sized companies together into one big network.

Funding:
2004-2011 by the EU with 6 million €, since 2011 through a foundation.

ELN structure



Project 6
Acute Lymphoblastic Leukemia (ALL)

European LeukemiaNet

Aims:

- Cooperation, pooling of data and information.
- Defining diagnostic standards and joint implementation of new procedures and methods.
- Documentation and harmonization of European ALL-studies.
- Development of joint prognostic models.
- International meta-analysis of rare ALL-subtypes.
- Joint phase-I/II-studies
- Research into the role of gender for development of ALL in grown-ups.
- Setting-up a virtual “center of excellence” for diagnostics and therapy.

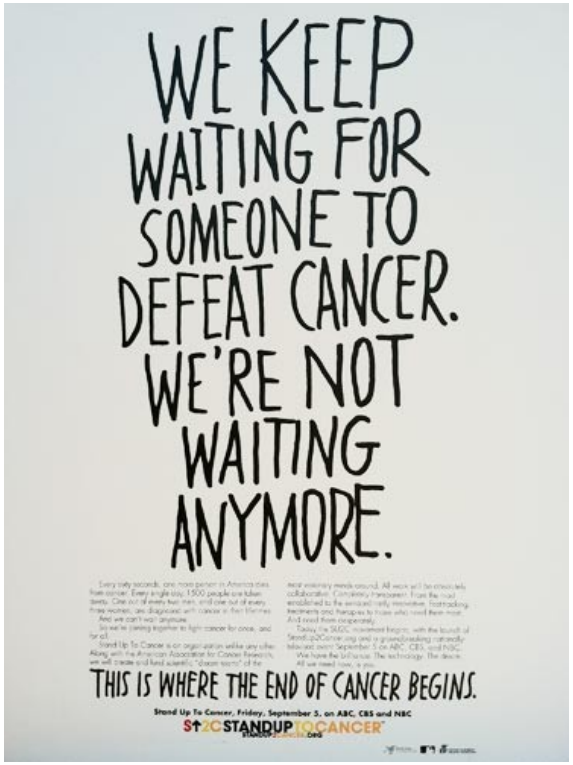
Lead Participants:
D. Hoelzer (G), N. Gökbuget (G), R. Foà (I), R. Willemze (NL), R. Bassan (I), H. Dombret (F), J.M. Ribera (ES)

STAND UP TO CANCER™

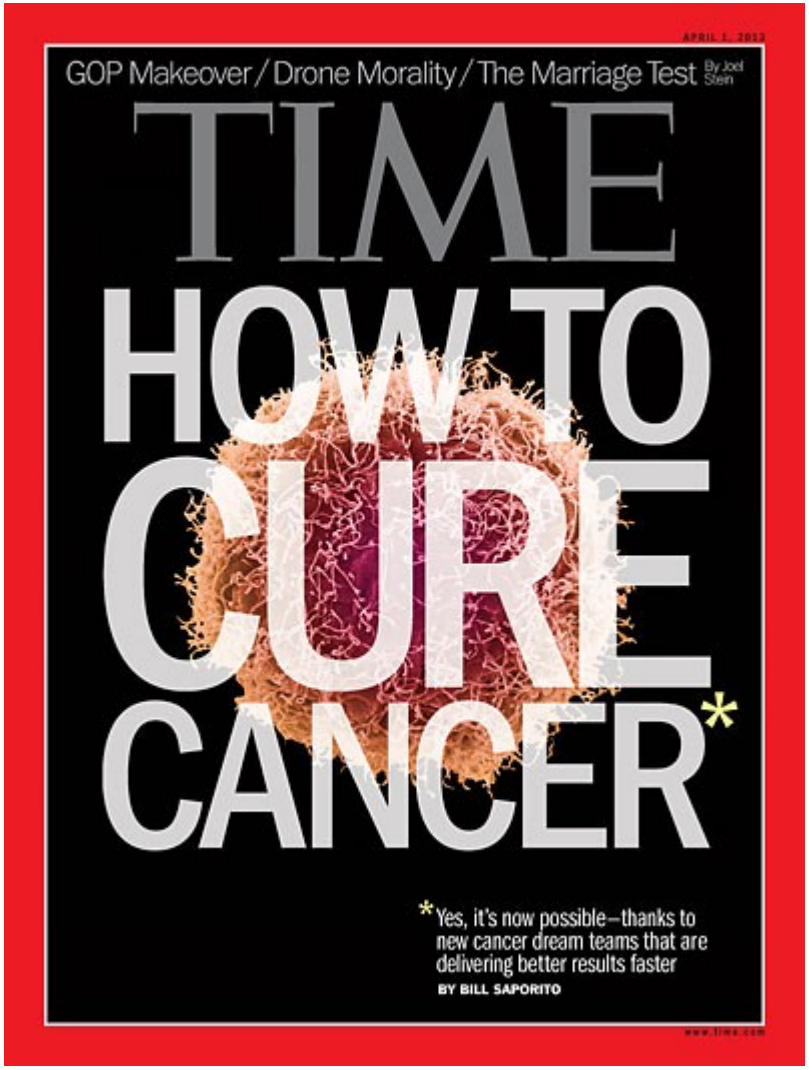
↘ THIS IS WHERE **THE END OF CANCER BEGINS**

“Like making a good Hollywood movie: Get the best people from all over – let ‘em brainstorm together – and give ‘em all the money they need.”

Traditional research takes a lot of time and is insufficiently funded.
The National Cancer Institute (NCI) and others took this idea. The campaign started in 2008.
Major, interdisciplinary programs with the best scientists and experts (“Dream-Teams“) are generously funded. For example the “Breast-Cancer-Dream-Team“ received 17.5 million $.



By now “SU2C“ over 244 articles were published, 48 clinical studies with over 3000 patients are started or planned.
In 2013 over 800 substances were under research.


APRIL 1, 2013
GOP Makeover / Drone Morality / The Marriage Test By Joel Stein
TIME
HOW TO
CURE
CANCER*
*Yes, it's now possible—thanks to new cancer dream teams that are delivering better results faster
BY BILL SAPORITO
www.time.com

SU2C Dream Team

Targeting the PI3K Pathway in Women's Cancers

Funding: 15 million $

Mutations of the phosphatidylinositol-3-Kinase (PI3K)

- Ovarian cancer
- Breast cancer
- Endometrial cancer

Results:

- Integration of all participating laboratories so that internal data can be accessed.
- >2000 tumor samples were analyzed for their specific mutation
- 14 clinical studies are underway or were conducted.

Team:

U.a. MD Anderson Cancer Center (Houston), Memorial Sloan-Kettering Cancer Center (New York), Dana-Farber Cancer Institute (Boston), Vanderbilt-Ingram Cancer Center (Nashville), Vall d'Hebron Institute of Oncology (Barcelona)

Strategie

I. Diagnostics

II. Gene-sequencing

III. Targeted Drugs

IV. Epigenetics

V. Bioengineering

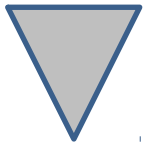
VI. Pattern Recognition

VII. Financing

I. Diagnostics

Smoking and lung cancer

US government panel 2013: CT for all older smokers!

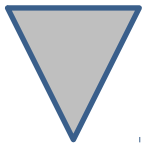


Mortality lung cancer 20%

Blood test: Biomarkers

II. Gene-sequencing

DNA analysis of tumor types and patients.



A personal and best fitting therapy.

III. Targeted Drugs

More than 800 substances for specific mutations are under research.

Even more are to be tested.

Many research facilities need to work together to do the neccessary clinical testing.

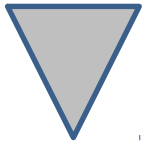
IV. Epigenetics

DNA-demethylation can neutralize genes, which would cause malignant cell proliferation.

Other therapies can reactivate genes which will stop cancer stem cells from self-renewal.

V. Bioengineering

Circulating tumor cells (CTC)
Chip with 78000 microposts on a surface the size of a bussiness card that can trap such cells.



Early recognition.
Easier and better analysis.

VI. Pattern Recognition

Studies for determining the genetic mutations and monitoring therapy success.

Management und analysis from resulting gene-sequenzing data.

VII. Finanancing

Traditional:
Results from research are not shared.
Reputation only for the “principal investigators“.

New:
Former rivals work together.
Many experts contribute their ideas.
Energizing and public campaigning for donations and funding.

Pharma-collaboration

BCRA1-mutated and triple-negative breast cancer (TNBC) can be cured in a mice model through a combination of a PI3K- and a PARP-inhibitor.

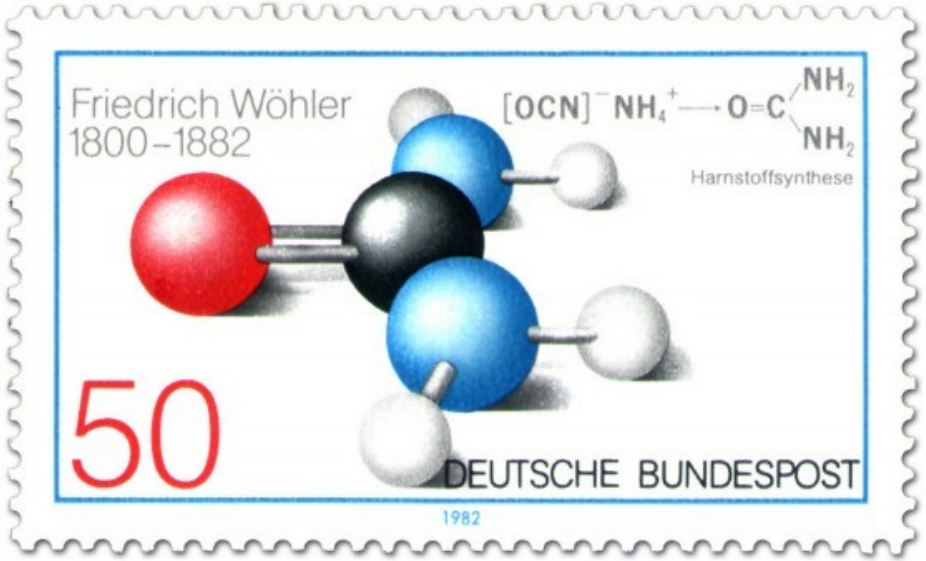
Clinical study:
Two not yet approved drugs:
Pi3K-inhibitor (Novartis)
PARP-inhibitor (Astra-Zeneca)

"Ich kann, so zu sagen, mein chemisches Wasser nicht halten und muß Ihnen sagen, daß ich Harnstoff machen kann, ohne dazu Nieren oder überhaupt ein Tier, sey es Mensch oder Hund, nöthig zu haben."
Friedrich Wöhler in einem Brief vom 22.Februar 1828

„Über künstliche Bildung des Harnstoffs" Annalen der Physik und Chemie, 87, 2 (1928), 253-256

Metaphysical storm:
An organic substance is made form anorganic salts!
The mythos of life (vitalism) is challenged.

Bridge between chemistry and biology.



THE EMPEROR OF ALL MALADIES

A BIOGRAPHY OF CANCER

SIDDHARTHA MUKHERJEE