

5.1 Hematopoietic Stem Cell Technology (Harvesting, Culture, Purging)

B. Deschler, C.I. Müller, C. F. Waller, M. Engelhardt

Def: Stem Cells

Hematopoietic stem cells are a small and predominantly dormant population of undifferentiated cells. They are characterized by the ability to self renew by continuous cell division and to differentiate into lymphoid, myeloid, erythroid, or megakaryocytic cells (► Chap. 1.3). Hematopoietic stem cells transplanted after radiotherapy (total body irradiation) and/or high-dose chemotherapy from autologous or allogeneic sources are subjected to intense proliferation and differentiation inside the recipient. The contribution of individual cell types to short- and long-term bone marrow recovery after transplantation has not been fully elucidated, however, stem cells are responsible for maintaining continuous hematopoiesis.

Meth: *Stem Cell Mobilization (“Stem Cell Harvest”)*

Hematopoietic stem cells can be harvested by various methods:

- Leukapheresis → peripheral blood stem cells
- Bone marrow aspiration → bone marrow stem cells
- Placenta / umbilical cord blood harvest → umbilical cord blood stem cells
- Embryonic / mesenchymal stem cells

Stem Cell Purging

Def: Removal of clonogenic neoplastic cells from a stem cell transplant product.

Phys: Tumor cell contamination of autologous stem cell preparations may limit curative potential of high-dose chemotherapy. The presence of malignant cells in stem cell transplants was shown in various diseases, via cytological, histological, immunocytological, and molecular analyses. The contribution of transfused tumor cells to the occurrence of relapse was demonstrated in gene marking studies. However, relapse rates with “purged” stem cell products have not been significantly reduced as compared to “unpurged” stem cells. Endogenous relapse, due to, e.g., insufficient elimination of malignant cells within the patient, occurring despite high-dose chemotherapy, appears to be of greater significance. The use of “purged” stem cell products is not recommended outside of clinical studies.

Meth: *Stem Cell Selection*

Reduction of tumor cells in the graft can be achieved in vivo (i.e., treatment of the donor) or ex vivo / in vitro (i.e., treatment of the harvested cell sample).

“Positive Selection”

Attempt to purify hematopoietic progenitor cells using specific surface markers, e.g., CD34 or AC133.

NOTE: This method is of limited use if malignant cells and stem cells express similar surface markers (e.g., CD34 on leukemia cells).

“Negative Selection”

Attempt to specifically eliminate tumor cells from the graft:

- In vitro use of monoclonal antibodies utilizing complement, immunotoxin, magnetic particles, or cytostatics (e.g., mafosfamide). *NOTE:* potential damage to healthy hematopoietic cells as well as malignant cells
- Experimental approaches: use of antisense oligonucleotides or specific tyrosine kinase inhibitors, in vitro differentiation induction in leukemias

“Ex Vivo Expansion”

Purification attempt involving in vitro expansion of hematopoietic cells and inhibition of malignant clones. In CML, but also in solid tumors and multiple myeloma, enhancement of the growth of non-malignant progenitor cells has been demonstrated under certain conditions, achieving a significant reduction in the number of malignant cells.

The most efficient methods as well as the clinical relevance of stem cell purging for specific disease entities can only be established by randomized trials.

Stem Cell Expansion

Def: In preclinical and clinical studies attempts have been made to grow (“expand”) cultured stem cells (ex vivo) in order to provide new treatment options. While it is possible to increase cell numbers, relevant stem cell expansion has not been achieved yet. In addition, clinical studies did not demonstrate a significant cost / benefit advantage despite the option of tumor cell depletion.

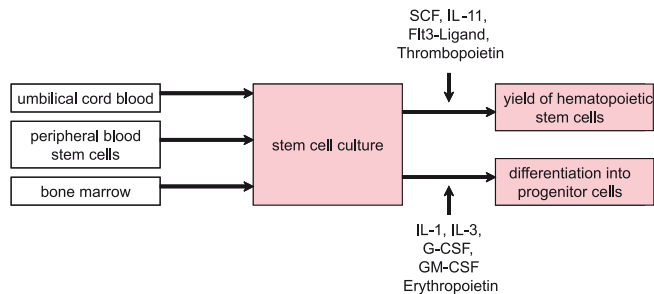
Possible Targets of Stem Cell Cultures

The following potential areas of use are being evaluated in clinical studies:

- Removal of contaminated tumor cells from autologous stem cell grafts
- Gene transfer into repopulating stem cells to correct hereditary enzyme deficiencies or for gene marking analyses
- Expansion of stem cells and partially differentiated progenitor cells from a single stem cell harvest for repeated clinical use in sequential therapy or tandem transplantation
- Expansion of lineage-determined progenitor cells (e.g., myeloid or megakaryopoietic postprogenitor cells) to accelerate hematopoietic regeneration or for differentiation into antigen-presenting dendritic cells
- Expansion of bone marrow repopulating stem and progenitor cells from umbilical cord blood samples for transplantation in adult patients
- Cell support in allogeneic transplantation
- De-/redifferentiation into other organ-type cells (“stem cell plasticity”)

Meth: ***Prerequisites***

- Preparation laboratory (equipped according to national and international guidelines) which allows production of cultivated and gene-transfected cells according to standards
- Central filing of all protocols of studies concerned with somatic cell and gene therapy

***Methods***

- Suspension culture using unseparated or CD34⁺-separated stem cells (advantage of reduced culture volume).
- Addition of recombinant growth factors. Optimal cytokine combinations are currently being tested. Stem cell factor (SCF), FLT3 ligand, thrombopoietin, and IL-11 seem to preserve

and expand the undifferentiated stem cell population, while IL-3, IL-1, G-CSF, GM-CSF, and erythropoietin lead to expansion of differentiated cells.

- Suspension cultures (from peripheral or umbilical blood) require no serum.
- The duration of the cultivation period remains the limiting factor of suspension cultures: safe interval approximately 3–5 days, while stroma-containing cultures have led to successful hematopoietic reconstitution even after 12–14 days of culture.

Quality Control

- Bacteriology, virology (EBV, CMV)
- Methyl cellulose culture assays for lineage-committed colony formation (CFU assay)
- Determination of progenitor cell/“stem cell” content, e.g., of CD34⁺ cells or subsets (e.g., CD34⁺/CD38⁻ cells) via flow cytometric analysis

NOTE: Phenotypic determination of repopulating stem cells is not possible. After cultivation, there is no definite correlation between CD34 antigen expression or the frequency of in vitro colony-forming cells (CFC), long-term culture initiating cells (LTC-IC), and the number of repopulating stem cells.

Storage

Cultivated cells are generally used immediately (without further storage). Successful cryopreservation (liquid N₂) has been described.

Stem Cell Transfusion

After 3–5 days in suspension culture or 12–14 days in bone marrow culture, cells are administered in the form of filtered single cell suspensions, analog to fresh or cryopreserved stem cells. Cytokines are removed by centrifugation and washing of the cell suspension. Commonly, premedication with corticosteroids and antihistamines is administered.

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5.2 Autologous Hematopoietic Stem Cell Transplantation

C.I. Müller, C.F. Waller, M. Lübbert, M. Engelhardt

Def: Hematological treatment aimed to accelerate bone marrow and blood reconstitution by use of autologous peripheral blood hematopoietic stem cells (PBSC) after intense (myeloablative) chemotherapy and / or radiotherapy (total body irradiation, TBI).

Ep: In 2004, 22,216 autologous transplantations in Europe (EBMT)

Phys: *Background*

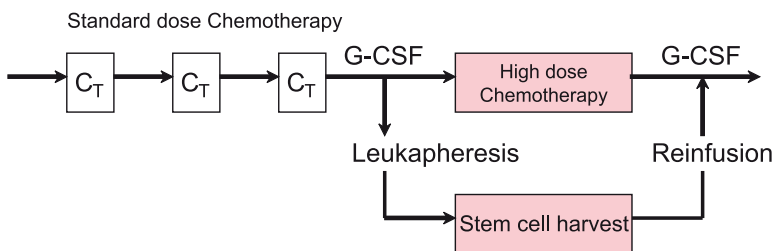
The intensity of conventional chemotherapy is limited, in particular due to hematotoxicity (myelosuppression) with neutropenia and thrombocytopenia. Dose-intensive myeloablative therapies require transplantation of hematopoietic stem cells from the patient (autologous transplantation) or a donor (allogeneic transplantation, ► Chap. 5.3).

- In early studies, hematopoietic stem cells (HSC) were obtained from the bone marrow by aspiration.
- Meanwhile, HSC are mainly harvested via blood cell separation (leukapheresis) after stimulation with colony-stimulating factors (CSF) and mobilization into the peripheral blood. Cells are cryopreserved and retransfused after high-dose therapy (peripheral blood stem cells transplantation, PBSCT).

In healthy donors (no stimulation with hematopoietic growth factors, i.e., steady-state conditions), HSCs are a rare population primarily located within the bone marrow. They do not circulate within the peripheral blood without mobilization.

- The number of circulating PBSC increases during the phase of hematopoietic reconstitution after conventional chemotherapy.
- The use of hematopoietic growth factors (e.g., granulocyte colony-stimulating factor, G-CSF) after conventional chemotherapy leads to a further increase and is used as a standard method for mobilizing and collecting PBSC.
- A sufficient PBSC yield is the prerequisite for high-dose chemotherapy with stem cell transplantation (PBSCT).

Peripheral stem cell transplantation after high-dose chemotherapy



Hematopoietic Stem Cells or Progenitor Cells

- Identification of hematopoietic progenitor cells by detection of CD34 antigen expression (1–4% of mononuclear cells in the bone marrow or mobilized blood are CD34-positive; however, recent data have shown the existence of CD34-negative hematopoietic stem cells, characterized by CD133, Thy-1, Oct-4, c-kit/CD117, SP phenotype).

- Over 90% of these cells are “committed progenitor cells” which have lost the ability to renew themselves. Only pluripotent stem cells hold this feature and have the potential for complete hematopoietic reconstitution (► Chap. 1.3).
- In mice, sufficient numbers of pluripotent stem cells for complete hematopoietic reconstitution can be attained with 100 highly purified cells. In humans after myeloablative chemotherapy (conditioning), $2\text{--}4 \times 10^6$ CD34-positive cells/kg body weight (BW) are regarded as sufficient. Optimal reconstitution of all cell lineages is achieved by transplantation of $\geq 4 \times 10^6$ CD34-positive cells/kg BW.

Mobilization of Autologous PBSC

Patients who are candidates for high-dose chemotherapy with peripheral blood stem cell transplantation (PB SCT) should initially be treated with conventional chemotherapy in order to combine maximum clinical response with early PBSC mobilization and harvesting. Myelosuppression generated by PBSC mobilizing chemotherapy should ideally be brief (without affecting the stem cell compartment) and have a maximum effect on the underlying disease:

- So far, there is no optimal mobilization protocol which could be used for the entire range of cancer patients. Most commonly, cyclophosphamide is used to facilitate PBSC mobilization, e.g., in the VCP-E Protocol (Etoposide (VP16), Cyclophosphamide, Cisplatin, and Epirubicin ► Protocol 13.1.1) followed by administration of recombinant human G-CSF.
- Administration of rh G-CSF increases the number of circulating multipotent progenitor cells by factor 10. Maximum PBSC mobilization occurs concomitantly with an increase in neutrophil granulocytes after the leukocyte nadir has been passed.

Meth:

Leukapheresis

PBSC are harvested as an outpatient procedure when the leukocyte count (WBC) is $> 5,000\text{--}10,000/\mu\text{l}$ and CD34⁺ cells are $> 10\text{--}20/\mu\text{l}$ blood, using a standard cell separator (“leukapheresis”). The procedure is well tolerated. Possible electrolyte imbalances can be compensated.

- In patients without prior chemo- or radiotherapy, sufficient numbers of PBSC can usually be harvested with 1–2 leukaphereses.
- For reasons of quality control, volume, differential leukocyte count, percentage of CD34-positive cells, viability, and sterility of every stem cell apheresis sample must be determined.
- Leukapheresis PBSC may be further processed (e.g., CD34-positive or -negative selection ► Chap. 5.1) or directly preserved in liquid nitrogen (at -196°C). Cryopreserved cells can be stored for many years prior to transplantation.
- Both processing and storage of the cell samples are carried out under GMP conditions (Good Manufacturing Practice, EU GMP guidelines) in accordance with national laws.

Ind:

Randomized studies have shown high-dose chemotherapy to be advantageous in the treatment of high-grade (and indolent) non-Hodgkin’s and Hodgkin’s lymphoma compared with standard-dose chemotherapy. EBMT (European Group for Blood and Marrow Transplantation) has published guidelines for autologous stem cell transplantation as follows:

- In certain patient groups with high-grade non-Hodgkin’s lymphomas (NHL), Hodgkin’s disease, multiple myeloma, and chemosensitive relapses of germ cell tumors, high-dose chemotherapy with autologous transplantation is effective and regarded as standard of care.
- In solid tumors (e.g., sarcoma, breast cancer, or ovarian carcinoma), high-dose chemotherapy should only be carried out within clinical studies.
- High-dose chemotherapy with stem cell support has been proven to be a potentially effective treatment for various other malignancies. However, the clinical benefit as compared to standard treatment has to be established in randomized clinical trials for each individual disease entity.

Recommendations on autologous hematopoietic stem cell transplantation in adults (EBMT, European Group for Blood and Marrow Transplantation)

Disease		Disease Stage	Degree of recommendation
Leukemias	AML	1st to 3rd CR, standard risk	Trials only
		Relapse	Not recommended
	Secondary AML / MDS	–	Not recommended
	ALL	1st or 2nd CR	Not recommended
	CML	Chronic phase	Not recommended
Acceleration, blast crisis		Not recommended	
Lympho-proliferative diseases	NHL	Lymphoblastic (high risk)	Recommended
		High grade: 2nd CR, PR, relapse	Recommended
		Low grade: ≥ 1st CR, relapse	Trials only
	CLL	–	Trials only
	Multiple myeloma	Stage I	Trials only
		Stage II–III	Recommended
	Hodgkin's disease	1st CR	Trials only
≥ 2nd CR, PR		Recommended	
Solid tumors	Germ cell tumors	Chemosensitive relapse	Recommended
		Refractory	Not recommended
	Sarcomas	Chemosensitive relapse / high risk	Trials only
	Breast cancer	Adjuvant, high risk	Trials only
		Metastatic	Not recommended
Ovarian carcinoma	Minimal residual disease	Trials only	
	Refractory	Not recommended	
Autoimmune diseases	Progressive sclerosis		Trials only
			Trials only
	Multiple sclerosis		Trials only
			Trials only
SLE		Trials only	
Amyloidosis		Trials only	

AML acute myeloid leukemia, *MDS* myelodysplastic syndrome, *ALL* acute lymphatic leukemia, *CML* chronic myeloid leukemia, *CLL* chronic lymphatic leukemia, *NHL* non-Hodgkin's lymphoma, *SLE* systemic lupus erythematosus, *CR* complete remission, *PR* partial remission

Co: Transplantation-associated mortality is 1–5%, depending on comorbidity factors and age of the patient. Normally, patients are discharged from hospital approximately 2–3 weeks after autologous PBSC transplantation.

Acute Complications

- *Bone marrow aplasia:* high-dose chemotherapy causes bone marrow aplasia, which is overcome within approximately 10 (granulocytes) to 14 days (thrombocytes) after autologous PBSC transplantation. Infections and hemorrhage may occur within this period, and most

patients require antibiotics and blood products for 4–8 days. Fungal infections are rare. Viral infections may occur due to reactivation (HSV, VZV, rarely CMV).

- *Gastrointestinal toxicity*: oropharyngeal mucositis, gastroenteritis
- *Pulmonary toxicity*: with use of certain cytostatics (e.g., busulfan, cyclophosphamide, thiotepa, BCNU), inflammatory changes (fibrosis, alveolar hemorrhage, infection), pulmonary edema, pulmonary damage, and “acute respiratory distress syndrome” (ARDS)
- *Cardiotoxicity*: cardiac damage due to cytostatics, e.g., cyclophosphamide (cardiac insufficiency, transmural hemorrhagic myocardial necrosis), anthracyclines (acute and chronic cardiotoxicity); cardiac complications may be intensified in cases of preceding radiotherapy or anthracycline treatment
- *Renal dysfunction*: renal insufficiency or acute renal failure due to cytostatic drugs, antibiotic treatment with aminoglycosides, insufficient hydration during treatment, tumor lysis, and blood pressure fluctuations; renal insufficiency is usually reversible
- *Hepatic dysfunction*: besides fully reversible short-term increases in hepatic enzymes, rare occurrences of veno-occlusive disease (VOD)

Long-term Side Effects and Recommendations for Follow-Up

- *Secondary malignancies*: particularly after high-dose chemotherapy with alkylating agents and after total body irradiation (TBI); the likelihood of secondary malignancies occurring after 15 years is up to 6% in conditioning protocols without TBI and up to 20% in protocols with TBI
- *Immunologic dysfunction*: monitoring of infections (CMV, VZV, *Pneumocystis carinii* pneumonia)
- *Vaccinations*: pneumococci, influenza, tetanus, diphtheria
- *Endocrine dysfunction*: monitoring of thyroid function, ovaries, testes, osteoporosis

Th:

Therapy Protocols: Mobilization

“VCP(E)” ▶ Protocol 13.1.1			
Etoposide phosphate	500 mg/m ² /day	i.v.	Day 1, infusion 1 h
Cyclophosphamide	1350 mg/m ² /day	i.v.	Day 1, infusion 1 h
Cisplatin	50 mg/m ² /day	i.v.	Day 1, infusion 1 h
Epirubicin	50 mg/m ² /day	i.v.	Day 1, bolus injection
Before leukapheresis: G-CSF 5 µg/kg daily s.c., from day 5			
“VIP(E)” ▶ Protocol 13.1.2			
Etoposide phosphate	500 mg/m ² /day	i.v.	Day 1, infusion 1 h
Ifosfamide	4000 mg/m ² /day	i.v.	Day 1, infusion 18 h
Cisplatin	50 mg/m ² /day	i.v.	Day 1, infusion 1 h
Epirubicin	50 mg/m ² /day	i.v.	Day 1, bolus injection
Before leukapheresis: G-CSF 5 µg/kg daily s.c., from day 5			
“IEV” ▶ Protocol 13.1.6 <60 years (>60 years)			
Etoposide phosphate	150 (120) mg/m ² /day	i.v.	Day 1–3, infusion 1 h
Ifosfamide	2,500 (1900) mg/m ² /day	i.v.	Day 1–3, infusion 18 h
Epirubicin	100 (75) mg/m ² /day	i.v.	Day 1, infusions 1 h
Before leukapheresis: G-CSF 5 µg/kg daily s.c., from day 5			

“Cyclophosphamide Mob-1d” ▶ Protocol 13.1.4

Cyclophosphamide	4000 mg/m ² /day	i.v.	Day 1, infusion 1 h
Before leukapheresis: G-CSF 5 µg/kg daily s.c., from day 5			

Therapy Protocols: High-Dose Therapy (Conditioning)

ATTENTION: High-dose therapy protocols must only be performed at adequately equipped transplantation centers, according to national and international guidelines.

“BEAM” ▶ Protocol 14.1

BCNU	300 mg/m ² /day	i.v.	Day -7, infusion 1 h
Cytarabine	2×200 mg/m ² /day	i.v.	Day -6 to -3, infusion 1 h,
Etoposide phosphate	2×100 mg/m ² /day	i.v.	Day -5 to -3, bolus 15 min,
Melphalan	140 mg/m ² /day	i.v.	Day -2, infusion 30 min
Day 0 stem cell transplantation			

“Melphalan” ▶ Protocol 14.2

Melphalan	100 mg/m ²	i.v.	Day -2, infusion 1 h (or 100 mg/day i.v., day -3 and day -2)
Day 0 stem cell transplantation			

“VIC” ▶ Protocol 14.6

Etoposide phosphate	500 mg/m ² /day	i.v.	Day -4 to -2, infusion 1 h
Ifosfamide	4000 mg/m ² /day	i.v.	Day -4 to -2, infusion 18 h
Carboplatin	AUC 6	i.v.	Day -4 to -2, infusion 18 h
Day 0 stem cell transplantation			

“BuCy” (autologous) ▶ Protocol 14.4

Busulfan	4 mg/kg /day	i.v.	Day -7 to -4
Cyclophosphamide	60 mg/kg/day	i.v.	Day -3 to -2, infusion 1 h
Day 0 stem cell transplantation			

Perspectives

Progress in PBSCT is to be expected in the following areas:

- Sequential transplantation (e.g., multiple myeloma)
- Use of new hematopoietic growth factors for stimulation of recovery of platelets and neutrophils to further shorten the cytopenic phase after chemotherapy
- Improved management of side effects of induction therapy
- Generation of immunocompetent cells for the treatment of minimal residual disease

- Mobilization and high-dose protocols
- “Graft engineering”, manipulation of stem cell product (elimination of tumor cells, ex vivo expansion of stem cells, dendritic cells, etc.)

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| 4. | http://www.bmtnet.org/ | Blood Marrow Transpl Net |
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5.3 Allogeneic Hematopoietic Stem Cell Transplantation

J. Finke

Def: Transfer of pluripotent hemato- / lymphopoietic stem cells from healthy donors to recipients.

Methods of Allogeneic Hematopoietic Stem Cell Transplantation (SCT)

- Bone marrow transplantation (BMT)
- Peripheral blood stem cell transplantation (PBSCT)
- Umbilical cord blood transplantation (UCBT)

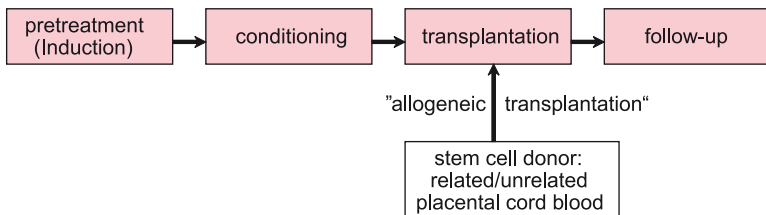
Ep: In 2004, 7407 allogeneic transplantations were performed in Europe (EBMT).

Ma: The success of allogeneic transplantation is based on two therapeutic principles which distinguish this method from conventional chemotherapy and autologous transplantation:

- *Conditioning:* immuno- and myeloablative high-dose chemotherapy and/or total body irradiation (TBI)
- *Graft versus leukemia effect (GVL effect):* immunological reaction of donor lymphocytes from the graft against malignancy in the recipient.

While cytostatic treatment largely reduces the malignant clone, the GVL effect seems to ensure long-term reduction of the relapse rate. Complete T-cell depletion of the graft leads to increased relapse rates.

Allogeneic Transplant Procedure



Meth: Donor

Suitable donors are a prerequisite for allogeneic transplantation. While HLA-identical donors and recipients are preferable, HLA antigen differences to the point of haploidentity can be accepted in certain situations:

- Matched related donor (MRD)
- Volunteer unrelated donor (VUD).

Donor Search

Allogeneic transplantation from HLA-identical siblings is preferable, however, sibling donors are available in only 25–30% of patients. Strategy:

- *Patients without related donor:* option of finding a volunteer unrelated donor; current success rate > 80%; 13 million registered voluntary donors worldwide. Blood group differences are of no importance when selecting a donor. After transplantation, the patient's blood group may be replaced by that of the donor.
- Early HLA typing of the patient, initiation of the donor search and contacting of allogeneic transplantation centers is pivotal for a successful strategy. Identification of a suitable donor may take 2–4 months. *Initiating the donor search only when relapse has occurred is often too late.*
- *Expanding the search to the extended family:* HLA-compatible additional donors in 6% of cases.

Donor Search Strategy

1. HLA-Typing of the patient and close family (siblings, parents)
2. In urgent cases, parallel search for donors among members of the extended family and unrelated donors

Selection Criteria for Related Donors

Of particular relevance are HLA alleles of the classes I A, I B and II DR.

- In patients with related donors, one allele difference in graft versus host direction (GVH direction) and three allele differences in host versus graft direction (HVG direction) are acceptable. This configuration provides for equally successful transplantation results as complete HLA identity.
- Class II DR alleles should be identified using genetic typing methods (DNA typing).
- The relevance of the class II loci DQ and DP is not yet known. When several HLA-A-, B-, and DR-identical donors are available, further selection criteria are considered (sex, age, CMV status, blood group).

Selection Criteria for Unrelated Donors

In the typing of unrelated donors, 8 HLA antigens (two alleles each of A, B, DRB1*, DQB1*) of the patient and the prospective unrelated donor are important. Recently, the relevance of HLA-C has been emphasized, especially for graft rejection and NK cell-induced GVL effects. DP seems to be of little importance in relation to alloreaactions.

- DR typing should be based on high-resolution DNA typing (4 digits), just as serological HLA-A, -B, and -C typing should be replaced by DNA methods (2 digits; recently 4 digits).
- Particularly in younger patients, minor differences may be acceptable.
- Additional HLA-DQB1* and/or HLA-DPB1* differences are at present no reason for exclusion of a donor. Allele differences in the A or B locus as well as minor DRB1* differences may also be acceptable, especially in patients with an aggressive underlying disease.
- Due to its low predictive value, mixed lymphocyte culturing (MLC) is no longer a routine procedure.
- The donor search should be coordinated by recognized immunogenetics laboratories together with a transplantation center.

Alternatives

Options in case of unsuccessful donor search:

- Transplantation from donor after T-cell depletion
- Transplantation of umbilical cord blood (UCB): of particular importance in pediatric patients and young adults.

Cord blood banks are currently being established. So far, over 2,000 UCB transplants have been carried out worldwide. If necessary, several HLA differences may be accepted, especially in pediatric patients. The main limiting factor is the cell content of the graft in relation to the patient's body weight.

Stem Cell Products**Stem Cell Compartments**

Hematopoietic stem cells are characterized by their expression of specific surface markers (CD34, CD133). The following hematopoietic stem cell compartments are used for transplantation:

- Bone marrow (BM) → bone marrow transplantation (BMT)
- Peripheral blood stem cells (PBSC, after stimulation with G-CSF) → peripheral blood stem cell transplantation ((PBSCCT)
- Cryopreserved umbilical cord blood stem cells (UCB) → UCB transplantation (UCBT)

Stem Cell Modification ("Graft Engineering")

Normally, the freshly collected grafts are transplanted without being manipulated or cryopreserved. However, in specific situations, the graft needs to be modified:

- T-cell depletion: prevention of graft versus host reactions
- Selection of CD34-positive cells (“stem cell selection”): reduction of immune reactions and elimination of malignant cells (autologous transplantation) (→ ► Chap. 5.1)

Bone Marrow Hematopoietic Cells

Bone marrow is collected under general anesthesia (approximately 60 min) by bilateral puncture and aspiration from multiple sites of the iliac crest.

- The bone marrow is anticoagulated and can be stored without cryopreservation for up to 1 day without significant stem cell loss (duration of transport with unrelated donor transplants).
- Potential disadvantages for the donor include blood loss, local pain and hematomas and side effects related to anesthesia.

Cytokine-mobilized Peripheral Blood Hematopoietic Cells (PBSC)

Recently, peripheral blood stem cells have increasingly been used for allogeneic transplantation.

- Pretreatment of the donor with 5–24 µg/kg/day G-CSF s.c. for 4–6 days.
- Stem cell harvest by one or more leukaphereses.
- Depending on the rate of yield, the graft may contain more CD34⁺ stem cells than comparable bone marrow products as well as 10 times more CD3⁺ T-lymphocytes.
- In randomized studies, allogeneic transplantation of peripheral blood hematopoietic cells conferred an increased risk of GVHD compared with allogeneic BMT.
- Hematopoietic engraftment is faster with PBSC than with bone marrow.
- PBSC grafts allow manipulation (stem cell enrichment, selective T-cell depletion, “graft engineering”), facilitating GVHD prophylaxis and transplantation despite HLA barriers.

Co: Myeloablative Therapy

Successful allogeneic transplantation is ultimately “myeloablative,” which is a result of both the conditioning therapy and the donor T-lymphocytes. Side effects:

- Toxic side effects of the conditioning therapy (chemotherapy, radiotherapy) depending on the therapy protocol
- Infections during the phase of bone marrow aplasia (until bone marrow reconstitution): bacterial infections, fungal infections (*Candida*, *Aspergillus*), viral infections (CMV, HSV)
- Long-term consequences: pulmonary fibrosis, bronchiolitis obliterans, gonadal insufficiency, hormonal deficiencies, cardiomyopathy, cataract, secondary neoplasia

Graft Versus Host Disease (GVHD)

- Lymphocytes in the graft play a major role in “alloreactions,” i.e., immunological reaction of transplanted immunologically active donor cells versus recipient organism → inflammatory immunological reactions in immunogenic structures, such as skin (dermatitis), intrahepatic bile ducts (cholestasis), and intestinal epithelium (enteritis); in extreme cases: destruction.
- The period of immunological adaptation and development of tolerance is divided into an acute phase (100 days after transplantation, acute GVHD) and a chronic phase (3–12 months after transplantation, chronic GVHD).
- Acute GVHD especially affects the skin, liver, and intestine.
- Chronic GVHD can potentially affect any organ. Particularly skin and mucosal dryness, generalized sicca syndrome with conjunctivitis, malabsorption syndrome, chronic cholestasis, weight loss, and increasing pulmonary obstruction may occur. In extreme cases, scleroderma-type skin symptoms and other autoimmune disorders may develop.
- GVHD prophylaxis with immunosuppressive drugs (e.g., cyclosporin A + methotrexate) is usually required in the initial weeks and months following transplantation. Unlike transplanted solid organs, an immune system developing from transplanted stem cells gradually becomes tolerant of the recipient.
- Especially in the first few months of this process, the patient is at risk of immune reactions and opportunistic infections (CMV, VZV, PCP, pneumococcal pneumonia) → close monitoring by the transplantation center in collaboration with the referring physician. A high degree of patient compliance is required.

Th: Principles of Treatment

Since different transplantation units may use center-specific protocols, consultation with experienced centers is always advisable. Variations may exist in relation to the following:

- Conditioning: based on high-dose chemotherapy with or without total body irradiation (IBI)
- Management of immunosuppression
- Stem cell graft engineering / manipulation

Furthermore, new protocols with less intensive conditioning regimens are currently being developed, for different indications and entry criteria (age). In protocols with reduced conditioning regimens, the age limit for allogeneic transplantation from related or unrelated donors has been raised from 55 years to approximately 70 years.

Indications

See Table (p. 308)

Pretreatment

Remission-inducing therapy (transplantation in complete remission generally leads to improved long-term outcome and cure rates)

Conditioning Therapy and Transplantation

- Chemotherapy \pm radiotherapy, high-dose or intensity-reduced (see below)
- Transplantation: transplantation of BM, PBSC, or cord blood from healthy donors via intravenous infusion; engraftment of hematopoietic stem cells in the bone marrow

Post-Transplant Period and Long-Term Follow-Up

- Supportive administration of antibiotics, erythrocytes and platelets during the neutropenic (10–15 days) and thrombocytopenic phase (12–25 days)
- Parenteral nutrition and pain therapy in cases of severe mucositis (5–15 days). Mucositis prevention (palifermin)
- Prophylaxis and, if necessary, treatment of graft versus host disease (GVHD), (cyclosporine levels). Monitoring for clinical signs of GVHD, exanthema, diarrhea, icterus, dryness of the mouth, conjunctivitis, mucositis
- Rehabilitation, outpatient follow-up, return to work after approximately 4–9 months

Signs of Toxicity

- Icterus, weight loss, ascites: VOD (veno-occlusive disease) \rightarrow immediate hospitalization, heparin, possibly steroids
- Dyspnea, cough: bronchiolitis obliterans as chronic GVHD \rightarrow high-dose steroids
- Palmoplantar erythema, dark pigmentation of the skin (busulfan, VP-16, thiotepea)
- Neuropathy, impaired vision, CNS disorders (cyclosporine, steroids)
- Hypertrichosis (cyclosporine)
- Opportunistic infections: *Pneumocystis carinii* pneumonia (PCP) \rightarrow prophylaxis with cotrimoxazole; *Candida* and *Aspergillus* infections, cytomegalovirus reactivation, varicella zoster virus (VZV), bacterial infections
- With chronic GVHD and long-term immunosuppression, risk of pneumococcal sepsis / meningitis
- From 6 months post-transplantation, patients should be revaccinated with inactivated vaccines (especially tetanus, diphtheria, Pneumovax, *Haemophilus influenzae* B; later: hepatitis B, possibly polio; seasonal: influenza vaccine)

Relapse after Transplantation

Relapse following allogeneic transplantation always constitutes a serious situation. Treatment options:

- Donor lymphocyte transfusion \rightarrow specific induction of GVL with immunotherapeutic effects (CML, plasmocytoma, AML, NHL, ALL, etc.)
- Second allogeneic transplantation from a different donor

Reduced Conditioning Prior to Allogeneic Transplantation

Terms such as “mini-transplantation,” “micro-transplantation,” or “non-myeloablative transplantation” have been used to describe reduced-intensity conditioning protocols which are based on dose modifications of traditional regimens (containing TBI 10–13.2 Gy or busulfan 16 mg/kg). However, these protocols represent a variation of allogeneic SCT with otherwise similar procedures and comparable immunological and infection-related problems (GVHD, opportunistic infections). Due to reduced acute toxicity, these regimens may preferably be used in patients with additional comorbidities or in older patients.

Background

- The immunotherapeutic benefit of the GVL effect was demonstrated in patients with relapse of CML (► Chap. 7.3.1) after allogeneic bone marrow transplantation: the transfer of immunologically active donor lymphocytes resulted in complete remission in > 50% of these patients.
- So-called myeloablative therapy (e.g., with 10–14 Gy TBI or busulfan 16 mg/kg in combination with cyclophosphamide) is an aggressive conditioning regimen with multiple side effects limiting use to younger patients without significant comorbidity. Older patients therefore do not equally benefit from allogeneic transplantation with standard conditioning regimens.
- Dose escalation studies with radiotherapy or chemotherapy in patients with aggressive, therapy-refractory leukemias have shown that despite excessive toxicity and mortality, the risk of relapse after allogeneic transplantation was not significantly reduced.
- Recent animal experiments have shown that stable lympho-hematopoietic engraftment of donor cells can be achieved with significantly lower irradiation doses of 2 Gy, causing less side effects (so-called immunoconditioning). Parallel to the GVL effect, the treatment induces “donor chimerism,” i.e., simultaneous existence of lymphatic and hematopoietic cells of the donor and the recipient.
- “Allogeneic transplantation with reduced conditioning” is aimed at utilizing the GVL effect without the disadvantages of maximum tolerable conditioning treatment. This therapeutic approach eventually constitutes a form of immunotherapy based on T-cell-mediated cytotoxicity.

Clinical Results

- Initial clinical studies, including patients over 60 years of age, have demonstrated the feasibility of this therapeutic approach. In individual cases, post-therapeutic complete and partial remission has been described. Its applicability in patients of > 60 years of age allows curative therapeutic attempts, particularly in patients with AML, MDS, and low grade NHL.
- Donor chimerism of lymphatic and hematopoietic cells without significant myelosuppression (leuko- or thrombocytopenia) was achieved.
- Other approaches use fludarabine in combination with alkylating agents to increase tolerance prior to allogeneic transplantation. Fludarabine has a particularly toxic effect on T-cells and has added benefit in the treatment of lymphomas.
- The long-term outcome (overall survival) after reduced-intensity conditioning has not been established yet. Randomized clinical trials for individual disease entities are necessary.

Possible Areas of Use

Treatment protocols for allogeneic transplantation with reduced conditioning are currently being developed for different disease entities and stages, giving rise to interesting prospects for a wider use of the therapeutic concept of allogeneic transplantation in patients with:

- Leukemia
- Myelodysplasia
- Multiple myeloma
- Chronic leukemias
- Lymphomas (especially low-grade NHL), multiple myeloma
- Various solid tumors

Ref:

1. Appelbaum FR. Dose intensity and the toxicity and efficacy of allogeneic hematopoietic cell transplantation. *Leukemia* 2005;19:171–5
2. Bertz H, Potthoff K, Finke J. Allogeneic stem-cell transplantation from related and unrelated donors in older patients with myeloid leukemia. *J Clin Oncol* 2003;21:1480–4
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5. Deeg HJ. How I treat refractory acute GVHD. *Blood* 2007;109:4119–26
6. Grathwohl A, Baldomero H, Frauendorfer K et al. Results of the EBMT activity survey 2005 on haematopoietic stem cell transplantation: focus on increasing use of unrelated donors. *Bone Marrow Transplant* 2007;39:71–87
7. Schoemans H, Theunissen K, Maertens J et al. Adult umbilical cord blood transplantation. *Bone Marrow Transplant* 2006;38:83–93

Web:

1. <http://www.ebmt.org> EBMT, Eur Grp Blood Marrow Transpl
2. <http://www.asbmt.org> ASBMT, Am Soc Blood Marrow Transpl
3. <http://www.bmtnet.org> Blood and Marrow Transplant Network
4. <http://www.nature.com/bmt/index.html> Bone Marrow Transpl Journal
5. <http://www.bmtinfonet.org> Blood and Marrow Transplant Information
6. <http://www.bloodline.net> Bloodline, Hematology Education
7. <http://www.marrows.org> National Marrow Donor Program
8. <http://www.ibmtr.org> International Bone Marrow Transplant Registry

Indications for allogeneic hematopoietic stem cell transplantation in adults

Disease	Stage / type	Transplantation type		Comments
		Related ^a	Unrelated ^b	
AML	First CR, intermediate risk	+	-(+)	Early search for potential donors
	First CR, high risk	+	+	
	First PR, second or later CR	+	+	
	Relapse, refractory AML or > first CR	+	+	
MDS	RA, RAS	+	+	With clonal markers or progressive cytopenia
	RAEB, RAEB-T, secondary AML, CMML	+	+	
	First CR: high risk t(9;22), t(4;11) pre T-ALL, first PR, > first CR	+	+	Clinical trials
MPS	CML: chronic phase, acceleration, blast crisis	+	+	High-risk patients after failure of targeted therapies (imatinib, dasatinib)
Lymphomas	OMF, progressive	+	+	Advanced disease, refractory
	Myeloma	+	+	Frequently as tandem transplant after first remission
	Follicular lymphomas, stage III–IV	(+)	(+)	Advanced disease, refractory, rapid progression
	Mantel cell lymphoma	+	+	First PR, relapse or second CR: clinical trial
	Aggressive NHL	(+)	(+)	Lymphoblastic lymphomas (high risk)
	Hodgkin's disease	(+)	(+)	High-risk situation, relapse after autologous Tx (study protocols)
	CLL	(+)	(+)	High risk, rapid progression, refractory

^a Related allogeneic transplantation

^b Unrelated allogeneic transplantation

+ indicated, (+) in studies only

Tx transplantation, AML acute myeloid leukemia (▶ Chap. 7.1.2), CML chronic myeloid leukemia, CMML chronic myelomonocytic leukemia, MDS myelodysplastic syndrome (RA, RAS, RAEB, RAEB-T) (▶ Chap. 7.2), ALL acute lymphatic leukemia (▶ Chap. 7.1.1), CLL chronic lymphatic leukemia, MPS myeloproliferative syndrome (▶ Chap. 7.3), NHL non-Hodgkin's lymphomas (▶ Chap. 7.5), OMF osteomyelofibrosis, CR complete remission, PR partial remission

5.4 Granulocyte Transfusion

H. Bertz, G. Illerhaus

Def: Experimental treatment procedure. Transfusion of donor granulocytes with the aim of correcting neutropenia (e.g., after chemotherapy).

The use of rhG-CSF for mobilization of donor granulocytes has been a prerequisite for successful development of granulocyte transfer. Several phase I/II studies have produced promising results in neutropenic patients with severe infections. Prospective phase III studies with comparison of granulocyte transfusion vs. standard of care are still pending.

Granulocyte transfusions must be carried out in accordance with transfusion guidelines and national laws.

Meth: *Preparation and Storage*

- Donors and recipients must be ABO and Rh compatible (granulocyte concentrates contain erythrocytes). CMV status has to be considered
- Stimulation and mobilization of granulocytes with rhG-CSF (5 µg/kg) s.c.
- After 12 h, leukapheresis → with HAES 6% (to accelerate sedimentation) and sodium citrate (anticoagulant)
- Granulocyte yield depends on donor WBC count, leukapheresis efficiency, and volume of processed blood
- Irradiation of granulocyte concentrates with 30 Gy to avoid graft versus host reaction (large numbers of lymphocytes and stem cells in the leukapheresis sample)
- Store concentrates at room temperature, without agitation for up to 24 hrs.

Transfusion

- Carry out transfusion as soon after collection as possible
- Therapeutic success depends primarily on the number of transfused cells → transfusion of $\geq 1.5 \times 10^8$ granulocytes per kg body weight of recipient

Ind: Clinical studies in patients with severe infections not responding to anti-infective treatment, with concomitant neutropenia (< 500 neutrophils/µl) and without foreseeable bone marrow recovery.

Ci: *Donor*

- Pregnancy, lactation
- Severe general illness or known malignancy
- Acute and chronic infections, autoimmune diseases
- The number of maximum granulocyte donations per year must conform with national regulations

Recipient

- Allergic or pulmonary reaction to previous granulocyte transfusions
- No CMV-positive donor for CMV-negative recipient

Se: *Potential Side Effects (Donor)*

- G-CSF: bone pain, myalgia, restlessness, insomnia, headache, splenomegaly (rare) (► Chap. 4.3).
- Leukapheresis: anemia, thrombocytopenia
- HAES: allergic reactions, pruritus
- Sodium citrate: citrate toxicity, arrhythmia, tetany, metabolic alkalosis

Potential Side Effects (Recipient)

- Allergic reactions, anaphylactic shock → premedicate with antihistamines and nonsteroidal antipyretics, e.g., paracetamol

- Direct pulmonary toxicity, dyspnea, hypoxemia → monitor O₂ saturation
- Transfusion-induced acute pulmonary insufficiency (TRALI: transfusion-related acute lung injury) (► Chap. 9.8)
- Alloimmunization against HLA class I antigens and granulocyte-specific antigens → inefficiency of further transfusions, fever, respiratory symptoms, anaphylactic reactions

Due to the risk of alloimmunization, granulocyte transfusions should be avoided prior to allogeneic bone marrow transplantation.

Th: Granulocyte Transfusion: Procedure

Requirements

- Inclusion in clinical study, information and signed consent of donor and recipient
- ABO and Rh compatible donor, check CMV serology of donor and recipient (no transfusion from CMV-positive donor to CMV-negative recipient)

Donor

- Clinical diagnosis: case history, physical examination
- Laboratory tests: full blood count with differential, blood group, hepatic and renal function parameters, coagulation parameters, serology (HAV, HBV, HCV, CMV, HIV, *Treponema pallidum*); female patients: pregnancy test where appropriate
- Cross-match (blood group testing) before each granulocyte transfusion
- ECG, optional chest x-ray, abdominal sonography (splenic enlargement)
- G-CSF 5 µg/kg s.c., 12 h before each leukapheresis
- Prior to each new leukapheresis as well as 5 and 30 days after the final donation: blood count with differential, urea and electrolytes, serum creatinine, bilirubin, ALT

Granulocyte Sample

- Leukapheresis sample irradiated with 30 Gy
- Administer as soon as possible (within 6 h)

Recipient

- Serological (erythrocytic) and leukocytic (lymphocyte toxicity test) compatibility must be assessed prior to each granulocyte transfusion. After administration of amphotericin B, wait at least 6 h before giving a granulocyte transfusion (pulmonary toxicity).
- Ten minutes prior to transfusion, premedication with antihistamines and antipyretics (e.g., paracetamol).
- Recommended transfusion rate: 1×10^{10} cells per hour. Use standard filters (pore size 170–230 µm).
- Monitor blood pressure, pulse, respiratory rate, and O₂ saturation from the beginning until 1 h after transfusion.
- Evaluate transfusion success by measuring the post-transfusion granulocyte increase.

- Ref:**
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- Web:**
1. <http://www.aabb.org/> American Association of Blood Banks

5.5 Immunotherapy

A. K. Kaskel, A. Mackensen, H. Veelken

Def: Specific or non-specific modulation of the immune system with the objective of immunologically mediated destruction of malignant cells.

Meth: Immunotherapy approaches to cancer treatment have been studied since the nineteenth century. Four different approaches can be distinguished:

- Active specific immunotherapy
- Active non-specific immunotherapy
- Passive immunotherapy
- Adoptive immunotherapy

T-cells

The T-cellular immune response is crucial to recognize and eliminate tumor cells. T-cell subtypes include:

- CD8-positive, cytotoxic T-lymphocytes [restricted by class I MHC molecules (MHC I)]
- CD4-positive, helper T-lymphocytes [restricted by class II MHC molecules (MHC II)]

A more recent classification is based on the cytokines produced by T-lymphocytes. CD4-positive T-lymphocytes are subclassified as:

- Inflammatory Th1-cells
- Helper cells of Th2-type
- Th0-cells (intermediate type)

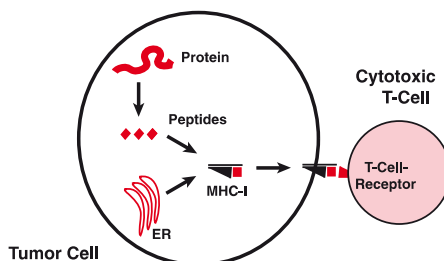
T-cell Activation: Antigen Presentation

T-lymphocytes do not recognize intact proteins, but peptides bound to MHC (major histocompatibility complex) molecules. Different types of proteins can be processed by intracellular proteases, the resulting peptides are bound to MHC molecules and expressed on the cell surface of antigen-presenting cells. Consequently, T-cell recognition is not limited to surface markers, but may include intracellular antigens, thus multiplying the diversity of T-cell target epitopes. Prerequisite for the recognition of peptides is the binding to MHC molecules:

- Peptides binding to MHC I: 7–14 amino acids
- Peptides binding to MHC II: 14–24 amino acids

The specifics of antigen recognition by T-lymphocytes are dependent on the interaction of the variable region of the T-cell receptor (TCR) molecule with the MHC-peptide complex. This binding site of the TCR is coded by a unique gene segment, which is formed by the recombination of a V-D-J segment for the β locus and a V-J segment for the α locus during T-cell differentiation. This combinatorial diversity is amplified by the random addition of nucleotides and is the basis for the diversity of the T-cell repertoire.

Specific recognition of tumor antigens by T-lymphocytes



Proteins (e.g. melanoma antigens tyrosinase, MAGE 1–3, Melan-A) are processed by intracellular proteases. Resulting peptides are bound by MHC molecules and presented at the cell surface. T-cell receptor (TCR) binds to MHC-peptide-complex. ER Endoplasmic Reticulum

Costimulatory Molecules

The binding of a TCR to a specific peptide–MHC complex alone is not sufficient for activation and proliferation of naive T-cells. Additional signals are required:

- Adhesion molecules which facilitate contact with the targeted cell
- Costimulatory signal: costimulatory molecules are the antigens of the B7-family (B7-1, B7-2), which are expressed by antigen-presenting cells (APC). “Professional APCs” play a central role for the initiation of the immune response. The antigens interact with suitable ligands on T-cells (CD28, CTLA-4). If a naive T-cell meets a non-professional APC (e.g., tumor cell presenting a peptide matching a specific TCR), a second costimulatory activation signal is missing. The result is the induction of anergy, i.e., the T-cell is refractory to further stimulatory signals.

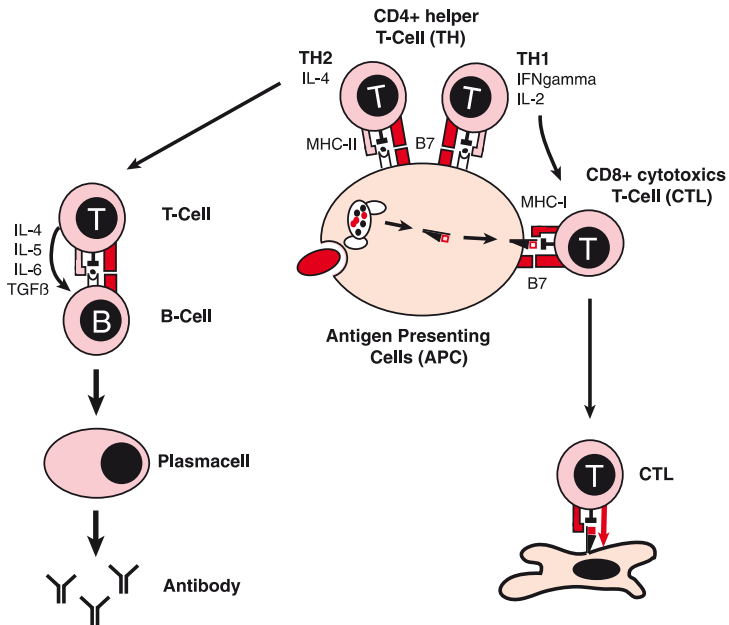
Immune Escape Mechanisms

The immune system of a tumor patient is rarely capable of inducing regression of manifest tumors and metastases. This observation supports the hypothesis, that tumor specific antigens lead to incomplete activation of the immune system, or tumor cells “escape” immunologic toxicity through induction of immunosuppression.

Various “tumor escape” mechanisms of neoplastic cells have been described:

- Lack of expression of costimulatory molecules on tumor cells
- Loss or downregulation of MHC molecules (β 2-microglobulin, HLA-A or -B) or receptors for apoptosis
- Loss of transport proteins (TAP) \rightarrow reduced presentation of tumor peptides with MHC molecules
- Selection of so-called antigen-loss variants, without expression of tumor-associated antigens (MAGE, tyrosinase, gp100)
- Induction of angiogenetic or antiapoptotic factors
- Secretion of immunoinhibitory cytokines, such as TGF- β and IL-10, by tumor cells

The effect of costimulatory molecules



Dendritic cells or monocytes (professional antigen-presenting cells, APC) ingest antigenic material and disintegrate and process it to peptides. Peptides are attached to MHC-I or MHC-II molecules and transported to the cell membrane where they are presented. The TCR binds to peptide–MHC complexes, and cellular inter-

action is supported by adhesion molecules and costimulatory signals such as B7 (on APC) and CD28 (T-cells). Depending on the antigen and the cytokine environment, cell types preferably induced are Th2 or Th1 helper cells. Th2 cells produce cytokines (such as IL-4, -5, -6), which are particularly important for the stimulation and differentiation of B-cells to antibody-producing plasma cells. Th2 cells also interact with B-cells, which can ingest and process the same antigen complex (e.g., bacterium, tumor cells) with the help of membrane antibody molecules or the B-cell receptor – similar to dendritic cells. The interaction of B- and T-cells leads to a coordinated antigen-specific B- and T-cell immune response. Th1 cells produce interferon- γ and IL-2, which particularly promote the maturation of MHC-I-peptide complex specific cytotoxic T-cells

Th: Active Non-specific Immunotherapy

The term “active non-specific immunostimulation” relates to the administration of modifiers, which can directly modulate the immune system. “Non-specific” indicates the lack of antigen specificity. Non-specific immunity is mainly based on activated macrophages, but also NK cells and neutrophils. The following are possible biological response modifiers:

- Cytokines such as interleukin-2 and interferon
- BCG (Bacille Calmette-Guérin)
- Lipopolysaccharides, immune complexes, muramyl dipeptide

Indications (► Chap. 3.4)

- Interferon α : malignant melanoma, renal cell cancer
- Interleukin-2: malignant melanoma, renal cell cancer
- BCG: bladder cancer

Active Specific Immunotherapy

Directed activation of the antigen-specific cellular immune response by vaccination. Possible vaccines include:

- Irradiated tumor cells, without further modification
- Irradiated tumor cells + immunostimulation (e.g., BCG)
- Modified tumor cells (after transfection with cytokines or costimulatory molecules)
- Defined tumor antigens (proteins, peptides)

Tumor antigens inducing a specific T-cell response:

- Tissue- / organ-specific antigens, differentiating antigens (Melan A, tyrosinase in melanocytes/melanoma, PSA in prostate cancer, HER2/neu in breast cancer)
- Overexpression of normal gene products (MAGE antigens in melanoma)
- Mutated cellular gene products of tumor suppressor genes or cell cycle genes (p53, cyclin)
- Viral gene products (EBNA-1 in Burkitt’s lymphoma, nuclear protein E6/E7 of HPV16 in cervical carcinoma)
- Rearranged normal gene products (bcr/abl translocation in CML, immunoglobulin idiotypes in B-cell neoplasia)
- Activated protooncogene products (p21 point mutation in colon carcinoma)
- Oncofetal antigens (CEA in colon / breast cancer)

Specific Immunotherapy with Defined Tumor Antigens

- Proteins \pm adjuvant
- Immunodominant peptides \pm adjuvant
- Peptide-loaded antigen-presenting cells
- Naked DNA coding for tumor-associated antigens
- Recombinant constructs in live vectors (viruses, bacteria)

Indications

- Colon carcinoma, stage III, adjuvant therapy
- Melanoma and renal cell carcinoma, in clinical trials

Passive Immunotherapy

Treatment with monoclonal antibodies directed against tumor antigens. Mechanisms of tumor cell lysis by monoclonal antibodies:

- Antibody-dependent cellular cytotoxicity; ADCC
- Complement-dependent cytotoxicity; CDC
- Intrinsic cytotoxic activity / induction of apoptosis
- Carrier of a cytotoxic substance (toxins, radionuclides, cytostatics)
- Antibody variants: murine antibodies, chimeric / humanized antibodies, bispecific antibodies, immunotoxins / radioconjugates

Indications (► Chap. 3.5)

- B-NHL: anti-CD20 monoclonal antibody, chimeric (rituximab)
- Breast cancer: anti-HER2/neu antibody, humanized (trastuzumab)
- CLL: anti-CD52 monoclonal antibody, humanized (alemtuzumab)
- Colorectal cancer, NSCLC: VEGF monoclonal antibody (bevacizumab)
- Colorectal cancer, HNC: EGFR monoclonal antibody (cetuximab)

Adoptive Immunotherapy

Passive Immunotherapy with Effector Cells (Cellular Therapy)

- Donor lymphocytes in HLA-chimeric patients (graft versus leukemia effect)
- Virus-specific T-lymphocytes (CMV, EBV, HIV)
- Tumor-specific T-lymphocytes
- Antigen (peptide)-specific T-lymphocytes
- Ex vivo expanded tumor-infiltrating lymphocytes (TIL) or ex vivo expanded and activated NK cells (lymphokine-activated killer cells, LAK)
- Antigen-presenting dendritic cells

Indications

- Adoptive transfer of donor lymphocytes in allogeneic transplantation (CML, AML, multiple myeloma)
- Adoptive transfer of virus-specific lymphocytes in allogeneic transplantation (CMV, EBV, EBV-associated lymphoproliferative disorders)
- Adoptive transfer of tumor- or antigen-specific T-lymphocytes (malignant melanoma)

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 2. <http://www.cancersupportivecare.com/immunotherapy.html> Cancer Immunotherapy
 3. <http://www.meds.com/immunotherapy/intro.html> Immunotherapy Training
 4. <http://www.cancerimmunotherapy.org> Assoc Immunother Cancer
 5. <http://www.meniscus.com/horizons/2-1.pdf> Cancer Immunotherapy

5.6 Gene Therapy

H. Veelken, F.M. Rosenthal

Def: Treatment of a disease by expression of one / multiple specific genes in a cell or group of cells. The production of the desired gene product corrects the genetic defect or alters cellular function.

Types

- Somatic gene therapy: expression of genes in differentiated somatic cells
- Germline therapy: expression of genes in fertilized human oocytes or embryonic stem cells

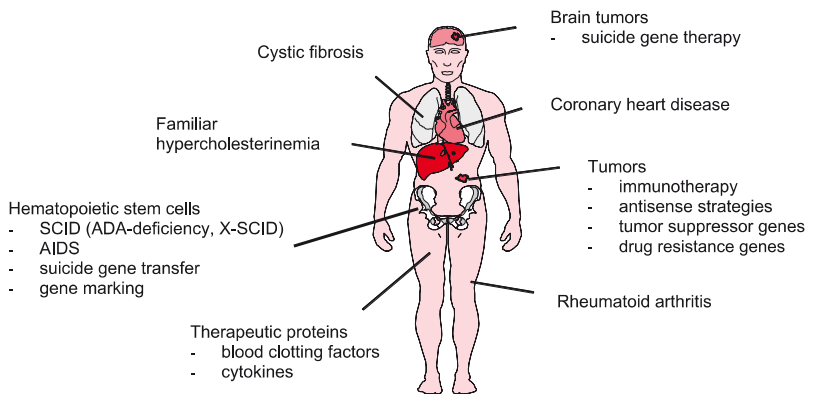
Meth: Gene Transfer

Adequate methods of gene transfer are a basic requirement for gene therapeutic approaches. In most cases, so-called vectors are used to transport the therapeutic gene construct into the target cells. Due to directed genetic deletions, viral vectors are usually unable to replicate after transfection of the target cell. Viral vectors are produced with the help of “packaging cells,” which provide the necessary structural proteins for replication. After transfection of the genetic construct, these cells produce the required vector.

The following are important criteria for the evaluation of gene transfer methods:

- *Efficacy*: transfection quality (transient or stable), transfection efficiency, tropism (potential of organ-specific gene transfers), biological efficacy (expression of gene products)
- *Safety*: tolerance, adverse effects, immunogenicity
- Production effort, cost and compliance with GMP / GLP / GCP criteria

Gene therapy studies: diseases, examples



Basics and Clinical Studies

The first somatic gene therapy was carried out in September 1990 at the NIH, USA, on a 4-year-old girl with adenosine deaminase (ADA) deficiency. Since then, more than 630 clinical gene therapy studies have been licensed worldwide and 3,500 patients have been treated. Besides monogenic hereditary diseases, gene therapy is used particularly in patients with advanced malignancy, AIDS, or multifactorial diseases such as coronary heart disease or rheumatoid arthritis.

Strategies for Gene Therapy of Malignancies

- *Induction of specific immune responses* by transfer of immunostimulating genes (e.g., interleukin or interferon genes) into tumor cells, bystander cells, or immunoeffector cells
- *Transfer of tumor suppressor genes* → correction of regulation defects in tumor proliferation
- *Blockade of oncogenic effects* via antisense strategies
- *Transfer of “suicide genes”* into tumor cells: suicide genes usually encode enzymes (e.g., HSV thymidine kinase), which by means of phosphorylation convert non-toxic prodrugs (e.g., ganciclovir) into toxic substances, thus selectively killing HSV-TK-expressing cells
- *Transfer of cytostatic drug resistant genes* (MDR-1, aldehyde dehydrogenase, O₆-alkylguanine-DNA alkyltransferase, cytidine deaminase) into hematopoietic stem cells to increase the in vivo resistance of the hematopoietic system to cytostatic drugs and alleviate the hematotoxicity of subsequent chemotherapy

Until now, mainly critically ill patients with short life expectancy were included in gene therapy studies. Therefore, the results of these studies focus primarily on safety and side effects, rather than on curative aspects. Initial data on the safety of gene therapy methods showed that close surveillance is required:

- After administration of high doses of adenoviral vectors, immune reactions to adenoviral proteins and pulmonary toxicity were detected in patients with cystic fibrosis.
- Severe adverse reactions also occurred during a clinical trial in patients with the X-chromosomal form of severe combined immunodeficiency (X-SCID), carried out in Paris. Newborn children suffering from this so far incurable disease were cured by transfer of the normal gene. However, approximately 3 years after treatment, 3 out of 11 children developed T-cell leukemia, the cause of which seemingly involved the insertion of the retroviral vector. The exact causes of this severe adverse effect are under intense medical and molecular biological investigation.
- A 17-year-old patient with a severe congenital metabolic disease died in September 2000 in the USA after infusion of high doses of genetically modified adenovirus into the liver artery.

The importance of strict adherence to the highest standards for production and quality control of gene therapy drugs as well as the conduct of controlled clinical trials was underlined by these cases.

Th: At present, gene therapy is used in clinical studies only (► Chap. 3.7). Besides legal aspects mentioned above, national and international guidelines and regulation for gene therapy products and studies have to be followed.

Clinical Gene Therapy Studies, Germany (as of May 2005, German Register for Somatic Gene Transfer Studies)

Target entities

- | | |
|-----------------------------------|-----|
| • Infections / parasitic diseases | 13% |
| • Cardiovascular diseases | 10% |
| • Malignancies | 77% |

Therapeutic approaches (number of trials)

- | | |
|-------------------------------|----|
| • Immunotherapy | 16 |
| • Tumor suppressor regulation | 9 |
| • Vaccination | 5 |
| • Suicide gene expression | 4 |
| • Regulation of angiogenesis | 3 |
| • Other approaches | 9 |

A total of 46 studies with 465 patients (361 with gene transfer, 104 in control groups)

Methods of gene transfer

Transfer method	Foreign DNA	Target cells	Transfection efficiency	Transfection	Cellular toxicity	Gene expression	Preparation	Use
Electroporation	150 kb	Mitotic / resting	Stable < 0.1–1%	1–20 copies	20–60% survival rate		Simple	In vitro
Microinjection	Unlimited	Mitotic / resting	Stable < 0.1–1%, transient up to 100%	Integration possible	30% survival rate		200–400 injections/h	In vitro
Naked DNA	Unlimited	Especially myocytes	10–30% of cells at injection site	Extrachromosomal	Lymphocytic infiltration	In myocytes: until cellular death	Simple, cheap	In vivo
Particle bombardment	10,000 copies	Mitotic / resting	Stable < 0.01–0.1%, transient ≤ 20%	Persistence / integration?	85–95% survival rate	2–12 months	Simple	In vitro, in vivo
Lipofection (cations)	Unlimited	Mitotic / resting	Stable < 0.1–1%, transient up to 80%	Integration possible	Membranotoxic, no antigenicity		Simple	In vitro, in vivo
Calcium phosphate coprecipitation	Unlimited	Mitotic / resting	Stable < 0.1%	Often multiple copies	High		Simple	In vitro
Receptor-mediated	> 48 kb	Mitotic / resting	Up to 50% in vitro, very variable	Extrachromosomal, variable	High	High, transient	Labor intensive / time-consuming	In vitro, in vivo
Retrovirus	Approx. 8 kb	Only mitotic	Up to 100% in vitro	Stable, one copy	Non-toxic	Relatively low	Labor intensive	In vitro, in vivo
Adenovirus	> 8 (7–36) kb	Mitotic / resting	High	Extrachromosomal	Direct toxicity, immune reactions	High	Homolog recombination, stable	In vitro, in vivo
Adeno-associated virus	4 kb	Mitotic / resting	Relatively high	Stable integration, tandem repeats	Non-toxic		High viral stability	In vitro, in vivo
Herpes simplex virus	10–100 kb, amplicon DNA: approx. 15 kb	Mitotic / resting / neurotrophic		Extrachromosomal, multiple copies	Low		Heat-resistant, lyophilization	CNS / PNS

Proof-of-concept has been established for the biological or clinical efficacy of gene transfer in studies:

- Induction or amplification of tumor-specific immune responses in tumor vaccination studies
- Occasional tumor regression or stable disease after transfer of tumor suppressor genes (p53)
- Decreased incidence of GVHD after allogeneic hematopoietic transplantation due to transfer of HSV-TK suicide genes into allogeneic donor lymphocytes after administration of ganciclovir
- Correction of the immunodeficiency in X-chromosomal severe combined immunodeficiency (X-SCID)
- In patients suffering from hemophilia, factor VIII use was reduced by 50–80% after intramuscular injection of AAV vectors carrying the wildtype gene

Furthermore, genetic marking of hematopoietic stem cells showed that while contributing to long-term hematopoietic reconstitution, transplanted stem cell products can potentially contain malignant cells which act as a starting point for relapse. Although this method is a diagnostic procedure, the results of these studies are seminal for future advancement of transplantation strategies and trends in gene therapy with respect to the hematopoietic system.

The clinical use of gene therapy is still in an early stage. In addition to the safety aspects discussed above, there are numerous technical issues to be resolved:

- In vivo transfection efficiency
- Duration and degree of expression of a therapeutic gene
- Regulation of gene expression
- Organ-specific gene transfer in vivo
- Immunogenicity of vectors and therapeutic gene products
- Industrial-scale production of viral vectors

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 5. <http://www.euregenethy.org> European Gene Therapy Network
 6. <http://www.cancer.gov/cancertopics/factsheet/Therapy/gene/> NCI, CancerNet

5.7 Inhibition of Angiogenesis

A. Müller, J.S. Scheele

Def: *Angiogenesis*: formation of new blood vessels; mostly formation of new capillaries from pre-existing blood vessels.

Inhibition of angiogenesis: through inhibition of endogenous angiogenic factors or administration of physiological / pharmacological angiogenesis inhibitors.

Phys: Physiological angiogenesis is essential for the development of embryonic organs as well as the regulation of the adult vascular system:

- Embryogenesis: vasculogenesis, i.e., formation of new angioblast-derived blood vessels
- Proliferation of uterine epithelia, menstruation
- Proliferation and vascularization of muscle tissue
- Wound healing, bone growth, nerve regeneration, hair growth
- Regulation of vascular permeability → homeostasis

In adult organisms, angiogenesis is typically strictly regulated and of limited duration (local duration 1–2 weeks maximum).

Endogenous Angiogenesis Promoters

- Angiopoietins (Ang1, Ang3, Ang4)
- Ephrines (Eph-A1, Eph-12, Eph-B2), VE-cadherin
- Fibroblast growth factors (aFGF, bFGF), hepatocyte growth factor (HGF)
- Platelet-derived growth factor (PDGF-BB)
- Transforming growth factors (TGF α , TGF β), tumor necrosis factor alpha (TNF α)
- Interleukin 8 (IL-8)
- Integrins $\alpha_3\beta_3$, $\alpha_5\beta_5$, $\alpha_5\beta_1$
- Prostaglandins E1 (PgE1) and E2 (PgE2)
- Matrix metalloproteinases (MMPs)
- Vascular endothelial growth factors (VEGF-A, VEGF-B, VEGF-C, VEGF-D)

Endogenous Angiogenesis Inhibitors

- Angiostatin, endostatin, protamine, vasostatin, angiopoietin 2 (Ang2)
- Thrombospondin-1
- Cartilage-derived inhibitor
- Interferons (IFN α , IFN β)
- Interleukins (IL-4, IL-10, IL-12, IL-18)
- Platelet factor 4 (PF4)
- Prolactin fragment, SPARC fragment, osteopontin fragment, antithrombin III fragment
- Soluble VEGF receptors (sVEGF-R1, sNRP-1)
- Tissue inhibitor of metalloproteinase (TIMP), MMP inhibitors, MMP2 fragment (PEX)

Pp: Diseases with pathological angiogenesis

Organ	Disease
Blood vessels	Atherosclerosis, hemangioma, hemangioendothelioma, vascular anomalies, retinopathies
Skin	Impaired wound healing, keloid formation, Kaposi's sarcoma, psoriasis, skin tumors, decubitus
Female reproductive organs	Follicular cysts, menstruation anomalies, ovarian hyperstimulation, endometriosis, tumors, preeclampsia, placental insufficiency

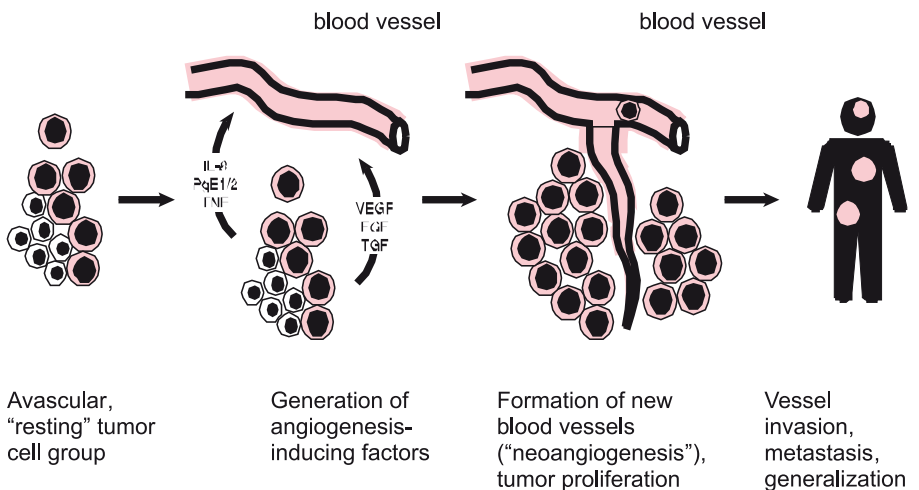
Pp: Diseases with pathological angiogenesis (*continued*)

Organ	Disease
Skeletal system	Rheumatoid arthritis, synovitis, osteomyelitis, pannus formation, osteophyte formation, tumors, aseptic necrosis, impaired wound healing
Internal organs, epithelia	Hepatitis, pneumonia, glomerulonephritis, asthma, hepatic regeneration, tumors, pulmonary hypertension, diabetes
Eye	Vitreous body disturbances, diabetic retinopathy, choroid neovascularization
Endocrine organs	Thyroiditis, hyperthyroidism, pseudocysts
Lymphatic system	Metastasis, lymphoma, lymphedema
Hematopoiesis	Hematological neoplasia, AIDS
Other	Solid tumors

Angiogenic Switch

In 1970, Folkman described the transition of solid tumors from an avascular resting state to a vascularized phase with optimal tumor oxygenation and nutrition. Only in the vascularized state, i.e., after the “angiogenic switch”, accelerated tumor proliferation, metastasis, and generalization can occur, as for example in prostate carcinoma, breast cancer, and renal cell carcinoma.

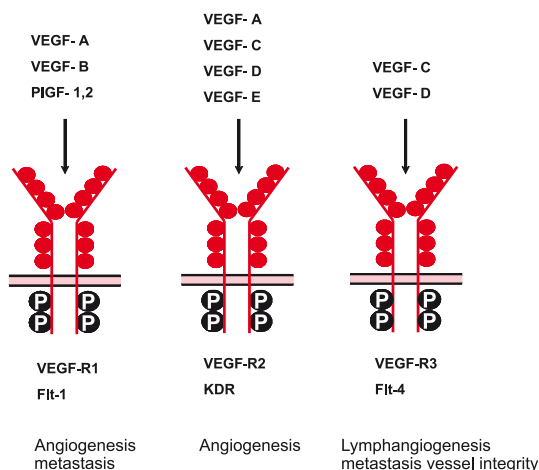
Similarly, increased bone marrow microvessel density has been described in proliferating hematological neoplasia, particularly in patients with leukemia (AML, ALL, CML) and myelodysplasia.

Tumor neoangiogenesis

Th: Inhibition of tumor-induced angiogenesis and the angiogenic switch of human tumors (transition from avascular state to vascularized proliferating tumor) was first demonstrated as an effective means of treatment with the VEGF-receptor antibody bevacizumab (► Chap. 3.5). Bevacizumab inhibits binding of VEGF to VEGF receptors (i.e. VEGF-R1 = Flt-1 and VEGF-R2 = KDR) → inhibition of tumor-induced neoangiogenesis → inhibition of tumor growth and metastasis. The

compound has been approved for treatment of metastatic colorectal cancer and non-small cell lung cancer (NSCLC). Similar approaches are followed with small molecule inhibitors of angiogenesis, e.g. sorafenib and sunitinib (► Chap. 3.6).

VEGF and VEGF receptors



Angiogenesis Inhibitors in Clinical Trials

- *Matrix metalloproteinase-inhibitors (MMPI)*: CGS 27023, COL-3, BMS-275291
- *Inhibitors of endothelial cell proliferation / migration*: 2-methoxyestradiol, combretastatin A4, farnesyltransferase inhibitors, thalidomide, lenalidomide, soy isoflavone, IM862, LY317615, ZD6126, AVE8062, ABT-751, TZT-1027, AS-1404
- *Inhibitors of angiogenesis-inducing factors / tyrosine kinases*: BAY 43-9006, PTK787/ZK222584, AMG706, SU6668, Neovastat (AE-941), VEGF-Trap, ZD6474, CP-547632, aplidine
- *Endothelin / integrin antagonists*: ABT-627, vitaxin, EMD121974

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5.8 Developmental Therapeutics

U. Martens

Def: Increasing understanding of the molecular mechanisms of cancer and hematologic malignancies led to new strategies in the development of therapeutic agents, and this has resulted in the introduction of new drugs such as tyrosine kinase inhibitors in the treatment of malignant diseases. Promising new approaches include:

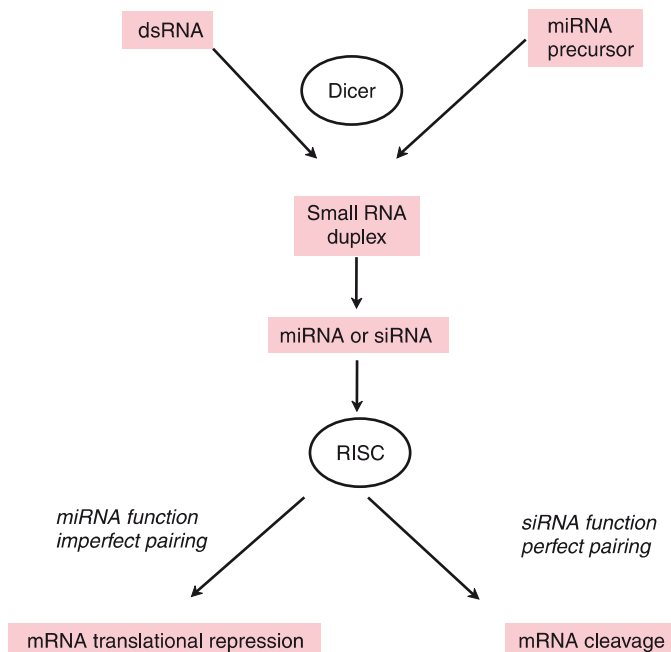
- RNA-targeted therapy
- Aurora kinase inhibition
- HSP (heat shock proteins) as targets for cancer therapeutics
- Telomerase therapeutics

RNA Technology

Small RNAs

In recent years the members of the RNA family have grown rapidly. In addition to the coding messenger RNAs (mRNAs) and transcriptional RNAs [ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs)], another subfamily, called small RNAs, has been discovered, each member of which has its own particular function. Small RNAs do not code for proteins, but instead control the transcription and translation of protein-coding RNAs. The small RNA subfamily contains small interfering RNAs (siRNAs), microRNAs (miRNAs), small nucleolar RNAs (snoRNAs), and small nuclear RNAs (snRNAs). siRNAs and miRNAs have attracted much attention due to their potential diagnostic and therapeutic applications in different diseases.

siRNAs and miRNAs are generated using the same pathway by processing long double-stranded RNA (dsRNA) or microRNA precursors with an endonuclease known as “Dicer”. Subsequently, the RNAs attach to an RNA-induced silencing complex (RISC) and are directed to the messenger RNA (mRNA) of interest which is marked for cleavage or inhibition of translation.



Small Interfering RNAs (siRNAs)

Def: These RNAs are 21–24 nucleotides in length, double-stranded, and have 3' overhangs of 2 nucleotides. siRNAs mediate the phenomenon of RNA interference (RNAi) which is a pathway for silencing the transcript of an active gene. RNAi, discovered in 1998, is now a standard laboratory tool for knocking down gene expression.

MOA: Exogenous synthetic siRNAs or endogenously expressed siRNAs attach to an RNA-induced silencing complex (RISC) and are directed to the messenger RNA (mRNA) of interest which is marked for destruction.

Th: The highly specific silencing effect of RNAi has emerged as an attractive way to allow specific inhibition of the function of any chosen target genes, including those involved in diseases such as cancer, AIDS, and hepatitis.

- The biggest obstacle to the development of RNAi-based therapeutics is the delivery. Trigger RNAs (dsRNAs from which siRNAs are derived by the action of Dicer) can be expressed from vectors or delivered as artificial siRNAs. Inserting foreign vector sequences (gene therapy) into chromosomal DNA includes the problem of insertional activation and inactivation of cellular genes. Direct administration of siRNAs would require siRNAs that are stable and modified to be resistant to nucleases.
- Drug therapies using siRNAs are now in clinical trials for treating age-related macular degeneration and respiratory syncytial virus infection (RSV).

MicroRNAs (miRNAs)

Def: MicroRNAs are short 20–22 nucleotide RNA molecules that are negative regulators of gene expression in a variety of eukaryotic organisms. miRNAs are involved in numerous cellular processes including development, differentiation, proliferation, apoptosis, and stress response. About 350 miRNAs have been identified in humans, with the total predicted to eventually reach 1,000 or more. miRNA mutations or altered expression correlate with various human cancers and indicate that miRNAs can function as tumor suppressors or oncogenes (“oncomirs”).

MicroRNAs currently associated with human cancer

MicroRNA	Cancer role	Cancer type	Mechanism
miR-15	Tumor suppressor	CLL	Bcl-2 inhibition
miR-16	Tumor suppressor	CLL	Bcl-2 inhibition
miR-155	Oncogene	Lymphoma, breast	Cooperation with myc
let-7	Tumor suppressor	Lung	ras inhibition
miR-21	Oncogene	Glioblastoma	Antiapoptotic

MOA: Like siRNAs, miRNAs are generated from long primary precursor RNAs before being processed by the Dicer protein in the cytoplasm and incorporated as mature miRNA into the RNA-induced silencing complex (RISC). Whereas siRNAs perfectly match with the target mRNA, most miRNAs do not match the target sequence exactly, enabling them to bind to multiple mRNAs. Instead of destruction of mRNA as in RNAi interference, imprecise matching results in inhibition of translation.

Th: About half of the annotated human miRNAs map within fragile regions of chromosomes in cancer genomes. Expression profiling of about 200 miRNAs has been shown to be a more accurate method of classifying cancer subtypes than using the expression of protein-coding genes.

Gene therapies using miRNAs might be an effective approach to restore tumor suppressor function or to block oncogene activation.

- Administration of synthetic antisense oligonucleotides that encode sequences that are complementary to mature oncogenic miRNAs—termed anti-miRNA oligonucleotides (AMOs)—might effectively inactivate miRNAs in tumors and slow their growth.

- Antagomirs, a novel class of chemically engineered oligonucleotides appear to be specific and effective silencers of miRNA expression in mice when conjugated with cholesterol.

Aurora Kinase Inhibition

Def: The serine-threonine kinases Aurora A, B, and C represent a family of mitotic regulators which are essential for mitotic progression, spindle formation, centrosome maturation, chromosomal segregation, and cytokinesis. Selectively inhibiting Aurora kinase activity by RNAi or small molecules leads to chromosome segregation errors and deregulation of the spindle checkpoint associated with cell death.

MOA: Aurora A localizes to centrosomes / spindle poles and is required for spindle assembly, whereas Aurora B is a chromosome passenger protein required for phosphorylation of histone H3, chromosome segregation, and cytokinesis. Elevated expression of Aurora A and B has been detected in many human cancers and overexpression of Aurora A has been shown to induce oncogenic transformation in vitro.

Tumor cells treated with Aurora kinase inhibitors show normal timing of expression of core cell cycle regulators, such as cyclins, and do not undergo arrest or delay transit through mitosis as classic antimitotic agents do. The antiproliferative effect is unique, in that tumor cells, especially those lacking functional p53 damage response, are catastrophically driven forward and out of an aberrant mitosis, which finally leads to cell death due to massive chromosomal instability.

Th: Although Aurora A has received most of the attention so far in terms of a link with human cancer, Aurora B might be the more suitable anticancer drug target, because inhibition of Aurora B rapidly results in a catastrophic mitosis.

- Small molecule inhibitors in development: Hesperadin, AZD1152 (phase I), MK0457(= VX-680, inhibits also Flt-3, phase I), MLN8054 (Aurora A, phase I), JNJ-7706621 (inhibits also cyclin-dependent kinases, CDKs)
- Aurora kinases are only expressed and active as kinases during mitosis, therefore, it is assumed that non-proliferating cells would not be adversely affected by Aurora kinase inhibitors
- Reduction or abrogation of histone H3 phosphorylation could serve as biomarker
- MK0457(= VX-680) is effective against imatinib- and dasatinib-resistant bcr-abl(T315I) kinase in vitro

HSP90 as Target for Cancer Therapeutics

Def: The heat shock protein (HSP) family of proteins has emerged as a target for cancer drug discovery because it is important for mediating the action of oncogenically relevant growth factor receptors and their downstream signaling elements.

MOA: Many HSPs form multimolecular complexes that act as chaperones binding other proteins, denoted as client proteins. HSP90 consists of two isoforms and is one of the most abundant cellular chaperone proteins. It is a cellular chaperone required for refolding of denatured proteins, cellular survival under stress conditions, and the maturation of a subset of proteins that play key roles in oncogenesis. Therefore, HSP90 does not catalyze a single reaction, but mediates the stability and function of multiple client proteins such as:

- Signaling protein kinases (e.g., PDK1, RAF-1, AKT, ZAP-70, IKK)
- Transmembrane tyrosine kinases (e.g., HER2, c-Kit, IGFR, MET)
- Mutated signaling proteins (e.g., p53, c-Kit, FLT-3, B-Raf)
- Chimeric fusion oncoprotein kinases (e.g., bcr-abl, NPM-ALK)
- Cell cycle regulatory proteins (e.g. CDK4, myc, Chk1, wee1)
- DNA repair (e.g., telomerase, DNA-PKcs)
- Steroid receptors (e.g., androgen, estrogen, progesterone receptor)

- Persp:** Natural products, including the ansamycin antibiotic geldanamycin and radiciol, that bind selectively to HSP90 and inhibit its chaperone function have been identified. However, geldanamycin is limited by its hepatotoxicity for clinical use, but a less toxic derivative 17-allylamino-17-demethoxygeldanamycin (17-AAG) has been identified.
- Due to poor chemical stability and bioavailability subsequent geldanamycin- and non-geldanamycin-based compounds are in development: KOS-953 (Tanespimycin, cremaphor-based formulation of 17-AAG, phase I/II), KOS-1022 (17-DMAG, Alvespimycin hydrochloride, orally active, phase I), CNF1010 (oil-in-water nanoemulsion of 17-AAG, phase I), CNF2024 (orally, phase I), IPI-504 (water soluble, phase I), SNX-5542 (orally active, preclinical).
 - Tumors which are dependent on a given client protein are particularly sensitive to degradation of HSP90 inhibitors. The proteins observed to be most sensitive to HSP90 inhibitor-induced degradation are the HER2 and MET receptor tyrosine kinases, RAF-1 kinase, and the estrogen and androgen receptors.
 - HSP90 inhibitors might be particularly effective in cancer cells in which Rb is mutationally inactivated (e.g., small cell lung cancer). Typically, HSP90 inhibition induces cell cycle arrest in G1 phase. However, in Rb-defective cells, tumor cells fail to arrest in G1 and enter a mitotic block with disordered prometaphase and unstable kinetochore assembly which is followed by apoptotic cell death.
 - HSP90 inhibitors enhance the activity of cytotoxics including taxanes, anthracyclines, hormonal agents, bortezomib, trastuzumab, and radiation. There is a schedule dependence in context with an intact retinoblastoma (Rb) function due to its growth arrest in G1 phase of the cell cycle.
 - Inhibitors of angiogenesis may also sensitize tumor cells to HSP90 inhibitors, because hypoxic tumor cells are under greater stress and HIF1alpha is also a client protein required for survival under these conditions.

Telomerase Therapeutics

- Def:** Maintenance of telomeres at the ends of chromosomes is essential for unlimited cellular proliferation and confers immortality in cancer cells. Since most cancer cells are reliant on telomerase for their survival, this enzyme represents an attractive mechanism-based target for the development of new cancer therapeutics.
- Ma:** Telomeres consist of repetitive double-stranded repeats of the sequence TTAGGG associated with telomere-binding proteins. Their major function is to cap the ends of chromosomes and to provide genetic stability. Telomerase is a ribonucleoprotein enzyme, which synthesizes telomere repeats de novo. In human cells, the telomerase holoenzyme consists of a high-molecular weight complex with a template-containing RNA subunit, hTR, and protein components including the catalytic subunit human telomerase reverse transcriptase, hTERT. In addition, several additional molecules might play a role in regulating in vivo activity of telomerase such as the chaperone HSP90/p32. In most normal somatic cells telomerase activity is absent and telomere repeats are lost with cell division and with ageing. Telomere attrition beyond a certain threshold is assumed to uncouple chromosome ends which subsequently induces DNA damage and onset of replicative senescence. In contrast, about 80–90% of cancer cells have detectable telomerase activity, which leads to stabilization of telomeres and unlimited growth potential.
- Persp:** Strategies targeting telomeres / telomerase in cancer cells:
- Oligonucleotide antagonists against hTR or hTERT (e.g., GRN163L, a thio-phosphoramidate oligonucleotide targeting the template region of hTR as a “template antagonist”; phase I/II). Like siRNA therapeutics (see above) there remains the issue of delivery and stability of antisense oligonucleotides.
 - Small molecule inhibitors of the catalytic component hTERT (e.g., BIBR1532; preclinical); the antiproliferative effect is not induced by inhibition of the enzyme itself but through consecutive telomere dysfunction. The lag period of telomere shortening limits the widespread use of this approach.
 - Heat shock protein 90 (HSP90) inhibitors which compromise telomerase assembly by targeting HSP90/p23 (phase II)

- Small molecules that stabilize the folding of the G-rich telomere strand into G-quadruplex structures (e.g., BRACO19; preclinical). Such folding is incompatible with telomerase function and may induce rapid telomere uncapping. The toxicity of such molecules is not yet clarified.
- Immunotherapy with vaccines targeting hTERT-specific epitopes on cancer cells (GV1001; PrimoVax and TeloVax trial for pancreatic cancer, phase II).
- Telomerase-directed gene therapy:
 - Suicide gene therapy: the hTERT promoter is linked to a proapoptotic gene or cytotoxic prodrug.
 - Oncolytic viral therapy: viral genes which are critical for replication are placed under control of the hTERT gene promoter. This results in virus vectors that are replicated only in telomerase-positive cells, and then spread to adjacent cells on cell lysis (e.g., GG5757 adenovirus which replicates only in retinoblastoma (Rb)-defective and hTERT-positive cells, preclinical).

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