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SEMINAR

Molecular Pathology of Tumor Metastasis III.

Target array and combinatorial therapies

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Therapy of tumor progression and the metastatic disease is the biggest challenge of clinical oncology. Discovery of the diverse molecular pathways behind this complex disease outlined an approach to better treatment strategies. The development of combined cytotoxic treatment protocols has produced promising results but no breakthrough in the clinical management of metastatic disease. The multiple – specific and non-specific pathways and cellular targets of tumor progression are outlined in this review. Such an approach, individually designed for various cancer types, may have a better chance to treat or even cure cancer patients with progressive disease. (Pathology Oncology Research Vol 9, No 1, 49–72, 2003)

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Introduction

Recently, advances in molecular cancer research have revealed numerous new therapeutic targets, some of which have already been tested in clinical settings. However, the clinical management of cancer patients is still based on surgical and/or radiological eradication of the primary solid tumor and chemical/radiological anti-proliferative/cytotoxic treatment of the disseminated disease. Despite a great deal of improvement in the control of loco-regional disease, only slow progress has been seen in systemic disease. As a result, mortality of cancer patients is still high due almost exclusively to the development of metastases. One obvious explanation for this discrepancy could be a difference between the molecular pathways controlling tumor growth and tumor progression (as analyzed in detail in the previous publications of this series).[1,2]

By definition, antimetastatic therapy covers all those available approaches which can prevent cancer cell dissemination or eradicate already disseminated and arrested tumor cells or their growing colonies outside the primary site, irrespective of the size of the cell population. Since metastatization (or tumor progression) is a complex phenomenon, its effective inhibition requires more than an anti-proliferative protocol.

Management of primary cancer and its eradication is primarily based on surgery and/or irradiation. On the other hand, chemotherapy is applied mostly as "anti-metastatic therapy", with the exception of the treatment of hematological malignancies and the primary chemotherapy of solid tumors. Since the process of metastasis is a complex interplay between the disseminating cancer cells and the host, rational and successful anti-metastatic interventions may target all the participants of these interactions in which anti-proliferative/cytotoxic interventions are only parts of a much more complex approach.

This review intends to describe all the possible targets in the metastatic cascade, which could be attacked pharmacologically, and to provide experimental and clinical examples for their therapeutic potentials. On the other hand, we will not review the currently available radio- and chemotherapeutic protocols (including endocrine therapy) of advanced cancer extensively documented in the current literatures.

Pathomechanism

Since chemical compounds with pharmacologically exploitable properties may act on pathobiological events and interfere with one of the relevant molecular mechanisms it appears highly important to analyze the series of steps in

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the metastatic process and to attempt to select their appropriate inhibitors. It is conceivable that antimetastatic therapy might not be limited to a priviledged drug but hopefully will cover multiple agents acting on molecular mechanisms implicated in the sequence of the metastatic cascade.[1] Certainly these pathobiological events represent only those sequential steps that are strictly related to the passage of the tumor cells from the primary tumor to the secondary site. For the full completion of metastasis the tumor cells must have high viability to survive the circulation and have the potential for growth and vascularization. These latter events however occur in the primary lesion as well and their biological consequences do not necessarily lead to metastasis. There are overlaps in the molecular mechanisms in the metastatic cascade (i.e. shedding and proteolysis, which occur at several steps), in addition, some features may be present in both the primary tumor and the metastatic lesions.

There are two unique features in the metastatic process that have come to light recently, namely its inefficiency (i.e., only a small proportion of the tumor cells escaped from the primary tumor can form metastatic lesions) and the transition of the carcinoma cells between the epithelial and mesenchymal pheno- or genotype.[3] Until now, it was assumed that most tumor cells entering circulation are killed by monocytes, lymphocytes or leukocytes. However, in experimental models it was shown that a great proportion of tumor cells can survive the hostile circumstances of the circulation and their viability and growth potential are controlled by the secondary site. This implies that in hematogenous dissemination the initial steps (intravasation and survival in the circulation) could be much more efficient processes than the subsequent ones (extravasation and survival at the secondary site).[4] Consequently, the fate of disseminated tumor cells is determined by the new environment which controls growth to allow persistence and the formation of micro- and macroscopic lesions, or death of the tumor cells.

Another principal question concerns the uniformity in the mechanism of tumor dissemination, which must be considered seriously when designing antimetastatic therapy. The prevailing model of metastasis holds that few tumor cells with metastatic potential (one in ten million) within the large primary lesions acquire metastatic capacity through somatic mutations.[5] The genotypic heterogeneity of the tumors characterized by identical histology indicates the existence of diverse mechanisms involved in the progression of the individual tumors. Therefore it came as a surprise that metastatic solid tumors of diverse histological types have been found to have a common gene expression profile.[6] Since in lung-, breast-, prostate adenocarcinomas and medulloblastoma (but not in lymphoma) a similar tendency of association between gene expression profiles and metastasis has been observed, a generic gene expression program related to metastasis rather than distinct mechanisms of metastasis in different tumor types has been proposed. The characteristic set of genes expressed are designated as a molecular signature of metastasis, and show a close relationship with overall survival of the patients. Perhaps more important is the finding that this gene expression profile can be identified in a subset of primary tumors with higher metastatic capacity, suggesting that propensity to metastasize is a consequence of the predominant genetic state of the primary tumor and making less plausible the emergence of rare cells with metastatic phenotype in large tumors. These results argue for the development of metastatic phenotype during malignant transformation and indicate that the metastatic potential may be encoded in the entire tumor, consequently providing guidelines to identify tumors with high propensity to metastasize before surgery. Nevertheless, it must be mentioned that the above gene expression profile associated with metastasis includes many less characterized genes not listed among those known to contribute to the invasive/metastatic potential of tumor cells. Rather, these genes are expressed by stromal components of the tumor tissue, supporting the important contribution of the host to the entire process.[7] There is a vast amount of clinical data indicating that the incidence of metastasis is not simply related to the size of the primary tumors. Micrometastasis can appear in patients with small, low stage tumors and also in the absence of clinically detectable primary tumors.[8] Thus the chronic and systemic features of malignant disease indicate the importance of planned patient control after surgery and the availability of an arsenal of pharmaceutical interventions for the prevention/treatment of disease progression.

Below, we will summarize all the possible pharmacological targets and approaches developed to date, which are tested at least in experimental metastasis models or in the clinic for the treatment of advanced metastatic disease. These approaches can be divided into metastasis-specific modalities targeting those molecular events that are specific for the metastatic cascade, and nonspecific modalities targeting the primary and the metastatic tumor tissue as well as the dissemination process itself. Furthermore, pharmacological approaches are also divided according to their targets, i.e. tumor cells or host cells (tumor-host interactions).

SPECIFIC ANTI-METASTATIC THERAPY

Target: tumor cells

Membrane receptors

Integrins

Tumor cell-extracellular matrix (ECM) interactions are key events of various repetitive steps in the metastatic cascade involving ECM recognition, concentration of proteases to the invadopodia and migration on matrix sheets.[7] In various cancer types a well-defined integrin receptor

pattern develops, which promotes the emergence of the invasive/metastatic subpopulation. In special circumstances individual integrins can even become predominant over other matrix receptors offering at least a marker of these invasive cells.[1] Once a predominant integrin is identified on the surface of invasive cancer cells it offers a target for therapeutical intervention.[9]

Since most ECM proteins share the consensus sequence, RGD, for integrin binding, ***small peptide inhibitors*** of invasiveness and metastasis have been primary options. *In vitro* data provided ample of evidences that RGD-ligand peptides are powerful inhibitors of cancer cell adhesion, migration on matrices and in experimental metastasis models.[10] Such peptides showed also anti-invasive, anti-metastatic activity, especially in the case of hematogenous dissemination.[11] Unfortunately none of these peptides have yet entered clinical testing. Recently, following the same concept, larger ***RGD-containing peptides of fibronectin with fibril-forming potential*** have been designed and tested in experimetal models of various human cancers.[12] On the other hand, natural RGD-containing inhibitors, called ***disintegrins*** have also been identified in the venoms of various snake species. These natural peptide inhibitors of integrins are powerful inhibitors of cancer cell ahesion to various matrices including basement membrane as well as of cell migration, and are active in vivo in experimental metastasis models, especially in the case of hematogenous dissemination.[13-16]

Neutralizing/inhibitory antibodies against integrin receptors have pharmacological potentials too. However, unlike anti-growth factor antibodies, only a few such antibodies have been applied as anti-cancer agents. ***Vitaxin*** is an $\alpha v\beta 3$ integrin inhibitor humanized antibody which was primarily designed as angiogenesis inhibitor.[17] However, several cancer types are characterized by prominent $\alpha v\beta 3$ integrin expression and in these cases this integrin has been shown to be involved in the complex process of invasion and metastasis.[1] Meanwhile, to date there are no experimental data available concerning the direct anti-metastatic potential of Vitaxin. Another humanized anti-integrin antibody, ***abciximab*** (ReoPro) was introduced to clinic as anti-thrombotic agent targeting the platelet integrin $\alpha IIb\beta_3$ and the endothelial $\alpha v\beta 3$.[18] Abciximab not only inhibits platelet aggregation with or without the presence of tumor cells but also angiogenesis.[19] Ectopic expression of the $\alpha IIb\beta_3$ integrin has been observed in various cancer types including melanoma (also known to express $\alpha v\beta 3$).[20] Using experimental human melanoma metastasis models, abciximab has demonstrated anti-tumoral and anti-metastatic effects, encouraging further investigation of this subject.[19]

There is another rationale to target surface integrins on invasive/metastatic cancer cells. *In vitro* data have indicated that matrix adhesion induces drug resistance through overexpression of certain integrins. Such a phenomenon can be observed both in the case of hematological malignancies as well as in solid tumors.[21] An anti-integrin approach to the treatment of metastatic tumors may have the long-awaited „side effect" of reducing the drug-resistance of cancer cells.

Growth factor (GF) inhibition

One of the earliest pharmacological agents discovered as a GF competitor was ***suramin***, a polysulfonated naftylurea.[22] The polyanionic molecule was able to sequester several growth factors with heparin binding domains including EGF, IGF, TGF-β, bFGF and PDGF. Several of these GFs are involved in the invasion and metastasis of various cancer types, and therefore it was expected that suramin would be an antimetastatic agent. However, suramin was too toxic and its non-specific binding to serum proteins limited its biological effect in vivo.[23] Accordingly, ***pentosan sulfate derivatives of distamycin A*** have been designed (heparinoids) and tested in experimental models. These compounds have anti-mitotic and anti-angiogenic effects and are able to compete with at least bFGF and IGF, suggesting that they could be developed as anti-metastatic agents too.

An alternative approach would be to use neutralizing anti-GF antibodies to suspend auto- or paracrine stimuli to cancer cell invasion. The feasibility of such approach is demonstrated in angiogenesis, where anti-VEGF antibodies have been demonstrated to be active *in vitro and in vivo* in preclinical models.[24] However, development of other anti-GF antibodies have not yet been reported in the literature.

Growth factor receptor (GFR) inhibition

The majority of growth factors involved in the malignant phenotype of cancer are also motogenic factors: frequently, the same mitogen regulates the migration of cancer cells through partially degraded matrix. Accordingly, targeting the receptors for these GFs can provide an approach to target the regulation of cancer cell migration too. Cancer cells frequently overexpress GFRs at their surface due to genetic alterations in their genome, i.e. amplification. Most frequently such genes are members of the EGFR family.[25] EGFR2 (c-erbB2) is amplified and overexpressed in a subset of breast cancer characterized by a more agressive, more metastatic phenotype[26,27] but it has also been demonstrated to be expressed and functioning in ovarian- lung-, prostate- and GI-tract cancers (adenocarcinoma) as well as in head and neck cancers (squamous cell cancers).[25] EGFR1 (c-erbB1), on the other hand, is overexpressed mostly in squamous cell cancers, and in some adenocarcinomas too (such as colon cancer).[25] Another reason to target GFR on invasive cancer cells is that there is a cooperation between the signaling pathways of integrins and growth factors, and parallel inhibition of the two initiators of cell migration promises more

success in shutting down the motogenic activity crucial for cancer cells. Although there are several alternative options for targeting EGFR (specific ligand-mimetics, and *inhibitory antibodies*) only the latter approach has proved to be successful clinically. The best example is *Herceptin,* a humanized anti-c-erbB2 antibody, which is active clinically in advanced breast cancer overexpressing c-erbB2. The molecular consequences of the application of anti-c-erbB2 antibodies indicated that they downmodulate the surface receptor, thereby inhibiting the signaling cascade, but also initiate complement-mediated cytotoxicity as well as antigen-dependent cytotoxicity against their target.[26,27] Its success could well be followed by *IMC-C225,* an anti-c-erbB1 antibody that already has a humanized version. This antibody proved to be very effective against cancer progression in preclinical models.[28,29]

It is a novel approach to inhibit GFR expression at the level of transcription. The c-met oncogene and its ligand HGF have been demonstrated to be important regulators of the invasive/metastatic phenotype of various cancer types including colon cancer and melanoma.[1,30] Furthermore, this receptor system has also been implicated in the liver metastatic potential of various tumors thereby offering another rationale for anti-receptor anti-metastatic intervention. There are two tested pharmacological approaches in the literature, one targets the ligand, HGF, and the other the expression of the receptor.

The HGF ligand competition approach has used two strategies, a „splice variant" recombinant HGF with receptor-inhibitory potential[31] and the exploitation of the heparin-binding potential of the ligand and its importance in the conformational activation. Both the *inhibitory ligand peptide NK-4* (either recombinant or incorporated in adenovirus vector [31,32]), as well as a *peptidomimetic ligand of the heparin binding domain of HGF*[33] exhibited anti-invasive/anti-metastatic activity in preclinical models including effects on liver metastasis. Furthermore, since HGF is also an angiogenic factor, both approaches resulted in the inhibition of tumor-induced angiogenesis,[31,34] where NK-4 seemed to be more potent.

The approach to transcriptional regulation exploited the unique potential of *geldanamycin* (a member of the family of anisamycin antibiotics). This drug exhibited very selective inhibitory activity on the expression of c-met in cancer cells resulting in the down-regulation of c-met signaling and loss of the invasive/motile phenotype in experimental systems.[35]

Transmembrane proteoglycans

Proteoglycans at the surface of cancer cells have been demonstrated to be involved in tumor progression in a Janus-faced manner.[36] In certain tumors downmodulation of their expression could be observed during carcinogenesis, while in other tumors overexpression occured when the tumor became invasive and metastatic.[36,37] The major players in this respect are the transmembrane type heparan sulfate proteoglycans (HSPGs: syndecans and CD44v3) and GPI anchored HSPGs of the glypican family. Their function is regulated by the glycanation process, which adds either heparan sulfate (HS) or chondroitin sulfate (CS) chains to the core protein. These proteoglycans, mostly through the HS-chains, are involved in cytokine/growth factor recruitment as well as in matrix adhesion processes, serving as co-receptors.[36] Furthermore, the transmembrane forms (syndecans and CD44) have been involved in important signaling processes such as vnt (syndecan-1), PKCα (syndecan-4) or motility signaling (CD44v3). These diverse functions of transmembrane HSPGs in the invasion process make them potential targets for therapeutic interventions.

It is well documented that in invasive/metastatic cancer cells glycanation of membrane proteoglycans is frequently shifted toward heparan sulfates from chondroitin sulfates offering a target for pharmacological interventions.[36,37] It was shown early on that using nonspecific glycosaminoglycan (GAG) biosynthesis inhibitors (β-xyloside, 2-deoxyglucose) it is not possible to alter invasive phenotype of cancer cells unless only the heparan sulfates are preferentially affected.[38] *5'hexyl-2'-deoxyuridine (HUdR)* preferentially inhibits HS biosynthesis in cancer cells.[39-41] Such treatment does not affect cell proliferation, but rather inhibits tumor cell-ECM interactions, thereby down-regulating the metastatic potential.

A unique approach for the inventional use of GAGs has been developed when neoglycans were prepared from albumin and CS or HS GAGs.[42] Among these new glycans neoCS demonstrated impressive anti-tumoral pro-apoptotic effects *in vitro* and *in vivo* in experimental breast cancer and myeloma. Further studies are required to explore the pharmacological potentials of these new GAG agents.

Cell adhesion molecules (CAM)

Local invasion (previously defined as shedding), representing the first step in metastasis, may occur if the intercellular links and – in the case of the epithelial cells – firm contact with the basement membrane have been drastically altered. Subsequently the detached tumor cells are not confined by their neighbours and are ready for invasive growth. Assuming that more detailed knowledge of these cell contacts could be utilized in drug development, the question has been raised whether loss or gain is the dominant feature in the molecular mechanisms implicated in shedding.

At present cadherins, through the formation of adherent junctions, are regarded as the critical molecules both in homophylic and heterophylic intercellular contacts.[43] It is noteworthy that MDA-MB-231 human tumor cells, after transfection with E-cadherin cDNA, lose their capacity to form osteolytic metastases.[44]

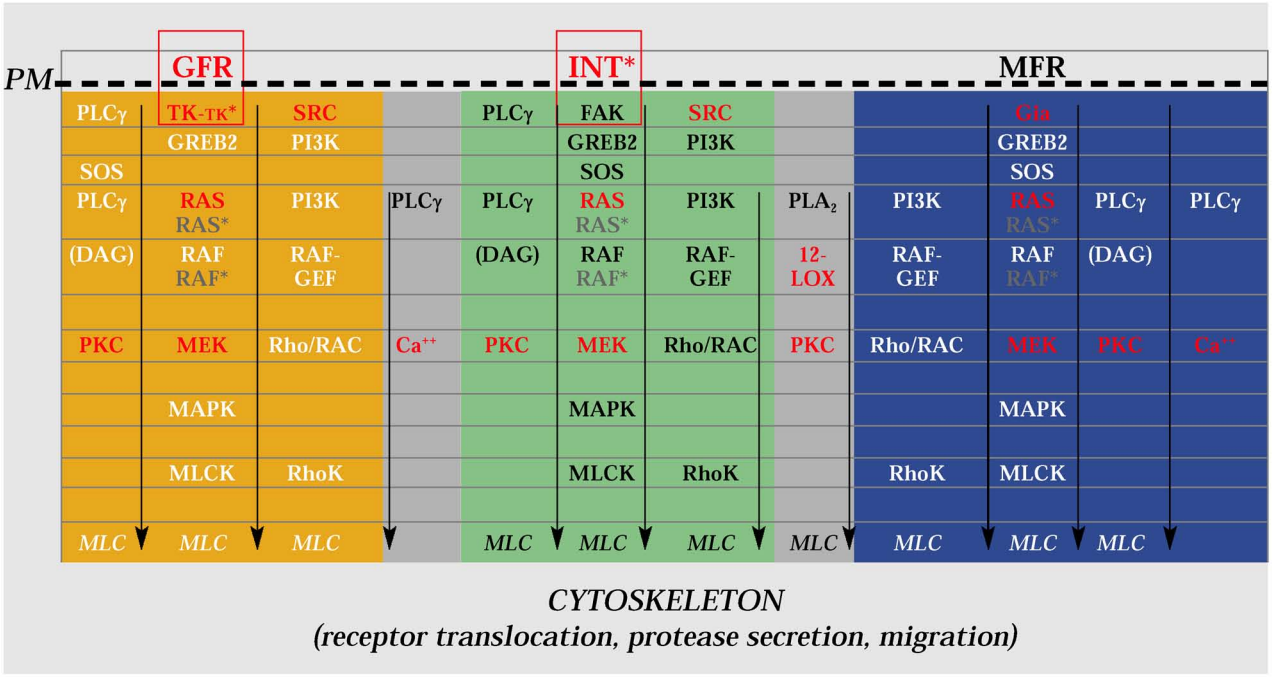


Figure 1. *Signaling pathways in tumor cell invasion. GFR: growth factor receptor; INT: integrin receptor, MFR: motility factor receptor; MLC: myosin light chain; PM: plasma membrane; TK: tyrosine kinase; INT*/TK*/RAS*/RAF*: activating mutations. Red: pharmacologically targeted signal elements*

Several clinical studies have concluded that E-cadherin expression is reduced in various malignant tumors (prostate, stomach, kidney, head and neck, etc.). This correlates with the metastatic potential of the tumors, therefore cadherins are considered as invasion/metastasis suppressor molecules.[44] Based on these observations it was suggested that administering agents that up-regulate E-cadherin expression may be a potential therapeutic approach to inhibit tumor spread.

Defects in E-cadherin functions can occur despite high levels of expression by steric hindrance of cell surface proteoglycans.[45] In this latter case experimental reduction of the glycanation of cell associated proteoglycans with β-D-xyloside resulted in the abrogation of tumor cell invasiveness without influencing E-cadherin expression.

Signal transduction

Tumor cell invasiveness and metastatic potential depend critically on the signaling events generated through the interactions with ECM and the autoparacrine regulatory factors of motogenicity *(Figure 1).* These signaling pathways are highly similar to those functioning in normal cells as far as their final downstream targets are concerned, but the upstream pathways are highly diverse in the various types of cancer depending on the specific genetic background (such as RAS mutations, oncogenic splice variants, deletions, fusions etc). Furthermore, these signaling pathways are differently controlled at receptor level since both the expression of adhesion receptors and cytokine receptors can be altered in cancer cells. Accordingly, signal transduction inhibitors could be potentially useful in controlling invasiveness and the metastatic potential of cancer cells. This idea is further corroborated by the recent findings on metastasis suppressor genes where a subset of these genes controls those signaling pathways that are critical for metastasis and does not affect the mitotic signaling, arguing for the existence of invasion-specific signaling pathways.[46]

Receptor tyrosine kinase (PTK) inhibitors

Although several PTK inhibitors have been recently developed to target the tyrosine kinase activity of EGFR,[47] VEGFR[48] and ABL/c-kit/PDGFR,[49] their effects on the regulation of tumor cell motility is largely unknown. Today several small molecular EGFR inhibitors are in *clinical trials*[50] and some of them have already demonstrated activity against head and neck-, ovarian- and non-small cell lung cancers.[50,51] It is interesting that c-ABL is one of the many downstream kinases of the integrin-linked signaling,[52] which already has a clinically successful inhibitor (*Gleevec*, imatinib mesylate, in chronic myloid leukemia[49]). Future studies will reveal if this drug has any anti-invasive potential in solid tumors. On the other hand,

there are a series of PTKs that could well be anti-invasive drug targets, including the integrin-associated FAK, c-met and c-src.[52] A small molecular inhibitor of src (PP2) has been shown to be active *in vitro* in various cancer types and even inhibited liver metastasis formation in a preclinical model, offering a promising pharmacological alternative.[53]

G proteins

Autocrine motility of tumor cells is regulated by two ectoenzymes, AMF/phosphoglucose isomerase and ATX/ecto-phosphodiesterase/lysophospholipase D.[54,55] While the receptor of the former was identified as a chemokine-type receptor,[56] it is still unkown in the case of the latter. The most upstream element of both signaling pathways contains *pertussis toxin (PTX)* sensitive G proteins.[57] PTX was shown to be a highly potent inhibitor of cancer cell migration *in vitro* and *in vivo* in experimental models and was tested recently in *clinical trials* againts bladder cancer as a local treatment. [58] On the other hand, a clinically available anti-thrombotic agent, *cilostazol*, targeting the phosphodiesterase activity of ATX has shown a potent anti-motile effect *in vitro* on cancer cells, suggesting that this autocrine loop could be specifically targeted in cancer. [59]

RAS

Downstream elements of the two signaling systems (adhesion and migration) involves RAS, which is a target of extensive investigations.[51,52] Since active RAS is anchored to the lipid bilayer by farnesyl transferase, *inhibitors (FTI)* of this process have been designed. [50,51] Although these inhibitors showed great promise initially in preclinical models, they turned out to be *clinically inactive*. A major problem with FTIs is that there is an alternative mechanism for RAS anchoring (geranylation) and the inhibitors act preferentially on H-RAS while in human tumors K-ras and N-ras are predominant. The antisense approach is also being tested clinically where both H-RAS and its downstream partner RAF-1 are used as target (results are yet unknown).[60-62] However, recently it was reported that activating BRAF mutations (but not RAF-1) are frequent in certain human tumor types, suggesting BRAF as a potential target in the RAS- (and motility-) signaling.[63] *BAY43-9006 is a non-selective RAF inhibitor,* which could well be the future drug for human tumors with BRAF activations and it is now in *clinical trials*.[51]

MAPK

A major downstream signaling target of the integrins is MAPK, involved in both matrix interactions and cancer cell motility.[50,52] Around one third of human cancers shows activation of the RAF-MAPK system, suggesting it as a feasible anti-invasive target. MAPKs (1,2) are phosphorylated by dual specificity kinases MEK1,2 and selective non-ATP-competitive antagonists already exist (PD98059 and U0126, see for review in reference).[51] These inhibitors affect cancer cell proliferation, survival and migration *in vitro* and *in vivo* in experimental models. Furthermore, *orally active variants of MEK inhibitors* have been developed and are now being tested in clinical trials.[51]

Lipid signaling

Activation MAPK in integrin signaling can be achieved through various pathways. A classical one is mediated through FAK/SOS/RAS pathway but alternatives exist and one of them is lipid signaling.[52] In this case integrin ligation or constitutive activity stimulates phospholipases-Cγ, D or A_2. Activated PLCγ pathway will result in PKC activation while PLA_2 activity will lead to the activation of the arachidonic acid pathway (COXs or LOXs).[52,64] Experimental data demonstrated that certain integrin signaling pathways involve the activation of 12-lipoxygenase providing bioactive lipid (12-S-HETE) for activation of PKC [65] as well as of cyclins.[66] Furthermore, the involvement of 12-LOX-PKC in the motility signaling of AMF was also documented.[67,68] Accordingly, both matrix adhesion as well as cancer cell migration dependent on the activity of 12-LOX enzyme of cancer cells.[64] Pharmacological inhibition of tumor cell 12-LOX inhibited tumor cell – matrix interactions (adhesion, protein degradation and migration) *in vitro* and metastasis formation *in vivo* in experimental models,[69,70] suggesting *lipoxygenase inhibitors* as feasible anti-metastatic agents.

COX-2 was demonstrated to be involved in GI tract carcinogenesis as well as in angiogenesis,[71] although the exact role for this enzyme in signaling is not known yet. Analysis of the autocrine signaling by AMF in cancer cells revealed the involvement of COX enzymes upstream of the target tyrosine kinases but downstream of the G proteins.[68] Recent studies on human colorectal cancer cell lines indicated that the *pharmacological inhibition of COX-2* by etodolac or JTE-522 results in the inhibiton of migration and secretion of MMP-2 *in vitro* and inhibition of liver metastasis in preclinical models.[72,73] These data indicate that at least in colorectal cancer COX-2 inhibition could be developed to form anti-metastatic regime specifically targeting tumor cells.

PKC

PKCs have been shown to be involved in the mitogenic and motogenic pathways, including integrin signaling and have been considered feasible drug targets for a long time. However, PKC is a family of serine/threonine kinases with variable structure and function. PKCs involved in cytoskeletal functions (adhesion and motility) are the, iso-

forms: these are therefore to be considered as anti-metastatic drug target. Several PKC inhibitors have been developed but few have yet *entered clinical trials*. The best known inhibitor is *bryostatin* which has been shown to be active *in vitro* and *in vivo* in preclinical models, though its clinical activity is minimal.[74,75] On the other hand, *selenocompounds* exhibited chemopreventive potential targeting PKC (at least the Ca-dependent isoforms) and resulting in sustained inactivation.[76] Although these molecules have shown promise in various carcinogenesis models they have not been tested in experimental metastasis models. On the other hand, it is a great challenge to know how these inhibitors would be able to discriminate between PKC in normal cells and those in the cancer cells since mutations or major structural alterations of these enzymes are not known.

Ca^{++} signaling

Ca^{++} is another important secondary messenger in the signaling pathways,[77] but in special circumstances it could well have a primary (signal initiator) function too. Classically, PLC activation in various signaling pathways leads to the generation of IP3, activation of the intracellular Ca^{++} stores and an inrease in intracellular calcium level. However, activation of certain membrane receptors can also directly induce influx of Ca^{++} into the cells through Ca-channels (voltage or ligand gated forms) and/or also liberate intracellular Ca^{++} from the intracellular stores (as a kind of third messenger function). Major intracellular downstream targets of Ca^{++} are Ca-dependent protein kinases (ie. PKC), but several EF-hand proteins should also be considered (calmodulin, calpain, Ca-dependent ATP-ase, aequorin etc.). Delineation of the molecular details of the RAS signaling identified that it is significantly modulated by intracellular Ca^{++}, suggesting another form of cross-talk between various signaling pathways.[78] The involvement of Ca-signaling in tumor cell adhesion-spreading-migration sequence was recognized early on, identifying it as a potential target for pharmacological interventions.[79] Experimental data already indicated an anti-metastatic potential of *classical Ca-channel blockers* of all classes (phenylalkylamines, benzothiazepins and dihydropyridine).[80] Treatment of cancer cells with Ca-channel inhibitors modulates integrins at the plasma membrane, rearranges cytoskeletal filaments and inhibits tumor cell-platelet interactions,[81] all critical events in dissemination.

Carboxyamido-triazol (CAI) was identified as a new pharmacological inhibitor of Ca^{++}-influx in the cancer cells, inhibiting the generation of secondary messengers, protein tyrosine phosphorylation and even gene transcription.[82] CAI was defined as a classical anti-metastatic agent inhibiting invasiveness *in vitro* and *in vivo* in experimental models. Furthermore, CAI was also identified as an anti-angiogenic agent.[79] CAI was and is being tested in *Phase I trials* in refractory solid tumors[83] and has shown frequent stabilization of the disease. In another setting CAI was used in combination with taxane in relapsed ovarian cancers showing some activity.[84] Collectively, these data suggest that Ca^{++} signaling in cancer cells can be considered as anti-metastatic target and further studies are warranted to identify clinical relevance of this approach.

Target: tumor cell-host interactions

Proteases and protease inhibitors

The fundamental role of matrix-degrading enzymes in cancer invasion and metastasis was recognized following the pioneer work of Liotta and their collegues back the 80s.[7] Since then the biological role of the three main protease classes (matrix metalloproteases: MMP, plasminogen activators and cathepsins) has become more and more complex. Initially MMPs were considered to have pure protein degrading function with their main targets in the ECM around the invasive cells, while later it turned out that they can be linked to the cell surface (some members are even transmembrane proteins), and can bind surface receptors such as integrins, CD44 or transmembrane HSPGs.[85] They are not only present at the cell surface of tumor cells but they also regulate proliferation and apoptosis. Studies also revealed that normal cells are equal contributors to the protease repertoire of invasive cancers. Major attention was attracted when the role of MMPs was revealed in tumor-induced neo-angiogenesis.[24] Pathological studies defined the protease patterns of major cancer types and identified uPA as the most common protease expressed and active in human cancer.[1] Meanwhile the expression of MMPs (MMP-2/9 – gelatinases, MMP-3/10 – stromelysin, MMP-7 – matrilysin, MMP-14 – MT-MMP) and cathepsin B and D in various cancers in association with progression was documented (colorectal-, breast- or prostate carcinoma[85,86]). Accumulating experimental data on the role of the natural protease inhibitors in the down-modulation of metastatic potential suggested proteases as the first molecular targets for metastasis-specific therapies.[86]

Since at molecular level MMPs were well-characterized, design of MMP inhibitors started early on and the experimental data supported the rationale that inhibition of MMP activity modulates the metastatic capacity of malignant cells. There are two major classes of MMP inhibitors, targeting the synthesis or the activity of the enzymes.[85] Inhibition of the biosynthesis of MMPs can be achieved by blocking their transcription by antisense technology,[87,88] using *ribozyme* (MMP-7/9),[85,86] or *downmodulating the signaling pathways* controlling it.[51] More recently *halofuginone* was identified as a fungal inhibitor of MMP expression and was shown to be biologically active in

experimental metastasis models.[89] The MMP activity inhibitors are usually small molecular inhibitors, although initially the use of the natural inhibitors was suggested, this concept did not prove useful. The three classes of MMP activity-inhibitors are *collagen peptido- and non-peptidomimetics and the tetracyclin analogues*.[86] The peptidomimetics are competitors for the cleavage site of the substrate. These include *Batimastat* and the new variant, *Marimastat* (both form British Biotech), both having been tested in phase III *clinical trials*.[85,86] Marimastat showed some promise in advanced pancreatic and gastric cancers by inhibiting progression of the disease as compared to conventional chemotherapies.[85,86] Small molecular inhibitors designed for the active site of the enzyme have also entered clinical testing, *Prinomastat* (Agouron/Pfizer) and *Tanomastat* (BAY). Among the experimentally active tetracyclin analogues *Metastat* (Col-3) has entered clinical testing in Kaposi's sarcoma (combining the anti-tumoral and anti-angiogenic potential against a malignant endothelial cell tumor).[85,86] Some unconventional MMP inhibitors have also entered clinical testing, even phase III trials including *Neovastat* (the shark cartilage extract)[90] and the green tea component *EGCG (epigallocatechin-3-gallate)*.[91] MMPs can be used also to target conventional cytostatics (e.g. Melphalan) to the tumor tissue (*collagen peptide-cytostatic drug conjugate*) as it was demonstrated in experimental models.[92]

However, the trial data do not support the overwhelming enthusiasm that developed around the MMP inhibitors as new anti-cancer/anti-metastatic agents,[86] but the design of the trials and the selection of cancer types to test their effects was and still is most probably the major cause. These invasion-inhibitors were tested clinically in advanced cancers already at a stage where invasion and metastasis had developed and angiogenesis was initiated. Secondly, the tumor types selected as target were not those reported to overexpress MMPs at advanced stage and where MMPs are prognostic factors.[1] Considering these facts, the clinical activity of some of these inhibitors must be regarded as encouraging.

The cysteine-protease system involved in cancer invasion is the cathepsin family,[93,94] and small molecular inhibitors have been designed and tested in experimental models. Of major importance is the newly developed selective *cathepsin B inhibitor* CA-074 since several cancer types overexpress cathepsin B at their surface.[95] A less selective inhibitor family was designed to the nucleophylic thiol residues in the active site of the enzyme.[96] Although these inhibitors are active *in vitro*, they have not yet been tested in experimental metastasis models. Even less development is reported in the field of the serine protease pharmacology though the significance of uPA and uPAR system in invasion and metastasis of solid cancers is widely accepted.[1,97] This family of proteases is part of the fibrinolytic system, a potent activator of the MMPs and regulator of the integrin functions, suggesting it as an attractive multipurpose anti-invasive target.

Extracellular matrix

Since the importance of a bidirectional relationship of tumor-host interactions in tumor progression has been widely accepted novel targeting of antimetastatic therapies toward molecular mediators of the tumor-host communication interface has been proposed.[7] Attachment of tumor cells to privileged host cells has been recently demonstrated showing that chemokine receptors expressed in tumor cells match the chemokines that are present in organs which are invaded by these tumor cells.[98] It is highly promising that blocking the relevant chemokine receptor can inhibit metastasis of breast cancer cells in experimental animals.[35,86] The fate of metastatic tumor cells depends on the interaction between the host cells and the tumor cells. Certain highly viable cancer cells may proliferate immediately after arrival into the new organ, sometimes even in the terminal capillaries before extravasation. More frequently, however, micrometastatic lesions are formed where the rate of apoptosis and cell proliferation is balanced.

The matrix milieu of the organs involved in cancer dissemination provides a strong selection factor for the entire process. Although this is evident from experimental data, the molecular details are not completely known, at least not to the point where clinical therapeutic modalities can be designed and tested. Bone metastasis provides an example how local ECM can influence metastasis formation and which kind of pharmacological targets could be used to be clinically successful in the selective treatment of metastasis.

Meticulous analysis of the pathomechanism of bone metastasis development revealed that osteoclasts are the key players in the bone resorption and that cancer cells are using them to initiate the process, while osteoblasts are also involved.[99,100] As a result, bone metastases are composed of lytic and plastic elements depending on the predominance of the key host cell types (osteoclasts and osteoblasts).

Data now indicate that active osteoclasts develop from a monocytic precursor upon the interaction with osteoblastic stromal cells. Key chemical mediators of this process are vitamin D3, PTH and PTHrP hormones (the common receptor is PTHR1), IL-6 and IL-11 cytokines as well as PGE2. Their molecular target is RANKL (member of the TNF ligand family) in the osteoblastic stromal cells.[100] This ligand activates its receptor RANK on mononuclear cells and initiate a cellular program of differentiation toward osteoclasts. This process is fine-tuned by a soluble TNF receptor, OPG, an inhibitor of the RANKL/RANK

interaction.[99,100,101] Bone-metastatic cancers are characterized by PTHrP expression,[2] believed to be responsible for the organ selectivity of the process and also for the priming of osteolysis (*Figure 2*). Bone matrix TGF-β further promotes the expression of cancer-PTHrP through a PKC-dependent transcriptional mechanism.[102-104]

The pathomechanism of osteoblastic bone metastasis is less well defined. It seems that endothelin-1 and its osteoblastic receptor, endothelin-A, are of primary importance in the activation process.[102] However, other mitogens such as IGF1, TGF-β or PDGF-BB may all be involved in various types of cancers metastatic to the bone. Certain proteases such as uPA and the serine protease PSA, have been reported to be involved in the development of osteoblastic metastases.

Therapeutic approaches to combat bone metastases are developing rapidly and have *entered the clinical arena*.[105,106] This is due partially to the success of the development and widespread use of *bisphosphonates*. These drugs targets the „soil" in the metastatic cascade of bone metastasis, covering the mineral components of the bone trabeculi, thereby inhibiting bone resorption by osteoclasts. Some experimental data, however, suggest that bisphosphonates have a direct anti-tumor effect as well.[100] Another target of bone metastasis is PTHrP produced by tumor cells. Its inhibition can be achieved by vitamin D analogues,[107] neutralizing antibodies[100] or even by new specific transcriptional inhibitors.[108] These approaches have shown conclusive results in experimental model systems and they are now under advanced clinical testing. The other molecular target is the RANKL/RANK system where the *natural inhibitor OPG,*[109] and a *chimeric ligand RANK-Fc*[110] have just entered clinical trials.

These approaches all target the lytic phase of bone metastasis, although osteoblastic mechanisms could be equally important. Unfortunately, only one therapeutic approach has been developed to date for this pathomechanism, and tested in experimental systems, an *antagonist of endothelin-A receptor signaling*.[100] When future therapies are designed for the more effective management of the prevention and tretament of bone metastases, both osteoclastic and osteoblastic processes should be considered.

Circulating tumor cells and hemostasis

Coagulation

Both circulating tumor cells and tumor tissues are in contact with the coagulation factors. In the latest case this is provided by the leaky new vessels produced during tumor-induced neoangiogenesis. Furthermore, it has been demonstrated in various cancer types that tumor cells can produce an array of pro- and anti-coagulation factors. However, cancer patients, especially those in advanced stages of the disease, are characterized by coagulation disorders, primarily a prothrombotic state.[111-114] Cancer cells

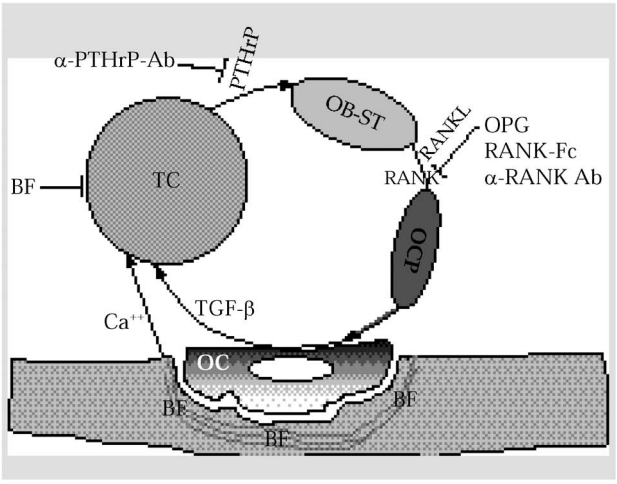


Figure 2. *Molecular targets in bone metastasis. BF: bisphosphonates, TC: tumor cell, OB-ST: osteoblast – stromal cell precursor, OPG: osteoprotegerin, Ab: antibody, OCP: osteoclast-progenitor, OC: osteoclast, PTHrP: parathormone-related protein*

produce tissue factor and/or cancer procoagulant, two major players responsible for this hypercoagulation.[112,113] Both factors activate the extrinsic coagulation pathway, cancer-TF must complexi with F-VII while cancer procoagulant directly activates F-X, ultimately leading to the production of thrombin. On the other hand, cancer cells can also express PAI-1,[112-113] which in turn promotes the expression of uPA, a physiological activator of the fibrinolytic system but also one of the most universal proteolytic enzyme of invasive cancer cells.[1]

Administration of *anti-coagulant therapy* to cancer patients revealed that such treatments not only eradicate thrombotic complications but also delay tumor progression.[115] However, such an anti-metastatic effect depends on the type of anti-coagulant applied. Agents that specifically inhibit thrombin, such as hirudin or warfarin, are less potent in this respect than the broad specificity heparin(s).[111,112,116] Recently *LMW-heparins* turned out to have interesting significant anti-metastatic effects in *clinical settings*.[116,117] Analysis of the potential targets of heparin(s) in tumor progression revealed that tumor cell-platelet interactions (mediated by P-selectin), tumor-induced angiogenesis or tumor cell proliferation and migration are all affected besides the coagulation system.[118-120]

Platelet-tumor cell interactions

The involvement of platelets in tumor progression and metastasis is a three decade-old story in which the molecular mechanism(s) responsible for these processes were gradually revealed and refined.[114,122] Tumor cell-platelet aggregates defend tumor cells from mechanical damage as well as from nonspecific or specific attacks by neutrophils,

monocytes or NK cells. Both experimental data and now clinical studies indicate that platelets promote hematogenous metastasis. The mediators of this specific intercellular communication are surface adhesion molecules and β3 integrins in particular.[122] In the case of platelets the αIIbβ3 integrin is involved, while in the case of tumor cells both classes of the β3 family could be considered (αvβ3 or αIIbβ3).[123] Integrins of tumor cells and platelets are engaged and bridged by soluble RGD-containing matrix molecules of the circulation, vitronectin, fibrinogen and fibronectin. Importantly, during the specific adhesion of platelets to tumor cells in the vasculature both participants become activated, resulting in the production of various pro-invasive and mitogenic agents including growth factors, cytokines, bioactive lipids and proteolytic enzymes.[114] In most cases tumor cell-platelet interactions are complicated by the involvement of endothelial cells (during the intra- and extravasation phases of hematogenous dissemination). Beside the integrins, this complex interaction involves selectins and other cell adhesion molecules. It is another aspect of this story that platelets, especially when activated by tumor cells, initiate or promote angiogenesis.[114,122] In this way activated platelets indirectly promote the establishment of the metastatic tumor foci.

Based on our knowledge of the molecular players involved in this interaction several therapeutic modalities have been tested in experimental and even in clinical settings. One of the most promising agents is the anti-platelet antibody, ***abciximab*** (ReoPro) targeting platelet integrin αIIbβ3.[122,123] This antibody was shown the be ***clinically active*** in disrupting thrombi in myocardial infarction. Under experimental conditions abciximab inhibited hematogenous dissemination of murine and human tumor cells and tumor growth, partially by its inhibitory effect on tumor-induced angiogenesis.

Much attention has been given to the bioactive lipids involved in platelet aggregation. Since platelet-COX as well as the LOX enzimes are involved in platelet aggregation, their inhibitors have been tested primarily in experimental metastasis models with controversial results. A possible explanation for such an inconsistency is that platelets rely on these enzymes differently depending on the concentration of the agonist(s) (in this case tumor cells). At low agonist concentration tumor cell-platelet interaction activates the COX pathway exclusively, whereas at high agonist concentration the lipoxygenase pathway is also activated.[124] This implies that COX (especially TBX) inhibitors can have inhibitory potential only at low tumor cell/platelet ratios, whereas both COX and LOX inhibitors are necessary at a higher tumor cell concentrations. As a result, such inhibitors have been frequently reported to have no effect on experimental metastasis since they were used alone and not in combination.[124] Prostacyclin, mostly produced in small amounts by platelets or endothelial cells, also has potent anti-platelet activity and was introduced early on as an experimental anti-metastatic agent.[114,122,125,126] However, further experimental studies on both PGI2 and their stable analogues indicated frequent failures of such treatments in inhibiting metastasis formation of various cancer types[125] – most probably due to similar problems as in the case of arachidonate metabolism inhibitors.

NON-SPECIFIC THERAPEUTIC TARGETS OF TUMOR METASTASIS

Target: tumor cells

Modification of apoptotic response

The inefficient apoptosis program is one of the most important factors in carcinogenesis. There are two main reasons for the lack of apoptotic response- both of which could be a target for therapy: *(a)* the apoptotic program is intact but it is inhibited by the continuous production of one (or more) survival factor(s); *(b)* the apoptotic program is damaged due to the underexpression of proapoptotic or overexpression of antiapoptotic signals. From therapeutic point of view, the activation of the apoptotic program either in the tumor cells or in those host cells which support tumor growth (e.g. endothelial cells in intra- or peritumoral vessels) could be an important contributor to therapy for both primary or metastatic tumors.

Induction or increase of the activity of proapoptotic molecules

The expression *of death receptors and/or death ligands* are key response elements to an outer apoptotic signal. Carcinogenesis (e.g. in the colon) can result in the loss of death receptor (e.g. Fas) expression.[127] It has been shown that chemotherapy can induce the expression of previously missing Fas in many neoplastic cells.[128] In cases when the lack of Fas receptor is the only deficiency along the apoptotic pathway, the responsiveness of tumor cells to FasL produced either by host cells (mainly lymphocytes)[129] or by neighbouring tumor cells (where the FasL production could also be induced by chemo- or any other therapy).[130] The latter effect may contribute to the „bystander effect" observed after gene therapy.

In colon cancer the ***Fas receptor*** can be induced by 5-fluorouracil through a p53-dependent pathway. [131] However, in many cancer types p53 is mutated or its pathway is damaged and it is not able to induce apoptosis. In that case agents as interferon-γ can „replace" p53 by activating Fas expression.[132] This is the reason why IFN-γ clinically supports the therapeutic effect of 5-fluorouracil.[133]

The ***clinical trials*** using the systemic administration of ***death ligands*** (TNF, FasL) have failed because of the tox-

icity of the ligands. [134,135] Nevertheless, there is a promising death ligand in oncology: *TRAIL*. TRAIL was found to be cytototoxic to many human tumor cell lines but did not cause significant toxicity in animal models.[136] The reason behind this selectivity against malignant cells is still unknown. The leading hypothesis is based on the observation that normal cells express more of the decoy receptors (DcR1 and DcrR2) than the tumor cells.[137] Nevertheless, treatment with TRAIL proved to be effective against glioma and colon cancer in preclinical models.[136,138] In breast cancer cells the effectiveness of TRAIL was dependent on the cytotoxic agent used in the combination. Doxorubicin or 5-fluorouracil - in combination with TRAIL – were synergistic, while methotrexate, melphalan or paclitaxel had no influence on TRAIL action.[139] The toxicity of TRAIL is still a question of debate,[140] but it is highly possible that the quality of the recombinant ligand from different sources is quite different.[141]

More and more agents are reported (lodinamine, arsenite, betulinic acid, CD437, and some amphipatic cationic α-helical peptides) which can *increase the permeability of the mitochondrial membrane*, acting either directly on the membrane or indirectly through the PTPC (permeability transition pore complex). This effect helps the escape of critical proapoptotic mitochondrial molecules (e.g. cytochrome c) into the cytoplasm and can induce apoptosis when other conventional anticancer agents are ineffective. *BCL2 family members* are important regulators of mitochondrial membrane permeability. In the case of decreased or missing expression of BAX or BCL-Xs the transfer of their genes into tumor cells could change the balance in favor of proapoptotic signals. In experimental models the introduction of an Ad-DF3-BAX destroyed 99% of tumor implants.[142]

Caspases could be reasonable targets to switch apoptosis on, however, it is very hard to ensure a selective toxicity against tumorous cells. In certain tumors (e.g. neuroblastoma, rhabdomyosarcoma, small cell lung cancer) the activity of caspase-8 is very low due to the hypermethylation of the promoter gene region. [143-146] There is hope that methylation inhibitors (e.g. 5-azadeoxicytidine) might restore caspase-8 expression.[147]

Inhibition of the activity of antiapoptotic molecules

Most strategies aim at inhibition of either antiapoptotic molecules or the inhibitors of proapoptotic molecules. *FAP-1* can bind to FAS, preventing signal transduction from the receptor.[148,149] An oligopeptide has been synthesized and used against FAP-1 re-establishing FAS sensitivity.[148] *FLIP* has a much wider inhibitory action than FAP-1 on FASL or TRAIL induced apoptosis in experimental systems.[150] An antisense oligonucleotide against FLIP made cholangiocarcinoma cells sensitive again to FAS mediated apoptosis.[151] Such approach could be a useful component in a schedule based on TRAIL administration.

The overexpression of *BCL2* has been considered as prototypic reason for the inhibited apoptotic response. Since such gene errors due either to translocation or to amplification are present in many human tumors, the inhibition of BCL2 became a central challenge. An antisense oligonucleotide targeting the first six codons of the coding sequences has reached clinical trials *(G-3139, Genta)*.[152] It seems that this antisense therapy is more effective in combination with cytotoxic agents than given alone. In lymphomas antisense-BCL2 has been combined with cyclophosphamide, in small cell lung cancer with paclitaxel, in hormone resistant prostatic cancer with mitoxantrone, in breast cancer with docetaxel, in colon cancer with irinotecan, in relapsing acute leukemias with fludarabine and cytosin arabinoside, and in melanoma with dacarbazine.[152] Furthermore, *antisense oligoncleotides* have also been made against BCL-XL. [153]

Another inhibitory family is the *IAP* (including *survivin*). Antisense oligonucleotides have been applied in lung cancer and in melanoma with reasonable success. [154]

In many tumors the apoptotic response is inhibited by *survival signals*. In most cases such signals are suggested but not identified. One of the most active survival protein is AKT which is the effector molecule of PI3K pathway.[155] AKT is able to inhibit a variety of proapoptotic proteins. Any agent that inhibits AKT expression has a potential to revitalize these proapoptotic molecules. *PI3K* could be inhibited e.g. by wortmannin. The PI3K pathway is stimulated by many signals, e.g. ABL or RAS. Overexpression of *BCR-ABL* fusion gene or its product can be inhibited by an antisense-oligonucleotide or by a specific kinase inhibitor (Gleevec, imatinib mesylate, is now on the market for use in the therapy of CML and GIST etc.). [156]

Target: tumor-host interactions

Tumor-induced angiogenesis and vascularization

Vascularization of tumor tissue is an essential event in the establishment of both the primary and the secondary tumor lesions and can also be considered a key feature of the dissemination process (the metastatic cascade).[24,157] This is due to the fact that intratumoral blood vessels are the key structures of the intravasation phase of the metastatic cascade and their density directly correlates with the metastatic potential of several cancer types. In this way tumoral blood vessels are important targets of metastatic therapies. Since vascularization of the tumor tissue can be realized by various biological mechanisms various forms of targeting can be designed. This way, neoangiogenesis is only one among several pathomechanisms of tumor vascularization, but is almost the only field of anti-angiogenic pharmacology.

Based on the basic pathomechanism of tumor-induced angiogenesis a wide range of specific pharmacological inhibitors have been developed and even tested in clinical settings. Major classes of these inhibitors are anti-angiogenic cytokine inhibitors (targeting primarily VEGF), inhibitors of their receptors (primarily VEGFR) or the key integrin receptor, αvβ3, and the coupled signaling pathway (flk-1/kdr tyrosine kinase inhibitors) or specific endothelial cell inhibitors of various endothelial molecular targets (see in details in[24]). However, many molecular targets are shared by angiogenesis and tumor invasion such as MMPs,[85,86] cytokines (HGF, VEGF, IFN) and signal transduction pathways (EGFR[48]), so that pharmacological agents can have significant anti-angiogenic effects in addition to anti-tumoral and/or anti-metastatic one (see earlier). Furthermore, several classical cytostatic and cytotoxic agents used in clinics (not surprisingly) turned out to have anti-angiogenic effects as well, almost as a non-specific „side-effect" (*Table 1*). [24,157]

Clinical trials are now on the way to see if the enormous amount of experimental data can be turned to clinical benefit.[24,157] It seems that *IFN-α and Thalidomide* are two commercially available leading pioneers of anti-angiogenic agents reaching phase III trials for various advanced malignant diseases. [157] Among the experimental agents the natural anti-angiogenic factor *Neovastat*[90] and a tyrosine kinase inhibitor *SU-5416*[158] have also reached phase III trials. Accordingly, it is too early to predict or even outline the potential new anti-angiogenic modalities. On the other hand, it is important to mention that several classical cytostatic drugs regularly used today in various regimes have significant anti-angiogenic properties which could be better exploited (*Table 1*).

The key issue is to determine the precise place of anti-angiogenic therapy of the future. When an anti-metastatic effect is the surrogate marker and clinical goal, it is obvious that these new therapies have to be applied in an early phase of the metastatic cascade, immediately after elimination of the primary tumor showing at least local invasiveness or early signs of systemic spread (prevention of the vascularization of an established microscopic tumor tissue[159]). Such a treatment must be applied for an extended period of time, and potential side effects are therefore of major significance.[157] From this point of view natural agents or their recombinant variants with high specificity toward proliferating endothelial cells must have selective advantage. Another issue is that these new anti-angiogenic drugs are first tested clinically in phase I-II-III settings in advanced malignancies. In these trials anti-angiogenic agents are used to delay or inhibit new vessels in metastatic tumors which are already in an advanced stage of vascularization (well beyond the avascular size of 1-2 mm). Accordingly, frequent lack of anti-tumoral effects of these new agents is not a surprise and stable disease is as significant as a decrease of the tumor size.[157]

It is now accepted that in certain tumors cancer cells utilize the pre-existing vasculature of the host tissue and tumor vascularization actually requires remodeling of these vessels.[24] In this form of vascularization only those agents that can specifically target vessel remodeling can have a pharmacological role. On the other hand, other cancer types can redirect their genetic program and embryonic angiogenic geno- and phenotypes are developing resulting in the emergence of vascular mimicry of tumor cells and vascular channels made entirely or partially by tumor cells.[24]

Considering these options as well as the fact that already vascularized secondary tumors are frequently the clinical targets, the established tumor vasculature is considered as an anti-metastatic target. These anti-tumor vessel therapies may involve fundamentally different agents compared with classical angiogenesis inhibitors. Unlike the anti-angiogenic agents, unique molecular determinants of the tumor vasculature have outstanding significance for this therapeutic strategy. Such a molecular determinant could be VA-cadherin[160] where an inhibitory antibody toward this epitope on tumor-blood vessels could serve as an anti-vascular agent. Tissue factor can also be targeted to tumor vasculature by using a toxic conjugate, which than induces infarction of the tumor tissue in experimental models.[161] On the other hand, both VEGF/VEGFR as well as αvβ3 integrin on the tumor blood vessels can serve as targets and the anti-vascular effects of the VEGF-toxin fusion proteins,[162] anti-αvβ3 antibody (Vitaxin)[17] or cyclic RGD peptides[163,164] can be exploited to cause direct anti-tumoral/anti-metastatic effects and even improve radioimmunotherapy as it has been demonstrated in various preclinical models.

Collectively, the expanding knowledge of tumor-induced neoangiogenesis and the process of tumor vascularization now provide a vast array of molecular targets for specific therapy, but the rationale must be driven by our understanding of the various steps of the metastatic cas-

Table 1. **Classical cytostatic agents with anti-angiogenic potentials**

	In vitro	*In vivo*
Cytotoxic for endothelial cells	Camptothecin/topotecan Taxanes Vinca alkaloids	Taxanes Vinca alkaloids
Cytostatic for endothelial cells	Cisplatinum Cyclophosphamide Doxorubicin Methotrexate	Cyclophosphamide

cade. Only these considerations can lead to clinically successful anti-metastatic application of these new anti-angiogenic or anti-vessel modalities.

Tissue hypoxia and anemia

Induction of neoangiogenesis in malignant tumors (both at the primary as well as at the secondary sites) is partly mediated by a hypoxia sensing mechanism.[165,166] The key molecular regulator of this system is HIF-1, a heterodimer of the HIF-1α transcription factor and ARNT/HIF-1β.[165] When O_2 is present HIF-1α binds VHL protein and the complex is ubiquitinated and degraded by the proteasome pathway. However, when O_2 is not present in the nucleus, HIF-1 can bind hypoxia-responsive elements (HREs) activating several target genes including pro-angiogenic ones like VEGF, bFGF, and other mitogens, pro-apoptotic genes, coagulation factors, genes involved in pH regulation or glycolysis, and even those involved in migration. However, a proportion of cancer cells is able to bypass hypoxic stress and develop a special phenotype involving radio- and chemo-resistance.[167,168] Importantly, the same tumor cell population is involved in the metastatic process, suggesting that hypoxia resistance could be an important factor that regulate the emergence of the metastatic phenotype. This is further corroborated by the fact that HIF-1α expression is increased in a wide variety of human cancers and serves as marker of poor prognosis.[166]

Anemia, the hallmark of the progression of various cancer types, develops on the basis of various pathomechanisms and results in general and local hypoxia.[169-172] Correction of anemia by rhEPO not only increased oxygen supply to normal tissues and improved quality of life of cancer patients but, surprisingly, improved response to radio- and chemotherapy, and even prolonged survival of patients.[168,173,174] However, regulation of hypoxia can serve as a double edged sword in cancer. Some aggressive tumors have a high oxygen consumption which generates hypoxia, but the HIF-1α pathway is abnormal and any correction of the O_2 level would simply further stimulate progression. In other tumors hypoxia in the tumor tissue generates the emergence of a more aggressive subpopulation through the involvement of the HIF-1α system. In this latter case correction of hypoxia will slow down the rate of generation of more aggressive cells (will turn off the hypoxic switch). Without the proper identification of function of the HIF-1 pathways in various cancers it will not be possible to identify those tumors where the correction of hypoxia can have an antimetastatic effect.[166]

Homeostasis: cancer cachexia

About one fifth of cancer patients die due to cachexia, a severe loss of body weight from all the tissue compartments except the viscera. The majority of these patients are in an advanced, metastatic stage of the disease. The loss of fat-free mass primarily involves muscle tissue. Since intracellular potassium is also lost, the process can be considered as bioenergetic deficit. However, unlike in starvation, liver mass is increased due to increased metabolic activities. On the other hand, cachexia not only characterizes the terminal stage of cancer progression but can also be present at an early stage of the disease and is a marker of poor prognosis which also affects response to therapy.

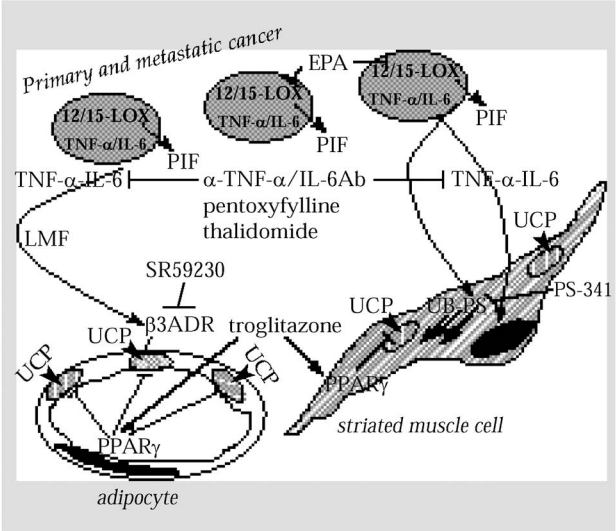


Figure 3. *Molecular pathways of cancer cachexia. PIF: proteolysis inducing factor, LMF: lipid mobilizing factor, UB-PS: ubiquitin-proteasome pathway, UCP = uncoupling protein*

Cachexia in cancer patients is due to central loss of apetite, increased resting energy expenditure (REE) as well as to increased muscle protein breakdown/decreased synthesis and is regulated by different mediators (***Figure 3***).[175] Decreased apetite is considered to be induced by proinflammatory cytokines such as TNF-α, and IL-6, acting through the blockage of the hypothalamic NPY peptide. Interestingly, in experimental models cachexia was also mediated through MSH receptor, MC4R.

In certain cancer types, such as lung- or pancreatic carcinoma, REE is significantly increased contributing to the development of cachexia. This process is mediated by the abnormal function of mitochondria in the skeletal muscle.[175] Uncoupling proteins (UCPs) are responsible for balancing heat over ATP production. Tumor-derived TNFα as well as lipid mobilizing factor (LMF) increases the expression of UCPs in skeletal muscle and in adipose tissue in cachexia. The expression of UCPs is regulated by PPARγ,[176] suggesting a potential phamacological approach to compete with this effect of tumor tissue. Increased function of the Cori cycle in cancer patients is also responsible for increased REE.[175] This is primarily due to hypoxia and to the increased production of lactate by the tumor tissue.

Lactate is metabolized by the liver in the Cori cycle. In cachexic patients gluconeogenesis is attenuated too due to increased lipolysis (fat) and proteolysis (muscle). LMF-induced lipolysis was shown to be mediated through β3-adrenoreceptor.[175]

The progressive loss of muscle tissue is mediated through three complementary mechnisms, ubiquitin-dependent proteolysis,[177] TNF-α-induced downregulation of MyoD (a cell-type specific transcription factor)[178] and probably also by the activation of myostatin (a member of the TGF-β family).[179] The proteolysis of muscle tissue is induced both by TNF-α as well as by a sulfated glycoprotein, proteolyis inducing factor, PIF.[180] The latter acivates PLA_2 and lipoxygenases, ultimately leading to the production of 15-HETE.[175]

Based on this complex mechanism of cancer cachexia, several feasible pharmacological targets have been identified (*Figure 3*). As it was mentioned above, central loss of appetite is one (but not essential) target, where *corticosteroids or progestogens* have been clinically used as stimulators of NPY production.[175] On the other hand, the identification of the role of the MSH/MC4R system as regulator provides an alternative to develop inhibitory therapies.

The increased REE in cachexic cancer patients is another key factor in this complex mechanism. Systemic *anti-hypoxic interventions such as EPO administration* are obvious approach (see in details earlier). The nutritional deficit is the easiest target which can be reverted by protein- and energy-dense supplement but this approach alone is clinically insufficient. [175]

Ubiquitin-mediated protein degradation induced by TNF- and IL-6 provides a specific target for intervention.[177] Unfortunately, *anti-cytokine therapies* (mostly antibodies) have frequently been ineffective clinically.[175] On the other hand, *eicosapentaenoic acid, EPA* (and its natural source, *fish oil*) has been shown to specifically downmodulate the ubiquitin-proteasome pathway and proteolysis.[181,182] The effect is thought to be mediated through the inhibition of PIF production of cancer cells and downmodulation of lipoxygenase activities. The new inhibitors of the proteasome pathway (such as the *dipeptide-boronic acid analogue PS-341)* may have increasing role in this respect too.[183] To modulate protein turnover unbalanced in cachexia, *hydroxymethylbutyrate* as well as *arginine and glutamine administration* have been tested successfully in the clinic.[175] It has been demonstrated in preclinical models that the excessive lipolysis in cachexia induced by LMF and mediated by UCPs of mitochondria can be specifically targeted by β3 adrenoreceptor antagonists (such as *SR59230*[184]) or *PPAR activators* (such as troglitazone[176]).

Collectively it is now evident that cancer cachexia can be specifically targeted pharmacologically and even treated clinically (in a combined modality), providing new approaches for supportive care of tumor progression.

Immunotherapy

The existence of tumor-associated antigens (TAA) and the detection of T lymphocytes recognizing these antigens, both systemically (in the peripheral blood) and locally (at tumor sites) in cancer patients, provides evidence that immune reaction can develop against metastatic cancer in these patients. However, the presence of tumor-specific cytotoxic T lymphocytes (CTL) most frequently does not translate into an effective antitumor immune response, as is reflected by the unarrested growth of tumors. Numerous mechanisms have been described that could contribute to the escape of tumor cells from immune recognition and destruction.[185] In order to boost immune responses against tumors, a variety of treatment modalities have been developed in preclinical and animal models, and tested in clinical setting. These include antigen-nonspecific approaches, as well as specific, active (stimulation of the host immune system) or passive/adoptive (transfer of effector cells or molecules) immunotherapeutical modalities. Some of these strategies were proved to be able to cause objective cancer regression, even of extensive metastatic disease, if only in a small percentage of patients, suggesting that a better understanding of the mechanisms of action of immunotherapeutical modalities may enhance the success rate of these strategies.

Nonspecific immunotherapy

The application of nonspecific immunotherapy involves the administration of *bacterial immunostimulants* (most frequently *BCG*), as well as cytokines such as *interferons* or *interleukins*. Having been tested in a variety of malignant diseases, BCG remained accepted as a treatment of choice for the adjuvant therapy of superficial bladder cancer, probably representing the most effective immunotherapy in the case of solid tumors, based on a long-lasting local immune activation.[186] As an adjuvant, it is involved in many immunotherapeutical protocols involving different tumor types.

Among the cytokines, *interferon-α* (*IFN-α*) and *interleukin-2 (IL-2*) are most frequently used in immunotherapy trials. IFN-α has proved to be effective against a range of malignant diseases including, among others, melanoma, renal cell carcinoma (RCC), and Kaposi's sarcoma. In metastatic melanoma and RCC, IFN-α yielded 10-20% overall response rates as well as survival advantage,[187] while its benefit in the adjuvant setting of high-risk melanoma or RCC is more controversial. The mechanism of action of IFN-α is not exactly known, since beside immunomodulatory effects, it also has direct antitumor and antiangiogenic potential. IL-2 plays central role in immune regulation, primarily via its ability to stimulate the growth of T cells, but its effects involve the stimulation of NK cells, B cells and

macrophages as well. Similarly to IFN-α, most clinical experience has been gained in patients with metastatic melanoma and RCC, yielding approximately 10-20% overall response rates with complete response (CR) in 3-10% of the patients.[188] In patients achieving CR in response to high-dose IL-2 the regression of tumors at multiple metastatic sites was observed, and in most cases this response was durable. However, the mode of action of this regimen is not fully understood; although it was assumed that IL-2 exerts its antitumor effect via its stimulatory activity on T-cell proliferation and activation, Marincola and colleagues recently suggested that systemic IL-2 administration may facilitate T-cell function by promoting their migration and indirectly, through the activation of antigen presenting monocytes.[189]

Active specific immunotherapy – tumor cell-based

Numerous forms of active specific anti-tumor immunotherapy have been investigated in clinical trials in the past few decades, utilizing whole tumor cells or lysates, recombinant viral and bacterial vaccines, peptides, nucleic acid- or dendritic cell- (DC) based vaccines. Initial studies using ***autologous tumor cells*** (generally with BCG as adjuvant) resulted in moderate response rates. One of the disadvantages of autologous preparations, the potentially low amounts or weak TAAs, was attempted to be overcome using hapten-modified tumor cells with increased immunogenicity.[190] In these trials clinical response was generally associated with delayed-type hypersensitivity (DTH) skin reactions to autologous cancer cells. The most intriguing finding was that immunization with DNP-modified tumor cells induced inflammatory response consisting mainly of $CD8^+$ lymphocytes at metastatic sites in a significant proportion of patients.

Another approach to improve the efficacy of tumor cell vaccines is ***transduction with genes encoding immunostimulatory cytokines***, which may enhance local immune response without causing systemic toxicity. The antitumor efficiency of these engineered tumor cell vaccines has been demonstrated in many animal studies. Clinical trials using *GM-CSF producing autologous tumor cell vaccines* in melanoma, RCC and prostate carcinoma patients[191,192] showed moderate clinical responses, and the development of DTH reaction to tumor cells, as well as a chronic inflammatory reaction in metastatic deposits in most patients, consisting of infiltrating T cells and plasma cells. Several forms of *allogeneic tumor cell vaccines* (whole cell-, lysate-, or shed antigen preparations) have also been tested in clinical trials, primarily in melanoma patients.[193] This type of vaccination was demonstrated to induce both cellular and humoral immune response in most patients, the extent of which was shown to be associated with clinical outcome. Survival data from phase II trials are promising, however, comparisons were made to historical controls only, and randomized phase III studies to confirm the therapeutic effect are either still ongoing or have demonstrated no statistically significant effects.

Active specific immunotherapy – antigen-specific

The identification of an increasing array of tumor-associated antigens and their respective HLA class I-restricted epitopes has opened new avenues for the antigen-specific immunotherapy of cancer. Of the potential forms of antigen delivery (recombinant viral or bacterial vectors, peptides, naked RNA or DNA, DC) the easy-to-produce, safe and reproducible ***peptide vaccines*** are the most studied. These peptides represent fragments of tumor antigens recognized by CTL in the context of a given HLA class I haplotype. In general, ***peptide vaccines*** (mostly administered without adjuvant or with weak adjuvants) induced the generation of cellular immune reaction against the peptide in about one half of the patients, but only limited clinical response rates, and there has often been no correlation between the immunological and clinical responses.[194,195] This dichotomy points to the inadequacy of currently used immunological assays for therapy monitoring and emphasizes the need for relevant intermediate endpoints measuring the activity of vaccines, which could predict clinical outcome.

Another potential problem arising from peptide vaccine trials is the selection of antigen-negative tumor cell populations due to down-regulation of the specific antigen and/or HLA molecule following immunization.[185,196,197] In theory, this could be overcome with the use of cocktails of peptides from different antigens. The application of whole antigen-vaccines (recombinant viral, bacterial, or naked DNA, etc.), including several epitopes presented by different HLA class I and class II alleles, would be available for a broader range of patients irrespective of their HLA haplotype, and would be able to target both $CD4^+$ and $CD8^+$ T cells. However, the documented effectiveness of these latter types of vaccines is low.[198,199]

Perhaps the most promising approach to improve antitumor immune response is the ***application of DCs as vaccines***. These cells are the most potent APCs capable of eliciting strong antigen-specific CTL response in murine models and in humans. DCs from cancer patients can be cultured *ex vivo* and loaded with many different forms of tumor antigens: peptides, whole proteins, or cell lysates, followed by re-administration to the patients. Alternatively, DCs can be transduced with DNA or RNA encoding a given antigen or purified from tumor cells. Most clinical studies have been performed using *DCs pulsed with peptides or loaded with tumor cell lysates*. These trials revealed that such vaccines are able to generate immunity to tumor antigens without significant side effects, and objective clinical responses have been seen in some cases.[200-205]

An interesting approach involving immunization of RCC patients with ***hybrids of autologous cancer cells and allogeneic DCs*** was reported by Kugler et al.[205] Such a vaccine combines the high MHC class I and II expression and costimulatory molecules on DCs and the antigenic repertoire of the tumor cells, and was able to induce immunological response (DTH) against the tumor in 11 of 17 patients, as well as clinical responses in 7 patients.

Adoptive immunotherapy

In addition to vaccination (active immunotherapy), the immune response against tumor antigens could be increased through the adoptive transfer of effector cells recognizing tumor antigens. Earlier attempts are exemplified by the administration of ***in vitro*** expanded ***tumor infiltrating lymphocytes (TIL), in combination with high-dose IL-2*** to sustain the proliferation of the infused T cells. This approach resulted in approximately 30-35% response rates in patients with metastatic melanoma and RCC, [206,207] however, in the case of RCC its benefit over IL-2 alone could not be confirmed in a randomized phase III study. [208] As a "revival" of this approach, Dudley et al. recently reported impressive results after ***adoptive transfer of TIL-derived, in vitro expanded, highly selected tumor reactive T cells***, combined with high-dose IL-2, in metastatic melanoma patients conditioned by prior lymphodepletion. They achieved partial response in 6, and mixed response in 4 of 13 patients; in two responders clonal repopulation by tumor-reactive T cells persisted for several months.[209] In a parallel study, Yee et al. infused ***PBMC-derived CD8$^+$ clones*** recognizing MAAs MART-1 or gp100, ***in combination with IL-2***, resulting in accumulation of the transferred cells at metastatic sites, and a few minor or mixed responses, together with the selective loss of the targeted antigen in 3 of 5 cases studied. [210]

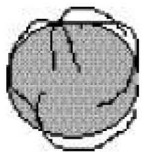
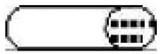
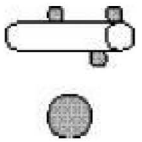
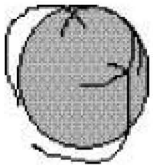
Comments to immunotherapy

Several conclusions can be drawn from the results of published immunotherapy studies. First, these modalities are able to induce durable complete tumor regressions, even of extensive metastatic disease, mostly with reasonable toxicity; however, generally only in a minority of patients. This points to the importance of appropriate patient selection. In the case of antigen- or peptide-specific vaccination strategies it is important to examine the presence of antigens the relevant and HLA haplotypes at protein level, if possible, at all accessible tumor sites. Mixed responses, i.e., different responses of individual metastases are often encountered in immunotherapy trials, and probably reflect heterogeneity in the expression of tumor antigens, HLA class I molecules, apoptotic signals or immunosuppressive factors that might influence the effectiveness of an immune reaction. Furthermore, most tumor vaccines involve patients with advanced stage (or end-stage) disease, and evaluating the general immunocompetence of the patients prior to treatment has been done only in the minority of studies. A successful antitumor immune response may take several months to develop,[193] therefore patients with only 3-4 months life expectancy are less likely to benefit from these therapies. Although in some cases the regression of bulky tumors has been demonstrated in immunotherapy trials, it is possible that greater clinical impact could be obtained in the post-surgical adjuvant setting. Second, monitoring immunological responses as alternative study endpoints showed a lack of correlation with clinical outcome in many cases,[198,199,211] emphasizing the need for more adequate surrogate markers. There are promising newer tools requiring less ***in vitro*** manipulation that could prove useful in this respect, including assays detecting antigen-specific T-cell frequency (MHC-peptide tetramers), as well as functional assays detecting antigen-specific cytokine production by T cells (ELISPOT, cytokine flow cytometry, real-time quantitative RT-PCR).[212,213] The presence (even at high frequencies) of vaccine-elicited tumor-reactive CTL in the circulation, or in the tumors expressing the relevant antigens does not guarantee an efficient immune response leading to tumor regression. It is not clear at present if these T cells are in an activated and functional state, and, on the other hand, tumors can develop multiple mechanisms to escape immune recognition.[185] The molecules participating in these processes are not routinely tested in the tumors of patients enrolled in immunotherapy studies, nor monitored during the treatment. Finally, in several single target antigen-based clinical studies a therapy-induced immunoselection of antigen-negative clones has been observed in nonresponding tumor deposits, leading to disease progression.[185,196,197] This could be overcome by the use of antigen (peptide) cocktails or whole tumor approaches, either by themselves or as a DC vaccine. Moreover, therapies based on tumor antigens related to the process of malignant transformation or critical to the growth of cancer cells may be more resistant to immunoselection, and therefore be more optimal targets for immunotherapeutical interventions.[185]

Final comments

At present metastatic tumors are principally treated with the currently available cytotoxic agents in clinical oncology which produce the most favourable responses. Alarmingly, there are experimental data suggesting the enhancement of metastatic potential after treatment with cytostatic drugs. Cylophosphamide was shown to enhance the formation of the metastatic nodules if the experimental animals were treated before the inoculation of the tumor cells. Unfortunately the elevated level of metastasis by cyclophosphamide could not be abolished by prostacyclin

Table 2. **Therapeutic approaches in the metastatic cascade. Target: metastatic tumor cell**

	Metastatic Cascade						
Therapies	*Primary tumor vascular, macroscopic*	*Local invasion ADM*	*Intra-vasation ADM*	*Circulation (Blood – lymph) A*	*Extra-vasation ADM*	*Dormancy / avascular micromets*	*Vascular macromets*
							
Cytotoxic (S/IR/CH)	+	(+)					**+**
„Anti-metastatic"							
Membrane receptors							
Anti-GF/GFR	+	+(M)	+(M)		+(M)	**+**	+
Anti-integrins		**+**	+	+	+	+	
Anti-tm-HSPG		**+**	+	+	+	+	
Signal transduction							
PTK-inhibitor	(+)	**+**	+	(+)	+	+	(+)
RAS-inhibitor (FTI)		**+**	+		+	+	
PKC-inhibitor		**+**	+		+	+	
Lipid signaling-inhibitor		**+**	+	+	+	+	
Ca-signaling inhibitor		**+**	+		+	+	
Survival/apoptosis	+	**+**		+		+	+

Abbreviations: A = adhesion (to matrix), D = degradation (of the matrix), M = migration (through the matrix), tm-HSPG = transmembrane heparan sulfate proteoglycan, GF/GFR = growth factor receptor, S = surgery, IR = irradiation, CH = chemotherapy

Table 3. **Anti-metastatic targets in tumor cell – host interactions (homeostasis)**

Therapies	*ECM (host)*	*„Angiogenesis"*			*Hemostasis*	*Metabolism (host)*	*Immune defense*
		Hypoxia	*Neoangiogenesis*	*Vascularization*			
Protease inhibitors	+		+		+		
ECM modifiers (bisphosphonates)	+		(+)			+	
Angiogenesis inhibitors							
Anti-hypoxic		+	+			+	
Anti-angiogenic		+	+	+			
Anti-tu-vessel				+			
Hemostasis regulators							
Anti-platelets	(+)		(+)		+		+
Heparins	(+)		(+)		+		+
Cachexia therapy							
Apetite						+	
Proteolysis						+	
Lipolysis						+	
Immunotherapy							+

which, when administered alone, has a remarkable antimetastatic action.[214] Recently the development of metastatic capacity was observed among the survivors of MCF-7 human mammary adenocarcinoma cell population treated alternatively with FUdR and adriamycin.[121]

Furthermore, chronic and especially, low dose therapies with cytotoxic agents can also have unwanted negative side effects on tumor progression. The proposed metronomic scheduling of these therapies[215-218] does not consider the existing experimental and clinical data on this issue.[121,219-221] A more fundamental experimental approach is required to analyse the effects of these protracted therapies on tumor progression before testing them in clinical trials.

Identification of various molecular mechanisms, involved in the late phases of tumor progression leading to the development of invasive/metastatic capacity and metastatic disease, have already identified an array of new and specific targets for pharmacological interventions (*Table 2*). From the clinical standpoint major features of metastatic disease are all therapeutic targets (*Table 3*). Furthermore, it is now evident that expanding the survival of cancer patients with metastatic disease requires a more „holistic" approach to the disease where an array of targets have to be treated individually to reach the ultimate goal.

Similarly to cancer prevention, anti-metastatic therapies have the best chance to be active when applied in the early (perhaps subclinical) stage of tumor dissemination. Although this approach is widely tested in experimental models, there are no data to date on their clinical applicability. These interventions will require safe and selective agents which can be used for an extended periods measured in years (perhaps for the lifetime of cancer patients), and therefore the chronic toxicity of any agent in this field will be of outstanding importance.

The therapy of tumor progression and metastatic disease is the biggest challenge in clinical oncology. Pharmacological approaches using single agents which were standards early on in clinical oncology have changed considerably with the development of combined treatment modalities. These have produced promising results but few breakthroughs in the clinical management of the metastatic disease. The multiple pathways and cellular targets all have to be identified (molecular diagnostics) and included into combinatorial therapeutic regimes as outlined in this review where none of the individual components have a replaceable role. It is our expectation that such an approach individually designed for various cancer types may have a better chance to treat or even cure cancer patients with progressive disease.

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