



Review article

Antimetastatic therapeutics according to metastatic cascade: An overview

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Abstract

More than 90% of cancer deaths are caused by cancer metastasis. Since cancer metastasis is the main cause of human deaths, antimetastatic treatment studies should play decisive roles for elongation of cancer patients' survival. Past three decades, despite cancer metastatic biological or pathological theories have been proved again and again by new biological techniques, translational or clinical studies against neoplasm metastasis are less fruitful. Facing these challenges, promoting the studies of all these new attempts might change the landscape of metastasis treatments. This overview, one of these attempts—optimizing antimetastatic drug efficacies by one possible strategy is highlighted.

Keywords: Neoplasm metastases, cancer chemotherapy, cancer treatment, cancer pathology, cancer pharmacology, medicinal chemistry, anticancer drug, antimetastatic drug, personalized cancer therapy, cancer hallmarks, drug sensitivity testing, drug combination

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1. Introduction

Two major obstacles in human cancer treatments—neoplasm metastasis and multi-drug-resistances (MDR) of cancer cells are major drawbacks of clinical cancer trials. Among these two thorny problems, treatment of neoplasm metastasis is especially difficult and unresolved. Furthermore, metastasized tumors often concomitantly manifest the characteristics of MDR. Last three decades, despite cancer metastatic studies become popular and progress a great deal [1-9], translational or clinical studies have progressed in small extents comparing with biological or pathological understanding of neoplasm metastatic processes [7-9]. Until now, no good antimetastatic therapy can be relied for late-staged cancer patients (having obvious remote macro-metastasis), especially for old aged cancer patients. Given this grim picture, any small breakthrough in metastatic therapeutic

study and clinical applications can make differences for improving mortality rate of late-staged cancer patients [10].

Current dilemma and pitfall for clinical cancer metastasis treatments

Overcoming the obstacles of cancer metastasis therapy in clinics needs in-depth understanding the biology and pathogenesis of neoplasm metastasis. Strangely enough, most cancer metastatic molecular and pathological mechanism hypotheses introduced more than three decades ago have been constantly proved by using updating bioscience techniques [4]. However, deep understanding biological or pathologic mechanisms do not improve cancer patients' survivals without excellent translational and clinical study. Moreover, no difference of clinical antimetastatic trials without developing or licensing more effective antimetastatic drugs

and updating clinical strategies can be usually made. So far no effective antimetastatic drugs are developed in clinical trials even although many biotherapies or chemicals are very sensitive to neoplasm metastasis in animal models [11-14].

Response to this dilemma

Recently, several strategies have been proposed to counter shortcomings and drawbacks of general cancer metastasis treatments in clinics. Relevant approaches are enlisted in the following avenues and scenarios

- (i) Acquire new visions of cancer metastatic biology and pathogenesis. Thus novel translational work can benefit from the fruits of metastatic biology and pathogenesis mechanism study and sizeable metastatic treatment outcomes can be achieved in clinics [5-6, 15-18].
- (ii) New generation of antimetastatic drugs must be developed by updating *in vitro* and *in vivo* experimental animal or human tumor models for which more effective agents can be stood out [10-14], transplanted in immune-deficient mice or genetic modified mice (GMM) and finally be licensed.
- (iii) Clinical treatment schedules or personalized cancer therapy can be satisfactorily invented and applied [19-22]
- (iv) Drug combination is a widely served way and effort to control cancer growth and metastasis and elongations of cancer patients' survivals in clinics. Nevertheless, this type of strategies is largely based on empirical and past references rather than technical-assistant or science-guided one [20-24]. In future, finding the law of drug combinations should be indispensable part of cancer treatment study and clinical trials [24].

Rethink possible solutions for these dilemmas

Transformation from knowledge of metastatic biology and pathogenesis into discovering highly effective treatment agents and schedules is conventional avenues of present thinking. The current vogue in antimetastatic study is to discover new metastatic-related genes and molecules, then screening for inhibitors of the metastatic-related genes or

molecules and finally seek drug licenses after clinical trials. These researches provide strong foundations from cancer metastasis biology and pathology study, but they are less fruitful and waste of money and slow in pace. Presently, only small amount of antimetastatic drugs are finally developed and entered into markets by aforementioned ways [6-14]. On the other hands, antimetastatic agents can be found by randomly drug screening tests in lab [11-15] or substituted with other drug categories [14].

Past three decades, despite rapid progressions of understanding biology or pathology cancer metastasis and several antimetastatic agents have been approved for clinical applications in US and Europe [12-15], wide-spectra and very effective antimetastatic drugs to majority of cancer categories in clinics have been insufficient developed, especially the late-staged cancer patients worldwide [7-10]. Some reports argued that many antimetastatic agents or therapies were even worse than none [25-27]. The possible reasons will be addressed in following paragraphs.

Avenue of developing new generations of antimetastatic drugs

Since 90% of cancer deaths are caused by cancer metastasis—especially for those cancer patients with formed metastatic nodules, only handful licensed antimetastatic drugs have been developed and provided for the healthcare of cancer patients. Promoting wide-spectra antimetastatic drug development is the key for the successes of cancer therapies in future. New drugs targeting against formed metastatic nodules remoted from primary tumors could be the focus of next generations of cancer therapy study. Facing the dilemma of shortage of wide-spectra effective antimetastatic agents or drugs, following three strategies are commonly pursued.

- (i) Reflecting drug screen systems might help us update anticancer or antimetastatic drugs development pipelines [11-12]. Renovation, replacing and utilization of new *in vitro* and *in vivo* experimental animal and human cancer models and tumor transplantation systems might promote new categories of anticancer or antimetastatic drugs [11-

- 12].
- (ii) More inhibitors or activators against metastatic-related genes or molecules will be testified and promoted. This antimetastatic agent development system is the conventional pathway, yet the hardest road.
 - (iii) In-depth study of anticancer or antimetastatic drug mechanisms of action and updating drug combination system and individualized cancer trials are avenues from clinical insights [19-24].

Improving drug screen and developing systems must reshuffle experimental animal or human cancer models. After reshuffling, are new *in vitro* and *in vivo* animal and human cancer model system more relevant to real clinical situations? Two major anticancer drug screening system in developed countries highlight with more human tumors transplanted to immune-deficient mice {athymic nude mice or severe complicate immune-deficient (SCID) mice} or humanized mice (genetic modified mice, GMM), such as Mouse Avatars [11-12, 28-30], and comparing and understanding what types of transplantation assay systems are more relevant or parallel to clinical situations of cancer patients with neoplasm metastasis. *In vivo* human experimental models today can be transplanted into aforementioned immune-deficient mice by intraperitoneal, subcutaneously, hollow-fiber, renal capsule or orthotopic ways. Different animal or human tumor transplantations may develop different positive agents of anticancer or antimetastatic potentiality [11-12]. Among these transplantation methods, human tumor orthotopic transplantation has been increasingly used as agent evaluations and drug response predictions for its possible parallel to clinical situations. Today's pitfall is incompetent to find and license enough effective drugs against solid human cancer, especially solid tumor metastasis. Although many new frontiers have been emerging with times, this era must be in the crossroad in development of antimetastatic drugs by changing the conventions of drug screening systems, preclinical or clinical evaluations [7-14].

The final solution for cancer metastasis treatment must depend on both discoveries of

effective anticancer or antimetastatic drugs and optimizing clinical cancer treatment protocols (such as drug sensitivity tests and personalized cancer therapy) [19-22]. No matter chemical agents or biotherapy, finding effective antimetastatic drugs is always the top priority. However, current antimetastatic drugs are still incapable to cure most of late-staged cancer patients. New waves of updating metastatic targets and drugs ought to be pursued [9-10].

Available antimetastasis targets or drug discoveries

Two antimetastatic therapeutic targets of currently prevailing

Primary tumors are embedded in surrounding matrix. Tumor cells and their surrounding matrix can secrete a spectrum of proteinases that will break up these surrounding matrixes and make tumor cells penetrate through these matrixes and finally initiate invasion and metastasis processes. These proteinases are mainly composed of matrix metalloproteinase (MMPs). So, MMPs inhibitors are proposed to inhibit tumor metastases. These agents have been licensing since 1990s in USA and they are one type of antimetastatic drugs [9].

Metastatic cells, after extravasation to remote organs, need new blood vessels to offer nutrients to transform the micrometastatic tumor to macrometastatic nodule. The formations of these blood vessels are controlled by vasculature growth factors, such as EGF, VEGF. Drugs that control the secretion or functions of these vasculature growth factors are known as potential antivasculature antimetastatic drugs [7, 9].

These two types of antimetastatic drugs including small molecular drugs and murine antibodies are the main source of current antimetastatic therapy in clinics. Yet only antibodies against vascular factors have widespread antimetastatic effects in clinical trials.

Drawbacks and pitfalls of present clinical antimetastatic therapy

Antivasculature (angiogenesis) and matrix metalloproteinase (MMPs) inhibitors, however, due to indiscriminative molecular inhibitions and survival benefits for only small fractions of

cancer patients, are far from satisfactory in clinics. Paradoxically to our efforts and expectations, only partial improvements and therapeutic benefits by present licensed antimetastatic drugs have been achieved [15-18]. However, therapeutic benefits in late-staged or aged cancer patients are still poor and for small categories of metastatic tumors [9-10]. More importantly, some unfavorable side-effects of these inhibitors in humans have been reported [25-27]. Moreover, no survival benefits of MMPs inhibitors for metastasis cancer patients—late staged cancer patients was found in phase III studies [25]. Since the pathogenic processes of neoplasm metastases are complicated, long term and not easy found in clinics [4-7], angiogenesis and MMPs are targeting only parts of them. Future clinical antimetastatic drug therapy study should focus each pathogenesis pathways. Owing to these characteristics, new metastasis-related targets or anticancer or antimetastatic drugs such as aberrant sialic acids biology [31-37] ought to be avenues for future systematic studies. (Table 1)

Table 1. Different pathways of antimetastatic drugs targeting neoplasm sialic acids

Compounds types	Proposed targets
Sia analogues or conjugates DNA chelating agents Sialyl transferase inhibitors	Pathologic sias DNA template Sia adding or releasing from antigens
Vaccines Antibodies Antimetastatic agents Sia-anticancer drugs	Human immune system Pathologic antigens Unknown mechanism Tumor affinity molecules

Adapted from ref 34-35

Antimetastatic targets under investigations

Cell adhesion molecule inhibitors

Cell adhesion molecules (CAM), such as E-cadherin, p-cadherin, integrin, selectin

plays important role in cancer progression and metastasis. Cell-cell interactions of cancer cells with endothelium determine the metastatic spread. In addition, direct tumor cell interactions with platelets, leukocytes and

soluble compounds significantly contribute to cancer adhesion, extravasation and the establishment of metastatic lesion. Alterations in these molecules are observed during tumor progression and metastasis [38-39]. Heparin can inhibit CAM related metastatic processes [40].

Plasma coagulation inhibitors

Plasma and tumor stroma fibrinogen play important roles in promoting neoplasm metastasis [41-42]. Almost 1/3 of solid tumor categories are the potential targets of anticoagulants or fibrinolytic agents [43]. These anticoagulants or fibrinolytic agents are assistant agents and they can improve the therapeutic outcomes only in combinations with cytotoxic anticancer drugs [44]. They will be useless when using alone.

Other types of antimetastatic targets

Apart from aforementioned antimetastatic agents, other new metastasis-related targets and antimetastatic agents have also been emerging and studied [45-48]. More therapeutic benefits will be expected if more new targets and inhibitors have been studied in clinical trials. Apart from development of new drugs, it is the time to create new visions towards optimizing utilizations of anticancer and antimetastatic drugs in clinics.

Antimetastatic treatments according to metastatic cascade

Background

Since no major breakthrough is achieved in antimetastatic drug development and licensing, creating something new from clinical respects might be not a bad idea. One of promising work is antimetastatic treatments according to metastasis cascade.

Introductions of metastatic cascade

Spontaneous tumor metastases involve a fixed course of pathophysiological processes, and is a lengthy pathogenic process, occurring on the order of months in humans and weeks in mice [49-51]. Is it appropriate and beneficial that cancer metastasis treatment according to the stage of metastasis and organs [8]? This argument can be hypothesized from following scenarios.

Anatomy and pathology of metastatic cascade

Spontaneous metastasis is a lengthy pathogenic process, occurring on the order of a month in humans—encompassing at least seven distinctive substages (1) invade locally through surrounding extracellular matrix (ECM) and stromal cell layers, (2) intravasate into the lumina of blood vessels; (3) tumor cells survive the rigors of transport through the vasculature; (4) arrest at distant organ sites; (5) tumor cells extravasate into the parenchyma of distant tissues; (6) initially survive in these foreign microenvironments in order to form micrometastases, and (7) reinitiate their proliferative programs at distant sites, thereby generating macroscopic, clinically detectable neoplastic growths [5]. From anatomical and physiologic points of view, may the long-evolving course of a metastasis involving transitions through multiple organs and other tissues trigger diversified biochemical or molecular pathways in each substages [5].

Pharmacology and treatment analysis for metastatic cascade

Owing to widely diversified organs or tissues through metastatic cascade processes, different anticancer or antimetastatic drugs might act differently in each metastatic cascade stages. Finally improving cancer patients' survival intervals and rates, especially for late staged cancer patients might be achieved by this type of treatment [7-10]. Thus the pharmacologic or treatment considerations of cancer metastasis individually might be future trend and importance worldwide.

Previously, the idea for different pharmacological or therapeutic study of cancer metastasis cascade was proposed and reported [53-54]. These reports were based on the pharmacological study by many famous antimetastasis agents or drugs, such as Bisdioxopiperazine compounds (Biz).

Biz compounds originally developed in UK are the first ever antimetastatic drugs worldwide [55-56]. A series of Biz compounds (Biz) developed in the UK and China, have been found to be effective against a model of spontaneous metastasis (Lewis lung carcinoma, 3LL) [55-59]. (Figure 1) It was showed that probimane (Pro) and bimolane

(Bim) significantly inhibited the pulmonary metastasis of 3LL both following day-2 and day-8 injections, but razoxane (Raz) only significantly inhibited metastasis in the same model following day-2 injections. Thus each drug act differently at different stages of metastasis. From our early data of ^{14}C -probimane tracing and autoradiography, an obvious greater accumulation of Pro was found in tumor tissues, especially in metastatic foci [60-61]. It can explain why Pro more effectively inhibits metastasis than Raz through a stronger antiproliferative or apoptotic efficacy to formed metastatic foci [62].

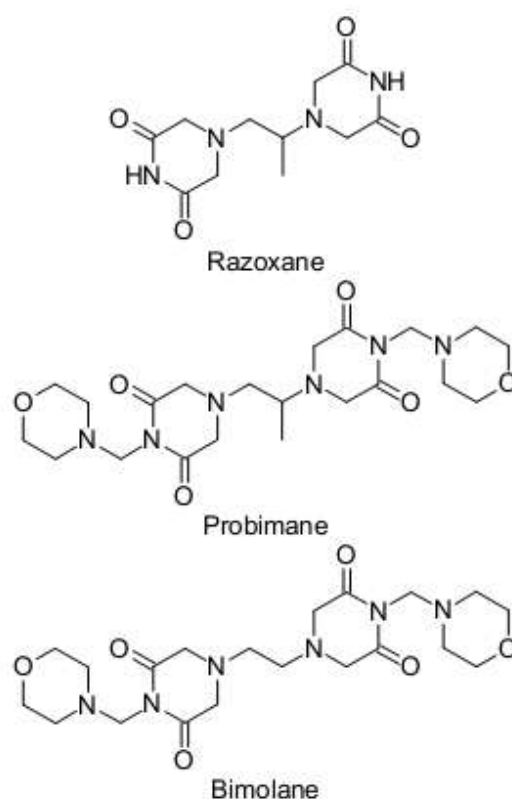


Figure 1. Structural formulae of three Biz compounds

Another important pathologic discovery is the early finding of organ-preference of metastases [63-64]. Similar results can also be found in early publications regarding differing cytotoxicities of drugs against tumor cells derived from various tissues [65]. Thus, tumor metastatic foci in different human organs might be targeted with different anticancer or antimetastatic drugs.

Molecular analysis of cancer metastasis cascade and related with therapy

Like aforementioned character of biology and physiologic of cancer metastasis cascade, aberrant biological molecules on cancer metastasis at different stages and organs might also be possible. This open question needs to be answered. Some recent findings may be useful for answering 安定 solving this question and are not understood in vain.

(i) Recently, it has been shown that there is a paradox feature of molecular aberrations between primary tumor lesions and metastatic foci formations. According to these authors, in the initial stage of metastatic cascade, primary tumors are transitioned from epithelial cells into mesenchymal cells (EMT). However, in the remote site of tumor metastatic formation, tumors are transformed from mesenchymal cells into epithelial cells (MET). The molecules of tumors Twist 1 and Prrx1 are up-regulated in primary tumors, yet down-regulated in metastatic nodules of same tumors [66-68]. **(Figure 2)** Thus a quick deduction is one anticancer or antimetastatic drug may produce contradictive actions between primary tumor lesions and metastatic nodules. This dilemma and pitfall need our further investigations, transcending and solved in clinical trials.

(ii) Other interesting question is the revisit "seed and soil" hypothesis [63-64]. Do anticancer drug sensitivities vary among different organ tumors from same primary tumor origin? How do these changes happen and can be overcome? Presently, no good clinical evidence leading to clinical successes are repeatable that dedicate to extend patients' survivals.

New insights into antimetastatic drugs for different stages and organs of metastatic cascades

Most antimetastatic agents finished in phase I or phase II clinical evaluations fail to become licensed drugs (proved to be effective after

phase III clinical treatment study). Many reasons may behind these scenarios. Some antimetastatic agents failing to inhibit neoplasm metastasis in phase III clinical investigations, as we may propose, only because of cursory or inaccurate design of therapeutic protocol. Many clinical drug evaluation failures might be due to antimetastatic treatment study not according to metastatic cascade. For this reason, good preclinical investigations in animals might improve clinical anticancer drug study by testifying this proposition. Improving antimetastatic compounds evaluation systems will increase the efficacies of antimetastatic drug development and clinical applications. In antimetastatic drugs targeting on circulatory tumor cells are even more difficult to clinical evaluations for assumptions of promoting human immunity [69]. Human immunity is however one of the most difficult evaluating criteria being measurable by algorithmic calculations. Owing to all these factors and drawbacks, the evaluation and clinical study of immune-promoters may be controversial. In future, animal models of both artificial and spontaneous can be borrowed to update clinical drug assessment and avoid false-positive or false-negative data of antimetastatic agents in clinical trials.

Relationship between pathology and therapy for metastatic cascade

To conclude, each drug or immuno-modulator might act differently within various stages of a metastatic course. In general, the MMPs inhibitors are proposed to be more active in preventing tumor cells from detaching from primary locations. Immuno-modulators might promote the activity of macrophages for killing tumor cells during the vascular and lymphatic circulation [69]. However, highly apoptic, angiogenesis or other potential agents such as probimane, might be more effective in treatment of formed metastatic foci in their

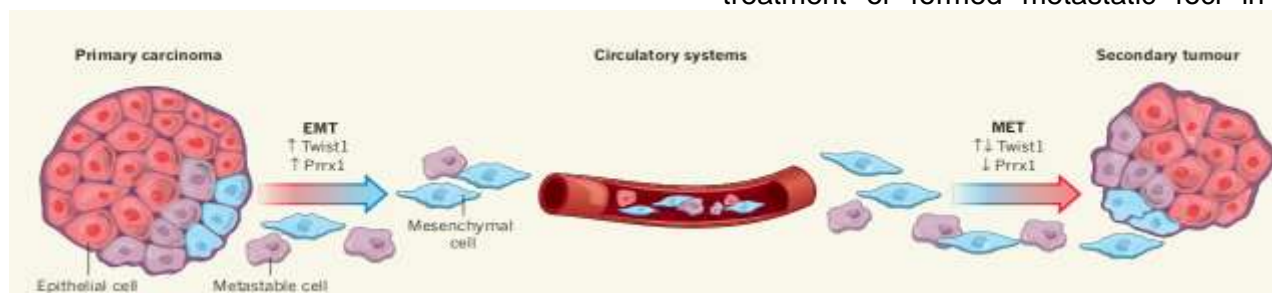
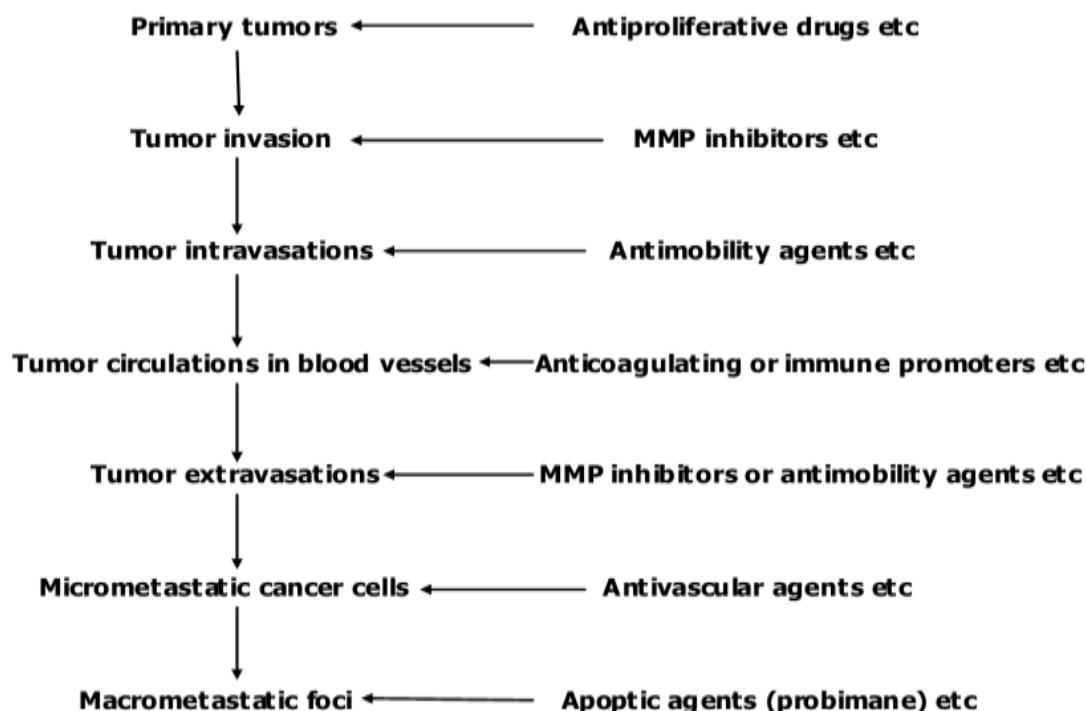


Figure 2. Diagram of neoplasm metastasis between primary tumors and remote metastasis formations (Copy from ref 68)

“preferred organs”[68-70].

Figure 3. Proposed strategy of using antimetastatic agents in clinics [8-10]



Discussion

Human tumors are mixed and intertwined with 6 different hallmarks of cancer (Table 2), and neoplasm metastasis is one of the hallmarks—the fatalist hallmark of cancer [71]. Hallmark of cancer invasion and metastasis is more related with the interactions between cancer and normal environment—human cells and tissues. Thus it is believed that good therapeutic schedules need different types of functioning, such as antiproliferative, apoptic or antimetastatic efficacies as a whole according to clinical bio- or pathological situations—metastatic cascade. Anticancer drugs combinations, a commonly applied way in clinical cancer trials must be adhered and updating strategies into scientific levels. Scientific rules of drug combination will be an emerging strategy of many personalized systems, such as drug sensitivity testing, cancer bioinformatics analysis and pharmacogenetic evaluation [19-22] and finally translating anticancer drug combination therapy from empirical into analytical data assistant strategies [24].

Table 2. Schematic diagram on biology and pathology mechanisms of cancer (Modified from Reference 71)

Hallmarks of cancer	Possible molecular or pathological mechanisms
Sustaining proliferative signaling	Oncogene mutation, cell or proliferative signal over working, environmental alteration etc
Resisting cell death	Apoptosis (caspases, Bcl-2, Bax etc) and autophagy
Inducing angiogenesis	Vascular or inflammatory factors (VEGF, TNF) etc
Evading growth suppressors	Tumor growth suppressors (RB, TP53) etc
Enabling replicative immortality	Telomerase
Invasion and metastasis	Tumor stromal or matrix (MMP), Immunological factors and function, angiogenesis, glycoproteins, blood coagulation, EMT and MET

Table 3. Roadmap for perfecting clinical antimetastatic therapy and improving therapeutic outcomes

Methods	Utilizations
Improvement of anticancer drug screening systems	Antimetastatic drug developments [10-15]
Drug administration or schedule analysis	Treatments with high efficiency by pharmacogenetics [21-22]
Mechanistic study of antimetastatic drugs	Better use of antimetastatic drugs [7-9]
Updating diagnostic studies in clinics	Find out if a patient needs antimetastatic drugs [72-77]
Metastatic cascade study	Properly use antimetastatic therapy in clinics [8]
Finding more active antimetastatic drugs—new generation of antimetastatic drugs	Against formed metastatic foci and cancer stages in patients [31-48]

Conclusion

Cancer metastasis is the key factor for cancer patient death. However, no good solution has been achieved until now for effectively control of cancer metastasis, especially for late-staged cancer patients. An ambitious roadmap for solving this dilemma is proposed (Table3). To conclude, optimizing clinical treatment protocols might be better based on the scenarios and staging of metastasis cascade rather than adherence on uniformed treatment schedule of present licensed antimetastatic drugs in cancer patients. This article proposes and reiterated on treatment of metastatic disease in the future according to pathological features in cancer patients and creating new type of individualized cancer therapy (ICT) [19-22].

Future direction

- About metastatic cascade, any uncharted cascade steps or aberrant molecules leading to drug responses to cancer metastasis should be future discoveries.
- Genetic study of the relationship between neoplasm metastasis and its therapies by next generation sequencing (NGS) techniques [78-80] might be an avenue for developing new generations of effective antimetastatic drugs.
- In depth study the molecular basis of drug targets and its applications for formed metastatic nodules.
- Despite anticancer drug combinations are mostly better than single anticancer drugs, most of these practice however lack systematic and in-depth therapeutic mechanism quests and this leads to clinical anticancer and antimetastatic drug combination therapies based on doctors' past experience and

references rather than scientific-based anticancer drug combinations [21-24]. Finding undiscovered law regarding combinations of anticancer and antimetastatic drugs and therapies in clinical cancer treatments is indispensable.

- Exploiting novel drug targets related with neoplasm metastasis [31-48]. Thus cancer patients with tumor metastases can be better treated.

- Updating relevant individualized or personalized cancer therapy systems and making clinical cancer therapies transformed from empirical to science-based therapeutic norms or strategies [19-22].

Metastasis cascade, as a possible therapeutic targets invite new insights and perspectives. If more these efforts and studies are undertook, marked difference in cancer treatments can be made in future. Let's do something great.

Conflict of interests

The authors declare there is no conflict of interests with other institutes and funds.

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