Brain Metastases: State of the Art and Innovative Targeted Therapies

Maria Caffo[1], Lucia Merlo[2], Valeria Barresi[3], Ema Tot[1] and Gerardo Caruso[1*]

[1]*Neurosurgical Clinic, Department of Neuroscience, School of Medicine, University of Messina, Italy*

[2]*Department of Neurosurgery, Trieste, Italy*

[3]*Department of Human Pathology, School of Medicine, University of Messina, Italy*

Abstract: Brain metastasis represents the most common intracranial tumor. The metastatic process involves the migration of a cancer cell from the bulk tumor into the surrounding tissue, extravasation from the blood vessels into the tissues, and formation of a secondary tumor. Patients affected by brain metastases are in need of a multidisciplinary approach that generally includes surgical treatment and radiation therapy. Conventional chemotherapies have generally produced disappointing results, possibly due to their limited ability to penetrate the blood-brain barrier. With new data regarding the biology of brain metastases, novel targeted therapies can be considered interesting and promising therapeutic options. Targeted therapies showed improved survival in patients with metastatic disease. The advent of new technologies such as graphene nanoparticles has led to the discovery of novel pathways that allow a better delivery of the therapeutic compounds to the brain.

Keywords: Angiogenesis, brain metastases, graphene, microRNA, nanoparticles, targeted therapy.

INTRODUCTION

Metastatic brain tumors are the most common intracranial neoplasms in adults occurring in up to 30% of patients [1-2]. Improved imaging techniques, a more frequent use of magnetic resonance imaging (MRI) in the upfront staging and follow-up have allowed a better detection of brain metastases (BM), thus increasing their incidence. The vast majority of BM occurs in the cerebral hemispheres (80%) with a smaller percentage appearing in the cerebellum (15%) and the brainstem (5%) [3]. In the adult, brain metastases commonly arise from primary tumors of the lung (40%-50%), breast (15%-25%), skin (melanoma) (5%-20%), kidney and gastrointestinal tract (4%-6%) [4]. Over two thirds of patients with BM show important neurologic symptoms, including headaches, focal weakness, cognitive dysfunction, and seizures.

Current therapeutic approaches for BM include surgery, whole-brain radiation therapy (WBRT), stereotactic radiosurgery (SRS), chemotherapy, or a combination of these. The use of radiation therapy and surgery represent a good option for the treatment of many BM. However, these treatments show various limitations caused by both their acute and delayed adverse effects and the peculiar location and characteristics of some tumor. Strong positive prognostic factors include good functional status, age < 65 years, no sites of metastasis outside of the CNS, controlled primary tumor, presence of a single BM, and a long time period from primary diagnosis to brain relapse [5]. Survival for patients treated with WBRT typically ranges from 4 to 6 months, but can be as long as 12-24 months for some of them [6]. The combination of radiotherapy and chemotherapy improves response rate and/or progression-free survival (PFS) but not overall survival (OS) [7-8]. Administration of therapeutic compounds is, however, limited by the presence of the blood-brain barrier (BBB) [9].

Recently, the introduction of targeted therapies in the management of cancer has showed interesting results. Targeted therapies block the activation of oncogenic pathways, either at the ligand-receptor interaction level or by inhibiting downstream signal transduction pathways, thereby inhibiting growth and progression of cancer [10]. Because of their specificity, targeted therapies should present a better efficacy and a reduced cytotoxicity than systemic chemotherapy or radiotherapy.

METASTATIC STEPS

Various biological processes participate to the development of BM including tumor cell survival in the vascular system and extravasation of those cells to a host organ [4]. The initial step is characterized by the migration of neoplastic elements away from the primitive tumor. This process is strictly regulated by the cell-cell interactions and cell-substrate adhesions [11]. This step alters the normal adhesion processes of the extracellular matrix (ECM) with E-cadherin-catenin

*Address correspondence to this author at the Neurosurgical Clinic, Department of Neuroscience, University of Messina, A.O.U. Policlinico "G. Martino", via C. Valeria 1, 98125, Messina, Italy; Tel: +39-090-2217167; Fax: +39-090-693714; E-mail: gcaruso@unime.it

ISSN: 1927-7210 / E-ISSN: 1927-7229/15 © 2015 Lifescience Global

complexes [12]. A switch in a tumor cell's expression of cadherins promotes the escape of neoplastic cells from the bulk tumor facilitating their binding to the surrounding tissues [12-13]. During disease progression, neoplastic cells activate resident fibroblasts and macrophages, and attract circulating monocytes and platelets to the primary tumor. Various enzymes including stroma-associated reactive cytokines, chemokines, growth factors and matrix metalloproteinases (MMPs) mediate angiogenesis and the degradation of basement membrane, promoting local tumor growth and invasion [14-16]. These events are checked by gene products of metastatic cells, and by paracrine interactions between cancer and stromal cells [14-16]. Before reaching the central nervous system (CNS), metastatic cells acquire mutations that lead to genetic instability, increased invasive potential, and resistance to hypoxia and cell death. Reduced expression of E-cadherin-catenin complex, a prime mediator of cell-cell adhesion, has been associated with tumor invasion, metastasis, and unfavorable prognosis [17]. Organ-dependent differences in growth factors, chemokines, cytokines, and matrix proteins, as well as stromal and immune components, may distinctly affect tumor cell growth and endothelial behavior in the mammary fat pad versus the brain and may synergize with or antagonize growth-promoting functions of specific activated tumor cells [18]. Of the various primary malignancies, lung, breast, renal, and colorectal cancers and melanoma are the main sources of metastasis to the brain [19].

The development of a BM is dependent on angiogenesis. An altered angiogenesis results in structural and functional abnormality of tumor associated blood vessels, characterized by defective endothelial cells, pericyte covering and basement membranes. The early steps of angiogenesis include degradation of the endothelial basement membrane and surrounding ECM, and directed migration of endothelial cells into the surrounding stroma. Although a plethora of molecules, such as acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), transforming growth factor alpha and beta (TGF-α and -β), and tumor necrosis factor alpha (TNFα), can act as inducers of angiogenesis, the main growth factors specific for vascular endothelium include members of the VEGF and angiopoietin families [11].

CURRENT TREATMENTS FOR BRAIN METASTASES

The treatment of BM requires a multidisciplinary approach tailored to each patient. The treatment algorithm for BM changes depending on factors such as primitive tumor histology and clinical characteristics of patients, as well as with the therapeutics options available. Many patients are treated with a combination of treatments, and therapeutic decisions must take into account factors such as patient's age, functional status, primitive tumor type, extent of extracranial disease, and number of intracranial lesions [11].

Surgery is used in patients with a single metastasis, and minimal or controlled systemic tumor. Surgical resection allows fast debulking of large, immediately life-threatening tumors, thus making it advantageous for patients with neurological signs and symptoms related to the metastatic disease. Additional surgical evaluations must include accessibility and size of the lesion, as well as its relative proximity to the eloquent brain. Surgery relieves mass effect and symptomatic intracranial hypertension, restores CSF flow, and lowers steroid dependence through peritumoral edema reduction. Pharmacoresistant seizures due to BM are an indication for surgical resection. Moreover, surgical resection allows a certain pathological diagnosis. When considering older patients with borderline performance status, multiple medical co-morbidities, uncontrolled and/or diffuse extracranial disease, and multiple metastatic lesions, the indications for surgical resection of BM are less clear. Patchell *et al*. prospectively evaluated 48 patients with a single brain metastasis who underwent surgical resection followed by WBRT or WBRT only. The median survival time of patients in the surgical group followed by WBRT was significantly longer than that of the radiotherapy alone group (40 versus 15 weeks, $P < 0.01$) [20]. Class I evidence in support of surgical resection followed by WBRT is currently available for patients with a newly diagnosed solitary brain metastasis, without advanced systemic disease [21]. Surgical technique has also been the object of various improvements. The use of "en bloc" techniques could reduce the risk of tumor recurrence and leptomeningeal tumor spread [22]. Recently, various advanced neurosurgical techniques have permitted a reduction of morbidity enhancing the benefit of surgical treatment. Additional intra-operative techniques include awake craniotomy and neurophysiological monitoring for functional assessment during resection, as well as other optical and molecular visualization technologies, including 5-aminolevulinic acid (5-ALA) fluorescence or fluorescein staining of malignant tissues within the operative bed [23]. The role of surgery in the management of multiple BM is still controversial. Traditionally, the most important factor to

consider when selecting patients for resection of multiple BM is the extent of the systemic cancer spread [24]. The identification of multiple BM is considered a contraindication to surgical intervention. In a retrospective study, Bindal *et al*. showed that patients undergoing excision of multiple BM had significantly longer survival than patients who underwent partial tumor resection [25]. In another study, the authors proved that, in patients with multiple BM treated, an age greater than 60 years, a Karnofsky performance score (KPS) of less than 70, an incomplete surgical removal, and the presence of extensive systemic cancer spread were significantly associated with shorter survival times [26].

The radiotherapeutic treatment doesn't show effects on the overall survival, but seems to be associated with a prolonged progression-free survival with better control of seizures [27]. Moreover, in many patients undergone to a WBRT has been evidenced a decline in the quality of life and a considerable neurocognitive sequelae [28]. The neurocognitive symptoms associated with WBRT is a moderate-to-severe dementia that occurs from several months to years after the treatment [28-29]. Based on class I and class II evidence, surgical resection followed by WBRT is an effective treatment for patients in good general condition with a single and surgically accessible BM [30]. In multiple BM, WBRT permits the control, in 70%–90% of cases, of presenting neurological symptoms, without causing acute neurological side effects [31]. Prophylactic cranial irradiation (PCI) in patients with small cell lung cancer (SCLC) has been investigated as a strategy to prevent dissemination to the brain. PCI resulted in a reduction in the incidence of BM from 18% to 8%, but did not impact overall survival [32].

Radiosurgery consists in the delivery of a high dose of ionizing radiation, usually in a single fraction, on a targeted volume. Stereotactic radiosurgery (SRS) uses a linear accelerator (LINAC) or multiple cobalt-60 sources (gamma-knife) directed to targets of maximum diameter of 3-3.5 cm. The local tumor-control rate was high after SRS, with an 85% control rate at 1 year, and a 65% control rate at 2 years [33]. The RTOG 9005 prospective study outlined the appropriate and safe doses of SRS, based on lesion size. This study showed that prescription doses of 15, 18, and 24 Gy to the tumor margin were the maximal safe doses for lesions with diameters of 31 to 40, 21 to 30, and 20 mm or less [34]. SRS is particularly useful for patients unable to tolerate surgery and for patients with lesions inaccessible to surgery. However, SRS as sole first-line treatment carries a risk of failure in non-treated brain regions, which has resulted in controversy around the indications for adding WBRT. SRS has been reported effective in the treatment of BM resistant to conventional radiation therapy, such as melanoma and renal cell carcinoma. Prognostic factors in patients receiving SRS were the KPS score, total intracranial volume and the presence of active systemic disease. Recent data established that the addition of WBRT to SRS significantly improves local tumor control, although an overall survival benefit has not been demonstrated. Pirzkall *et al*., in a retrospective analysis of 236 patients, reported no significant difference in median survival times between SRS alone and SRS plus WBRT [35]. A randomized trial evaluating the use of SRS with or without WBRT showed no difference in survival (8.0 months for SRS versus 7.5 months for SRS plus WBRT; P = 0.42); however, the trial was not designed to detect a significant difference in OS [36]. Cyberknife (CK) is a radiotherapeutic treatment that can give higher therapeutic doses directly to the tumor. CK shows the natural technical advantage in fixation, real-time authentication and dynamic tracing. Chang *et al*. using CK, radiated single-fractionated doses of 10-36 Gy in 84 BM in 72 patients, obtaining a tumor control rate of 95% and a radiation damage rate of 4% [37]. In 40 patients affected by BM, one week after CK treatment, symptomatic improvement occurred in 90.0% of patients, with a 77.8% local control rate at three months, a therapeutic effective rate of 94.1%, and a one-year survival rate of 67.5% [38].

Chemotherapy show a limited role in the treatment of BM. Treatment efficiency is determined by the sensitivity of tumor cells to the chemotherapeutic agents. In patients with BM from breast cancer, cisplatin and etoposide show a high objective response rate of 55% [39]. Topotecan penetrates the BBB and has been evaluated in 20 SCLC patients with asymptomatic BM after failure of first-line chemotherapy suggesting that topotecan induces a high response rate in SCLC BM [40-41] Temozolomide (TMZ) is an orally administered alkylating agent. Clinical activity of TMZ is closely linked to the activity of O6-alkylguanine-DNA alkyltransferase, a DNA repair protein which removes O6-alkylguanine adducts from DNA. TMZ, because of its small molecular weight, crosses the BBB, and, being also associated with a low incidence of adverse events. Paul *et al*. [42] reported that, among 40 patients with advanced melanoma treated with TMZ, the incidence of CNS recurrence was

lower in patients treated with TMZ. In a recent review of published clinical trials, the authors evidenced that TMZ, when used as a single agent usually achieved only a small number of good outcomes. On the other hand, the combination of TMZ with WBRT or other therapeutic compounds showed the potential to improve clinical outcomes of patients with BM [43].

MOLECULAR TARGETED THERAPY

Targeted therapies block the oncogenic pathways inhibiting the growth and progression of cancer. Recent progresses in the tumor biology have led to the identification of specific molecular steps such us, insertions and/or deletions within the EGFR gene and EML4–ALK chromosomal translocation in lung cancer, BRAF mutation in melanoma, as well as amplification of the ERBB2 gene (encoding HER2) and HER2 protein over expression in breast cancer.

Human epidermal growth factor receptor 2 (HER2) overexpression stimulates the outgrowth of neoplastic cells of breast cancer in the brain [44]. Trastuzumab is a humanized monoclonal antibody that targets the extracellular domain of HER2. In a retrospective study, Nam and colleagues reported, in patients affected by BM from HER2-positive breast cancer and treated with trastuzumab, a median overall survival (OS) of 13 months. This data reduced to 4 months in patients not treated and to 3 months in patients HER2-negative [45]. Trastuzumab may act synergistically with radiotherapy in patients HER2 positive. [46]. Intrathecal administration of trastuzumab in monkeys showed no related effects evidencing possible new application of this compound [47].

Lapatinib can cross the BBB and inhibits the EGFR and HER2. A recent study demonstrated that lapatinib represents a valuable option in Japanese patients HER2 positive affected by BM and after cranial irradiation [48].

Erlotinib and gefitinib are reversible inhibitors of the tyrosine kinase domain of EGFR. These compounds are efficacy in patients with recurrent NSCLC and for patients with advanced NSCLC. The use of Gefitinib has evidenced interesting results as a first-line and second-line treatment in terms of overall response rate and progression-free survival for advanced NSCLC patients harboring sensitive *EGFR* mutations [49]. Some initial case reports have showed activity of gefitinib and erlotinib on BM from NSCLC, suggesting a potential role of TKI in the treatment of NSCLC patients with BM [50-52].

Sorafenib is an orally active multikinase inhibitor that blocks intracellular kinases involved in tumor proliferation and angiogenesis. A recent study reported five cases of intracerebral hemorrhage in patients affected by metastatic renal cell carcinoma (RCC) with BM following treatment with sorafenib or sunitinib. This data confirms that antiangiogenic treatments, in patients affected by BM, may increase the risk for CNS hemorrhage [53]. Sorafenib has also been used, with interesting results, in the treatment of patients with BM of thyroid carcinoma [54]. Sunitinib is a small molecule that inhibits VEGFRs types 1 and 2, PDGFR-α and PDGFR-β, the stem cell factor receptor c-KIT, the FLT3 and RET kinases. Sunitinib exhibits potent antiangiogenic and antitumor activity. Interesting results have been evidenced in patients affected by metastatic renal cell carcinomas and gastrointestinal stromal tumors. In an open-label expanded study for the use of sunitinib in metastatic RCC, a positive response was demonstrated also in patients with BM. In addition, 52% of patients had stable disease for at least three months [55]. In a phase II study, antitumor activity and safety of sunitinib were evaluated with promising results in patients with previously irradiated BM [56].

Dabrafenib is a reversible adenosine triphosphate-competitive inhibitor that selectively inhibits the BRAFV600E kinase. Dabrafenib decreases phosphorylated ERK and causes cell cycle arrest. A phase I study showed a good tolerable and safe of dabrafenib in patients affected by BM of melanoma [57]. The results of a phase II BM trial (BREAK-MB)11 [58-59] suggest that dabrafenib may be an effective adjunct for the treatment of BM, and that it is worthy of consideration as first-line therapy in patients with BM and advanced extracranial disease [59]. Dabrafenib was also evaluated in a multicentric, open-label, phase 2 trial, in patients with Val600Glu or Val600Lys BRAF-mutant melanoma and BM. The results showed that dabrafenib is well tolerated and can represent a valid therapeutic strategy in patients with BRAF-mutant melanoma with BM [60].

Vemurafenib has a strong antitumor effect against melanoma cell lines with the BRAFV600E mutation [61-62]. A phase I study in patients with BM showed intracranial activity, [63] and a phase II study is currently underway (NCT01378975).

Bevacizumab is a monoclonal antibody that binds VEGF-b, inhibiting angiogenesis. Inhibition of VEGF by bevacizumab will not only affect endothelial cells but

also the tumor vasculature, suppressing both new blood vessel growth and the existing vasculature. The evidence of intracranial hemorrhage was reported in a phase II trial on bevacizumab for NSCLC in patients developing cerebral metastasis [64]. Findings from a prospective phase II trial showed that the addition of bevacizumab to various chemotherapy agents in patients with NSCLC and BM is safe, even though a low incidence of CNS hemorrhage was reported [65]. De Braganca *et al*. suggests that bevacizumab administration for the treatment of BM may be consider safe and effective, especially for small lesions [66].

DISCUSSION

BM currently represents an important cause of cancer morbidity and mortality. Although many patients with BM die as a result of extracranial disease progression, a significant amount suffers from local tumor progression in the CNS. Surgery, WBRT, stereotactic radiotherapy and systemic methods have been integrated for a multimodal treatment. Delivering concomitant chemotherapy may improve local tumor control but does not improve overall survival and is thus not recommended for the routine treatment of BM patients. Class II evidence suggests that larger lesions (>3 cm) or those causing significant mass effect (>1 cm midline shift) may have better outcomes with surgical resection, whereas radiosurgery may offer slightly better local control rates for radioresistant lesions. Tumors are biologically heterogeneous and contain subpopulations of cells with different angiogenic, invasive, and metastatic properties. Additionally, some primary and secondary tumors may have unique biological signatures that may respond to targeted agents and biological modulation. Considering the multitude of signaling pathways regulating the proliferation and cellular survival/cell death, the inhibition of a singular target could not be sufficient to suppress neoplastic progression [9].

Recent studies have demonstrated the ability of microRNAs (miRNAs) to distinguish normal from cancerous cells, primary tumors from secondary tumors, and characterize the tissue of origin of BM [67]. This data suggests that miRNA profiling may be used to identify cancer cell origin and its response to therapy. MiRNAs are a large class of small non-coding RNAs, 19-25 nucleotides long, produced naturally in cells after being cut into segments from larger strands of RNA. They negatively regulate gene expression at the post-transcriptional level by base pairing to the 3' untranslated region of target messenger RNA (mRNA). MiRNAs can function either as tumor suppressors or as oncogenes and initiate tumor growth, invasion, metastases, the process of epithelial to mesenchymal transition (EMT), as well as regulate the overall stemness of cancer cells [68]. A recent study successfully identified the tumor of origin in 84% of BM using a quantitative real-time polymerase chain reaction (qRT-PCR) of 48 different miRNAs [69]. The role of miRNAs in the biology of brain metastases has been established in studies investigating a number of primary tumor types [70-71]. In breast cancer, miR-1258 alterations were directly related to the expression of heparanase, a prometastatic enzyme found in brain metastatic breast cancer cells that permits to neoplastic cells to cross the BBB [72-73]. MiRNA-328 in non-small cell lung cancer (NSCLC) regulated cell migration and formation of BM through altered expression of the *PRKCA* genes [74]. MiRNA-200 family members were elevated in the CSF of patients with metastatic brain lesions [75]. It has been proved that one of the first steps in tumor progression may be mediated through the acquisition by cancer cells of an EMT phenotype, a process during which epithelial cells lose their cell-to-cell contacts and, subsequently, attain characteristics of a mesenchymal phenotype. These cells detach from the primary tumor site and enter the vascular district, a step believed to be responsible for tumor cell metastasis [76]. The acquisition of an EMT phenotype could be regulated by deregulation in the expression of miRNAs in the context of metastasis [77].

Recently, nanotechnology has provided a interesting opportunity to treat cancer through careful engineering of nanomedicines that specifically interact with neoplastic cells. This modality permits the use of lower doses of drugs and of a selective drug delivery to target tumor cells [78-79]. Nanomedicine shows the ability to target multiple tumor markers and deliver multiple agents simultaneously thus providing the chance to obtain a combined effect in addressing the challenges of cancer heterogeneity and adaptive resistance. Although nanotechnologies show a great potential, there are some questions about the nanoparticles' possible adverse effects on human health. Little is known about the long-term effects of exposure to nanomaterials, especially in clearance organs such as the liver, spleen, and kidneys. The develop of graphene nanoparticles platforms addressed to different pathways can be of great help for a more efficient treatment of BM. Graphene is an innovative and versatile two-dimensional nanomaterial whose properties are the focus of increasing

researches [80]. In recent years, graphene has becoming widely applied in targeting controlled drug/gene delivery as well as photothermal and photodynamic cancer therapy, biosensing and bioimaging [81]. New treatments barely defeat malignant brain tumors, which represent, among all, the most important challenge: the particular environment they develop being hard to be reached by most of the molecules known today due to the presence of the BBB. A compound structured by transferrin (Tf) conjugated with PEGylated nanoscaled graphene oxide and loaded with doxorubicin (Dox) was recently evaluated [82]. The complex Tf-PEG-GO-Dox showed a better intracellular delivery, efficiency and stronger cytotoxicity against C6 glioma cells. The life span of tumor bearing rats after the administration of Tf-PEG-GO-Dox was significantly extended when compared to rats treated with saline, Dx, and PEG-GO-Dox. Moreover, an innovative complex, characterized by a nanometer-scale magnetic GO drug carrier (NMGO-mPEG) that can carry Epirubicin (EPI) to form NMGO-mPEG-EPI and be monitored by magnetic resonance imaging and be guided by magnetic targeting to reach local toxic drug concentrations, was evaluated [83]. This compound combined targeted chemothermal therapy using MRI-guidance and irradiation by low focused ultrasounds. The study evidenced a highly efficient hyperthermia, a high drug capacity, a specific targeting ability, and molecular imaging, all aspects important in the process of overcoming problems associated with current chemotherapy and hyperthermia glioma treatments. Chlorotoxin, a 36-amino acid peptide isolated from the venom of the giant Israeli scorpion, has showed particular interest due to its high selectivity for gliomas through the binding to matrix metalloproteinase-2. In a recent study [84], a complex characterized by chlorotoxin and GO was developed for delivery of the anticancer drug doxorubicin. The cytotoxicity study demonstrated a higher glioma cells death induction rate when compared to GO/Dox and free doxorubicin. The mechanisms of graphene-mediated photothermal killing of brain cancer cells apparently involved oxidative stress. Despite lower NIR-absorbing ability, a suspension of polyvinylpyrrolidone-coated graphene sheets exposed to NIR radiation generated more heat than carbon nanotubes under the same conditions. Subsequently, graphene NP performed significantly better in inducing photothermal death of U251 human glioma cells *in vitro*. The superior photothermal sensitivity of graphene sheets could be explained by their better dispersivity [85].

CONCLUSION

The most important problem in the development of new and more efficient approaches to the management of brain metastases is the lack of information about the biology of the metastatic disease of the brain. Nowadays only surgical treatment and radiation therapy show important roles in the management of metastatic brain tumors. In the past two decades important progresses have been made only for patients with single or oligometastatic brain metastases and a well-controlled systemic disease. The use of targeted and biological therapies for brain metastasis is relatively uncommon because few therapeutic compounds are available for the treatment of patients affected by BM. Various experimental studies are trying to identify gene expression profiles in various cancers for the acquisition of new data on tumor biology. These new information will permit the comprehension of how some tumors metastasize to the brain. Promising biologically targeted agents, based on new data on tumor molecular biology, include the crizotinib in NSCLC with the EML4–ALK translocations, and especially to second generation ALK inhibitors such as LDK378. In addition, the combination of trastuzumab and lapatinib has been proven efficient in HER2-positive breast cancer. Finally, a very interesting treatment option, in patients with intracranial melanoma metastasis, is the use of dabrafenib and vemurafenib, potent inhibitors of BRAF-mutant melanoma cell function, and the immunomodulatory agent ipilimumab. Also of interest are chemoprevention strategies and methods for enhancing CNS drug delivery through manipulation of multidrug resistance pathways in the brain. In this field nanotechnologies could represent a valid tool. In addition, microRNA and even exosomal analysis might allow the genetic profiling of the metastatic disease and the prediction of its response to therapy through blood-based screening protocols.

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Received on 14-04-2015 Accepted on 16-05-2015 Published on 07-09-2015

http://dx.doi.org/10.6000/1927-7229.2015.04.03.4

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